



**THEME [KBBE.2013.1.2-09 KBBE.2013.1.2-09]  
[Diversification of fish species and products  
in European aquaculture Diversification of fish  
species and products in European aquaculture]**

Grant agreement for: Collaborative project\*

<b>Annex I - "Description of Work"</b>
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Project acronym: DIVERSIFY

Project full title: " Exploring the biological and socio-economic potential of new/emerging candidate fish species for the expansion of the European aquaculture industry "

Grant agreement no: 603121

Version date: 2018-08-08

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# A1: Project summary

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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One form per project

## General information

Project title <sup>3</sup>	Exploring the biological and socio-economic potential of new/emerging candidate fish species for the expansion of the European aquaculture industry		
Starting date <sup>4</sup>	01/12/2013		
Duration in months <sup>5</sup>	60		
Call (part) identifier <sup>6</sup>	FP7-KBBE-2013-7-single-stage		
Activity code(s) most relevant to your topic <sup>7</sup>	KBBE.2013.1.2-09: Diversification of fish species and products in European aquaculture	KBBE.2013.1.2-09: Diversification of fish species and products in European aquaculture	

## Abstract <sup>9</sup>

The European aquaculture is a modern industry employing 190,000 people, with a €7 billion ex-farm value. This sector is well situated to be among world leaders in the efficient and sustainable production of safe seafood of the highest quality and nutritional value, taking into account consumer preferences and the large diversity of aquatic products from the wild. DIVERSIFY identified a number of new/emerging finfish species, with a great potential for the expansion of the EU aquaculture industry. The emphasis is on Mediterranean or warm-water cage culture, but also addressed are cold-water, pond/extensive and fresh water aquaculture. These new/emerging species are fast growing and/or large finfishes, marketed at a large size and can be processed into a range of products to provide the consumer with both a greater diversity of fish species and new value-added products. DIVERSIFY focuses on meagre (*Argyrosomus regius*) and greater amberjack (*Seriola dumerili*) for warm-water marine cage culture, wreckfish (*Polyprion americanus*) for warm- and cool-water marine cage culture, Atlantic halibut (*Hippoglossus hippoglossus*) for marine cold-water culture, grey mullet (*Mugil cephalus*) a euryhaline herbivore for pond/extensive culture, and pikeperch (*Sanders lucioperca*) for freshwater intensive culture using RAS. These species were selected based both on their biological and economical potential, and to cover the entire European geographic area and stimulate different aquaculture types. In collaboration with a number of SMEs, DIVERSIFY will build on recent/current national initiatives for species diversification in aquaculture, in order to overcome the documented bottlenecks in the production of these species. The combination of biological, technological and socioeconomic research planned in DIVERSIFY are expected to support the diversification of the aquaculture industry and help in expanding production, increasing aquaculture products and development of new markets.

# A2: List of Beneficiaries

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## List of Beneficiaries

No	Name	Short name	Country	Project entry month <sup>10</sup>	Project exit month
-35	Hungarian Aquaculture Association	MASZ	Hungary	1	43
-17	NASJONALT INSTITUTT FOR ENAERINGS-OG SJOMATFORSKNING	NIFES	Norway	1	49
1	HELLENIC CENTRE FOR MARINE RESEARCH	HCMR	Greece	1	60
2	FUNDACION CANARIA PARQUE CIENTIFICO TECNOLOGICO DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA	FCPCT	Spain	1	60
3	INSTITUT DE RECERCA I TECNOLOGIA AGROALIMENTARIES.	IRTA	Spain	1	60
4	ISRAEL OCEANOGRAPHIC AND LIMNOLOGICAL RESEARCH LIMITED	IOLR	Israel	1	60
5	THE UNIVERSITY COURT OF THE UNIVERSITY OF ABERDEEN	UNIABDN	United Kingdom	1	60
6	STICHTING WAGENINGEN RESEARCH	DLO	Netherlands	1	60
7	HAVFORSKNINGSINSTITUTTET	IMR	Norway	1	60
8	INSTITUTO ESPANOL DE OCEANOGRAFIA	IEO	Spain	1	60
9	Université de Lorraine	UL	France	1	60
10	TECHNISCHE UNIVERSITEIT EINDHOVEN	TU/e	Netherlands	1	60
11	AARHUS UNIVERSITET	AU	Denmark	1	60
12	ASOCIACION EMPRESARIAL DE PRODUCTORES DE CULTIVOS MARINOS - APROMAR	APROMAR	Spain	1	60
13	UNIVERSITA DEGLI STUDI DI BARI "ALDO MORO"	UNIBA	Italy	1	60
14	INSTITUT FRANCAIS DE RECHERCHE POUR L'EXPLOITATION DE LA MER	IFREMER	France	1	60
15	UNIVERSIDAD DE LA LAGUNA	ULL	Spain	1	60
16	UNIVERSITE DE NAMUR ASBL	FUNDP	Belgium	1	60
17	HAVFORSKNINGSINSTITUTTET	IMR/NIFES	Norway	50	60
18	FUNDACION CENTRO TECNOLOGICO ACUICULTURA DE ANDALUCIA	CTAQUA	Spain	1	60
19	CONSELLERIA DO MAR - XUNTA DE GALICIA	CMRM	Spain	1	60

# A2: List of Beneficiaries

No	Name	Short name	Country	Project entry month <sup>10</sup>	Project exit month
20	SKRETTING AQUACULTURE RESEARCH CENTRE AS	SARC	Norway	1	60
21	DANMARKS TEKNISKE UNIVERSITET	DTU	Denmark	1	60
22	STERLING WHITE HALIBUT AS	SWH	Norway	1	60
23	ICHTHYOKALLIERGEIES ARGOSARONIKOU ANONYMI ETAIRIA	ARGO	Greece	1	60
24	AZIENDA AGRICOLA ITTICA CALDOLI	ITTICAL	Italy	1	30
25	DOR DGEY YAM LTD	DOR	Israel	1	60
26	VAS. GEITONAS & CO LTD EE	GEI	Greece	1	60
27	AQUACULTURE FORKYS AE	FORKYS	Greece	1	60
28	CANARIAS EXPLOTACIONES MARINAS SL	CANEXMAR	Spain	1	60
29	ASIALOR SARL	ASIALOR	France	1	30
30	CULMAREX SA	CULMAREX	Spain	2	16
31	IRIDA AE-PRODUCTS FOR ANIMAL PRODUCTION-SERVICES	IRIDA	Greece	1	60
32	Ayuntamiento de A Coruna	MC2	Spain	1	60
33	SYNDESMOS ELLHNIKON THALASSOKALLIERGEION SOMATEO	FGM	Greece	1	60
34	BUNDESVERBAND DER DEUTSCHEN FISHINDUSTRIE UND DES FISCHGROSSHANDELS E.V.	BVFi	Germany	1	60
35	MAGYAR AKVAKULTURA ES HALASZATI SZAKMAKOZI SZERVEZET	MAHAL	Hungary	43	60
36	ASOCIACION NACIONAL DE FABRICANTES DE CONSERVAS DE PESCADOS Y MARISCOS-CENTRO TECNICO NACIONAL DE CONSERVACION DE PRODUCTOS DE LA PESCA	ANF	Spain	1	60
37	EUROPEAN FOOD INFORMATION COUNCIL AISBL	EUFIC	Belgium	1	60
38	KENTRO MELETON AGORAS KAI KOINIS GNOMIS ANONIMI EMPORIKI ETAIRIA	HRH	Greece	1	60
39	FISH 2 BE NV	F2B	Belgium	33	60
40	GALAXIDI MARINE FARM AE	GMF	Greece	35	60

# A3: Budget Breakdown

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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One Form per Project

Participant number in this project <sup>11</sup>	Participant short name	Fund. % <sup>12</sup>	Ind. costs <sup>13</sup>	Estimated eligible costs (whole duration of the project)					Requested EU contribution
				RTD / Innovation (A)	Demonstration (B)	Management (C)	Other (D)	Total A+B+C+D	
-35 (UTRO)	MASZ	50.0	S	0.00	0.00	6,000.00	13,800.00	19,800.00	19,800.00
-17 (UTRO)	NIFES	75.0	A	192,033.00	0.00	8,400.00	0.00	200,433.00	152,424.00
1	HCMR	75.0	A	1,427,700.00	0.00	237,841.00	92,972.00	1,758,513.00	1,401,588.00
2	FCPCT	75.0	T	871,430.40	0.00	11,640.00	0.00	883,070.40	665,212.00
3	IRTA	75.0	A	942,943.00	0.00	27,235.00	26,211.00	996,389.00	760,653.00
4	IOLR	75.0	T	556,945.60	0.00	12,352.00	0.00	569,297.60	430,061.00
5	UNIABDN	75.0	T	276,416.00	0.00	9,696.00	0.00	286,112.00	217,008.00
6	DLO	75.0	A	375,944.00	0.00	18,000.00	0.00	393,944.00	299,958.00
7	IMR	75.0	A	740,347.00	0.00	14,999.00	22,001.00	777,347.00	592,260.00
8	IEO	75.0	T	538,267.20	0.00	9,803.20	19,200.00	567,270.40	432,702.00
9	UL	75.0	T	264,056.00	0.00	14,780.80	21,120.00	299,956.80	233,942.00
10	TU/e	75.0	A	336,702.00	0.00	15,353.00	0.00	352,055.00	267,879.00
11	AU	75.0	T	381,137.60	0.00	12,260.00	0.00	393,397.60	298,113.00
12	APROMAR	50.0	F	100,048.80	0.00	8,349.60	29,024.40	137,422.80	87,397.00
13	UNIBA	75.0	T	210,104.00	0.00	11,499.20	19,312.00	240,915.20	188,389.00
14	IFREMER	75.0	A	127,135.00	0.00	8,000.00	0.00	135,135.00	103,351.00
15	ULL	75.0	T	305,888.00	0.00	10,606.40	0.00	316,494.40	240,021.00
16	FUNDP	75.0	T	291,344.00	0.00	8,208.00	0.00	299,552.00	226,716.00
17 (UTRO)	IMR/NIFES	75.0	A	0.00	0.00	0.00	0.00	0.00	0.00
18	CTAQUA	75.0	T	207,257.60	0.00	9,520.00	88,656.00	305,433.60	253,619.00
19	CMRM	75.0	T	185,065.60	0.00	8,500.80	0.00	193,566.40	147,299.00



# A3: Budget Breakdown

Participant number in this project <sup>11</sup>	Participant short name	Fund. % <sup>12</sup>	Ind. costs <sup>13</sup>	Estimated eligible costs (whole duration of the project)					Requested EU contribution
				RTD / Innovation (A)	Demonstration (B)	Management (C)	Other (D)	Total A+B+C+D	
20	SARC	50.0	A	140,450.00	0.00	14,900.00	0.00	155,350.00	85,125.00
21	DTU	75.0	S	288,058.00	0.00	8,703.00	0.00	296,761.00	220,997.00
22	SWH	50.0	A	235,272.00	0.00	9,616.00	0.00	244,888.00	127,252.00
23	ARGO	75.0	T	475,734.40	0.00	9,000.00	0.00	484,734.40	365,800.00
24 (TERMINATED)	ITTICAL	75.0	T	96,424.00	0.00	2,771.20	0.00	99,195.20	75,087.00
25	DOR	75.0	T	75,000.00	0.00	2,240.00	0.00	77,240.00	58,490.00
26	GEI	75.0	T	42,400.00	0.00	3,200.00	0.00	45,600.00	35,000.00
27	FORKYS	75.0	T	67,040.00	0.00	5,328.00	0.00	72,368.00	55,608.00
28	CANEXMAR	75.0	T	183,200.00	0.00	9,040.00	0.00	192,240.00	146,440.00
29 (TERMINATED)	ASIALOR	75.0	S	116,926.00	0.00	2,678.00	0.00	119,604.00	90,372.00
30 (TERMINATED)	CULMAREX	50.0	A	17,084.00	0.00	1,498.00	0.00	18,582.00	10,040.00
31	IRIDA	75.0	T	63,992.00	0.00	8,960.00	0.00	72,952.00	56,954.00
32	MC2	75.0	T	43,072.00	0.00	8,480.00	0.00	51,552.00	40,784.00
33	FGM	50.0	F	0.00	0.00	6,000.00	13,200.00	19,200.00	19,200.00
34	BVFi	75.0	T	0.00	0.00	7,560.00	23,902.40	31,462.40	31,461.00
35 (UTRO)	MAHAL	50.0	F	0.00	0.00	0.00	0.00	0.00	0.00
36	ANF	75.0	F	0.00	0.00	5,554.80	15,104.40	20,659.20	20,658.00
37	EUFIC	50.0	F	0.00	0.00	1,800.00	37,680.00	39,480.00	39,480.00
38	HRH	75.0	T	324,772.00	0.00	8,000.00	0.00	332,772.00	251,579.00
39	F2B	75.0	T	171,240.00	0.00	1,920.00	0.00	173,160.00	130,350.00
40	GMF	75.0	A	106,848.00	0.00	1,800.00	0.00	108,648.00	81,936.00
<b>Total</b>				<b>10,778,277.20</b>	<b>0.00</b>	<b>582,093.00</b>	<b>422,183.20</b>	<b>11,782,553.40</b>	<b>8,961,005.00</b>

Note that the budget mentioned in this table is the total budget requested by the Beneficiary and linked Third Parties.

**\* The following funding schemes are distinguished**

Collaborative Project (if a distinction is made in the call please state which type of Collaborative project is referred to: (i) Small of medium-scale focused research project, (ii) Large-scale integrating project, (iii) Project targeted to special groups such as SMEs and other smaller actors), Network of Excellence, Coordination Action, Support Action.

**1. Project number**

The project number has been assigned by the Commission as the unique identifier for your project, and it cannot be changed. The project number **should appear on each page of the grant agreement preparation documents** to prevent errors during its handling.

**2. Project acronym**

Use the project acronym as indicated in the submitted proposal. It cannot be changed, unless agreed during the negotiations. The same acronym **should appear on each page of the grant agreement preparation documents** to prevent errors during its handling.

**3. Project title**

Use the title (preferably no longer than 200 characters) as indicated in the submitted proposal. Minor corrections are possible if agreed during the preparation of the grant agreement.

**4. Starting date**

Unless a specific (fixed) starting date is duly justified and agreed upon during the preparation of the Grant Agreement, the project will start on the first day of the month following the entry into force of the Grant Agreement (NB : entry into force = signature by the Commission). Please note that if a fixed starting date is used, you will be required to provide a detailed justification on a separate note.

**5. Duration**

Insert the duration of the project in full months.

**6. Call (part) identifier**

The Call (part) identifier is the reference number given in the call or part of the call you were addressing, as indicated in the publication of the call in the Official Journal of the European Union. You have to use the identifier given by the Commission in the letter inviting to prepare the grant agreement.

**7. Activity code**

Select the activity code from the drop-down menu.

**8. Free keywords**

Use the free keywords from your original proposal; changes and additions are possible.

**9. Abstract**

**10. The month at which the participant joined the consortium, month 1 marking the start date of the project, and all other start dates being relative to this start date.**

**11. The number allocated by the Consortium to the participant for this project.**

**12. Include the funding % for RTD/Innovation – either 50% or 75%**

**13. Indirect cost model**

**A: Actual Costs**

**S: Actual Costs Simplified Method**

**T: Transitional Flat rate**

**F :Flat Rate**

# Workplan Tables

Project number

603121

Project title

DIVERSIFY—Exploring the biological and socio-economic potential of new/emerging candidate fish species for the expansion of the European aquaculture industry

Call (part) identifier

FP7-KBBE-2013-7-single-stage

Funding scheme

Collaborative project



# WT1

## List of work packages

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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### LIST OF WORK PACKAGES (WP)

WP Number <sup>53</sup>	WP Title	Type of activity <sup>54</sup>	Lead beneficiary number <sup>55</sup>	Person-months <sup>56</sup>	Start month <sup>57</sup>	End month <sup>58</sup>
WP 1	Project management	MGT	1	84.01	1	60
WP 2	Reproduction and Genetic-meagre	RTD	3	40.65	1	36
WP 3	Reproduction and Genetics - greater amberjack	RTD	13	197.40	1	54
WP 4	Reproduction and Genetics - pikeperch	RTD	1	5.00	1	16
WP 5	Reproduction and Genetics - Atlantic halibut	RTD	7	20.50	1	48
WP 6	Reproduction and Genetics – wreckfish	RTD	8	34.16	1	54
WP 7	Reproduction and Genetics – grey mullet	RTD	4	23.30	1	60
WP 8	Nutrition – meagre	RTD	2	27.00	1	48
WP 9	Nutrition – greater amberjack	RTD	2	46.80	1	58
WP 10	Nutrition – pikeperch	RTD	21	38.00	4	48
WP 11	Nutrition – Atlantic halibut	RTD	17	14.20	1	48
WP 12	Nutrition - wreckfish	RTD	19	6.60	1	57
WP 13	Nutrition – grey mullet	RTD	4	68.15	1	55
WP 14	Larval husbandry - meagre	RTD	3	3.70	1	18
WP 15	Larval husbandry - greater amberjack	RTD	2	79.50	5	48
WP 16	Larval husbandry – pikeperch	RTD	9	34.70	1	57
WP 17	Larval husbandry - Atlantic halibut	RTD	7	20.00	1	48
WP 18	Larval husbandry - wreckfish	RTD	8	14.81	1	48
WP 19	Larval husbandry - grey mullet	RTD	4	16.00	1	58
WP 20	Grow out husbandry - meagre	RTD	3	91.50	6	42
WP 21	Grow out husbandry - greater amberjack	RTD	1	111.40	8	57
WP 22	Grow out husbandry – pikeperch	RTD	16	61.00	8	48
WP 23	Grow out husbandry - grey mullet	RTD	4	43.80	9	40
WP 24	Fish health - meagre	RTD	1	148.40	5	57
WP 25	Fish health - greater amberjack	RTD	5	95.10	6	57
WP 26	Fish health - Atlantic halibut	RTD	7	4.16	4	40
WP 27	Socioeconomics – Institutional and organizational context	RTD	6	23.65	1	12
WP 28	Socioeconomics – New product development	RTD	3	77.85	13	58
WP 29	Socioeconomics – Consumer value perceptions and behavioural change	RTD	38	75.48	1	44

# WT1

## List of work packages

WP Number <sup>53</sup>	WP Title	Type of activity <sup>54</sup>	Lead beneficiary number <sup>55</sup>	Person-months <sup>56</sup>	Start month <sup>57</sup>	End month <sup>58</sup>
WP 30	Socioeconomics – Business model and marketing strategy development	RTD	10	55.19	43	60
WP 31	Dissemination	OTHER	18	40.21	1	60
			Total	1,602.22		

# WT2: List of Deliverables

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## List of Deliverables - to be submitted for review to EC

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D1.1	Kick-off meeting	1	1	3.10	O	RE	2
D1.2	Consortium Agreement	1	1	3.00	O	CO	3
D1.3	Annual Coordination Meeting for Y2	1	1	4.00	O	RE	13
D1.4	Periodic Report, including financial and administrative reports for Mo 1-12	1	1	7.00	R	CO	14
D1.5	Interactions with other projects	1	1	0.25	R	RE	18
D1.6	Annual Coordination Meeting for Y3	1	1	4.00	O	RE	25
D1.7	Mid-term evaluation of progress	1	1	3.00	R	PU	30
D1.8	Periodic Report, including financial and administrative reports for Mo 13-30	1	1	12.00	R	CO	32
D1.9	Annual Coordination Meeting for Y4	1	1	4.00	R	RE	37
D1.10	Annual Coordination Meeting for Y5	1	1	4.00	R	RE	49
D1.11	Periodic Report, including financial and administrative reports for Mo 31-48	1	1	10.27	R	RE	50
D1.12	Annual Coordination Meeting (Final)	1	1	4.00	R	RE	60

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D1.13	Periodic Report, including financial and administrative reports for Mo 49-60	1	1	11.00	R	PU	60
D1.14	Final Report	1	1	14.39	R	RE	60
D2.1	SNP library and chip to genetically characterise meagre or to use in marker assisted breeding programs (M18)	2	1	8.00	R	PU	18
D2.2	Genetic characterisation of different meagre captive broodstocks and evaluation of available variability (M12)	2	2	7.00	R	PU	12
D2.3	Protocol for paired spontaneous tank spawning of meagre	2	3	8.65	R	PU	21
D2.4	Construction of a genetic linkage map in meagre	2	1	5.00	R	PU	36
D2.5	Identification of genetic markers related to growth for use in marker assisted breeding programs for meagre through QTL mapping	2	1	5.00	R	PU	36
D2.6	Description of sperm characteristics and cryopreservation protocol of meagre sperm	2	14	3.75	R	PU	36



# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D2.7	Protocol for the strip spawning of meagre females and in vitro fertilization	2	3	3.25	R	PU	36
D3.1	Establishment of quantitative PCR assays to measure transcript levels of target genes in greater amberjack (i.e., LH $\beta$ , FSH $\beta$ , leptin, Vg and Vg receptor)	3	4	6.00	R	PU	12
D3.2	Establishment of hormone specific ELISAs for measuring LH, FSH and leptin in greater amberjack	3	4	6.00	R	PU	18
D3.3	Identification of possible reproductive dysfunction of gametogenesis of greater amberjack reared in captivity based on the comparative evaluation of fish sampled in the wild, in terms of proliferating	3	13	20.50	R	PU	24
D3.4	Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of greater amberjack sperm	3	14	5.50	R	PU	32
D3.5	Description of the process of oogenesis in	3	13	35.00	R	PU	46

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of ovarian development, (c) pituitary levels of FSH						
D3.6	Description of the process of spermatogenesis in captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of testicular development, (c) pituitary level	3	13	35.00	R	PU	46
D3.7	Comparative effectiveness of a GnRHa injection vs GnRHa implant treatment for the induction of spawning of greater amberjack in the eastern Atlantic	3	2	18.00	R	PU	48
D3.8	Dose response of GnRHa implant therapy for the induction of spawning in F1 generation broodstock of greater amberjack in the eastern Atlantic	3	8	10.50	R	PU	54
D3.9	Development of a spawning induction therapy for	3	1	44.90	R	PU	54

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	captive reared broodstock in the Mediterranean Sea based on the use of GnRHa in the correct mode of administration (hormone/ implant), dose and timing of						
D3.10	Method for inducing spawning and collecting greater amberjack eggs in sea cages	3	1	16.00	R	PU	54
D4.1	Genetic analysis of domesticated pikeperch broodstocks	4	1	3.00	R	PU	12
D4.2	Population genetic analysis of wild and comparison with domesticated pikeperch populations to be applied in future breeding programs of the species	4	1	2.00	R	PU	16
D5.1	Documentation of reproductive performance in wild-captured vs cultured female Atlantic halibut	5	7	7.00	R	PU	30
D5.2	An optimised GnRHa therapy protocol to improve spawning performance of F1/F2 Atlantic halibut, and to increase availability of	5	7	6.50	R	PU	30

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	eggs of stable and predictable quality						
D5.3	Identification of potential disturbances in reproductive development in F1/F2 Atlantic halibut females	5	7	7.00	R	PU	48
D6.1	Computer Assisted Sperm Analysis (CASA) for wreckfish sperm	6	14	2.00	R	PU	24
D6.2	Cryopreservation method for wreckfish	6	8	3.50	R	PU	24
D6.3	Spawning induction methods with in vitro fertilization of wreckfish	6	8	8.76	R	PU	36
D6.4	Establish reliable collection methods and protocols to form new wreckfish broodstocks	6	19	3.10	R	PU	36
D6.5	Description of the reproductive cycle of wreckfish	6	8	10.40	R	PU	48
D6.6	An in vitro fertilization protocol to be employed by the industry to spawn wreckfish	6	8	3.00	R	PU	48
D6.7	Spawning induction method for spontaneous spawning of wreckfish in large tanks	6	1	3.40	R	PU	54
D7.1	Establishment of a Computer	7	14	0.50	R	PU	12

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	Assisted Sperm Analysis (CASA) for the evaluation of grey mullet sperm						
D7.2	Production of recombinant bioactive LH and FSH assay for grey mullet	7	4	2.00	R	PU	18
D7.3	Comparative effectiveness of hormonal treatments for spawning induction in captive grey mullet	7	4	2.00	R	PU	24
D7.4	Protocol for shipping grey mullet eggs	7	4	3.00	R	PU	24
D7.5	Description of the process of oogenesis in captive-reared vs hatchery-produced grey mullet, including aspects of growth, body indices, and histological evaluation of ovarian development	7	13	6.70	R	PU	48
D7.6	Culture procedure that identifies the on-growing period for the production of grey mullet roe (bottarga) from wild and hatchery juveniles	7	4	6.60	R	PU	54
D7.7	Development of a breeding protocol for	7	4	2.50	R	PU	60

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	captive reared grey mullet broodstock based on optimized hormonal treatment, group structure and photo-thermal regime						
D8.1	Improvement of larval weaning diets	8	2	11.00	R	PU	24
D8.2	Recommended essential fatty acids contents in diets to promote meagre growth, welfare and health	8	2	16.00	R	PU	48
D9.1	Optimum levels and ratios of essential fatty acids in relation to Tau and combined PUFA-carotenoids in greater amberjack enrichment products	9	2	20.00	R	PU	24
D9.2	Lys requirements of greater amberjack juveniles	9	1	6.00	R	PU	36
D9.3	Performance of grow-out diets for greater amberjack developed in order to maximize growth potential	9	28	14.50	R	PU	58
D9.4	Recommended protein, carotenoids, Tau and EFA levels in greater	9	8	6.30	R	PU	58

# WT2: List of Deliverables

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	amberjack broodstocks						
D10.1	Recommended Ca/P, vitamins and phospholipids to improve larval development and reduce skeleton alterations in pikeperch	10	21	17.00	R	PU	36
D10.2	Protocol for optimal early fatty acid enrichment to reduce stress sensitivity in pikeperch	10	21	11.50	R	PU	36
D10.3	Formulation for a diet better adapted to pikeperch requirements	10	39	9.50	R	PU	48
D11.1	Report on nutrient profile of Artemia nauplii and ongrown Artemia from IMR and SWH	11	7	1.00	R	PU	24
D11.2	Report on optimal characteristics of feed particles and feeding environment for early weaning of Atlantic halibut larvae	11	7	2.70	R	PU	36
D11.3	Report on the nutrient retention and digestive physiology in Atlantic halibut larvae fed Artemia nauplii and on-grown Artemia	11	17	4.00	R	PU	36

# WT2: List of Deliverables

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D11.4	Report on the nutrient retention and digestive physiology in Atlantic halibut larvae reared in RAS vs FTS	11	17	4.50	R	PU	36
D11.5	Report on the effect of dietary phospholipids on Atlantic halibut juveniles	11	17	2.00	R	PU	48
D12.1	Effect of live prey enrichment products on wreckfish larval performance	12	19	3.30	R	PU	54
D12.2	Recommendations for wreckfish broodstock feeds	12	19	3.30	R	PU	57
D13.1	Determine changes in the essential fatty acid requirement as a function of developmental stage and ambient salinity in grey mullet	13	4	11.00	R	PU	18
D13.2	Determine a developmental stage ability to synthesize key enzymes in Tau and bile acid synthesis in grey mullet	13	4	12.00	R	PU	18
D13.3	Determine the effects of pigments, essential fatty acids and Tau in grey mullet broodstock diets on egg quality, fecundity, hatching success, larval	13	4	14.50	R	PU	36



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Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	first feeding and vitellogenin expression accumulat						
D13.4	Determine the effects of essential fatty acids and Tau in non-fish meal feeds on flesh and bottarga quality in grey mullet	13	4	20.35	R	PU	48
D13.5	Evaluate and maximize the dietary incorporation of a non-GMO genetically selected soybean meal that will increase nutrient absorption and reduce DT inflammation	13	13	10.30	R	PP	55
D14.1	Improved larval rearing protocol for meagre that includes weaning at an earlier age leading to reduced cost in live feed production and better quality juveniles	14	3	3.70	R	PU	18
D15.1	Effective greater amberjack larval stocking densities	15	2	18.50	R	PU	16
D15.2	Efficient prey density and protocol of using immune modulators in greater amberjack larval rearing	15	8	10.00	R	PU	27

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D15.3	Optimum hydrodynamics and light conditions during greater amberjack larval rearing	15	2	18.00	R	PU	27
D15.4	Ontogeny of greater amberjack larval visual and digestive system	15	1	13.00	R	PU	27
D15.5	An industrial protocol for greater amberjack larval rearing	15	8	20.00	R	PU	48
D16.1	Determine effect of environmental factors on pike perch larval rearing	16	9	4.00	R	PU	12
D16.2	Determine effect of nutritional factors on pikeperch larval rearing	16	9	4.00	R	PU	24
D16.3	Determine effect of population factors on pikeperch larval rearing	16	9	4.00	R	PU	36
D16.4	Identification of optimal combinations of factors for pikeperch larval rearing	16	9	4.00	R	PU	48
D16.5	Evaluation of selected rearing combinations for pikeperch on farm condition	16	9	3.50	R	PU	57
D16.6	Proposition of an industrial protocol for pikeperch rearing	16	29	15.20	R	PU	57

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D17.1	Production protocol of on-grown Artemia	17	7	3.50	R	PU	24
D17.2	Determine if RAS is a more effective protocol than FT for Atlantic halibut larvae	17	7	6.50	R	PU	36
D17.3	The effect of probiotics on Atlantic halibut larval microbiota and survival	17	7	4.00	R	PU	36
D17.4	Comparison of feeding on-grown Artemia versus Artemia nauplii on Atlantic halibut larval performance	17	7	4.00	R	PU	36
D17.5	Development of an industrial protocol for probiotic treatment of halibut larvae	17	7	2.00	R	PU	48
D18.1	Development of the digestive system of wreckfish	18	1	3.00	R	PU	36
D18.2	Determine optimum temperature conditions for rearing wreckfish larvae	18	8	3.40	R	PU	36
D18.3	Develop a feeding protocol for wreckfish larvae	18	1	3.21	R	PU	36
D18.4	Determine the most effective culture system (RAS vs	18	8	5.20	R	PU	48

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	flow-through) for wreckfish larvae						
D19.1	Determine most effective type and concentration of algae used in grey mullet larval rearing	19	4	3.00	R	PU	24
D19.2	Determining the effect of co-feeding ciliates and rotifers on digestive tract maturation and enzyme production	19	4	3.00	R	PU	36
D19.3	Determining the effect of co-feeding copepods and Artemia nauplii on digestive tract maturation and enzyme production	19	4	3.00	R	PU	58
D19.4	Evaluate the effectiveness of replacing live algae with lyophilized algae during grey mullet larval rearing	19	3	4.00	R	PU	48
D19.5	Evaluate an improved grey mullet larval rearing protocol in a commercial hatchery	19	25	3.00	R	PU	55
D20.1	Methodology to avoid size variability in meagre juveniles	20	3	12.00	R	PU	24
D20.2	Definition of the optimum conditions for	20	1	36.50	R	PU	39

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	cage culture of meagre (Report)						
D20.3	Methodology for meagre feeding	20	1	43.00	R	PU	42
D21.1	Definition of optimum feeding methods for greater amberjack grow out	21	8	49.40	R	PU	42
D21.2	Definition of optimum conditions for cage culture of greater amberjack	21	2	62.00	R	PU	57
D22.1	Effects of multiple variables on stress, immune response and growth performances and recommendations of optimal conditions for pikeperch grow out	22	16	31.00	R	PU	24
D22.2	Validation of optimal rearing variables under commercial farm conditions	22	29	12.00	R	PU	42
D22.3	Effects of domestication level and geographical origin on stress, immune response and growth performances and strain recommendation	22	16	18.00	R	PU	48
D23.1	Cost-effective weaning strategies for wild-caught grey mullet grow out	23	3	3.00	R	PU	18

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	and their effect on growth and health status						
D23.2	Stocking protocols for pond monoculture grow out of F1 and wild caught grey mullet	23	4	28.00	R	PU	30
D23.3	Comparison of the project's improved grey mullet grow-out feed under the different environmental and water conditions in Israel, Greece and Spain	23	4	12.80	R	PU	40
D24.1	The effect of vitamin D inclusions in diets in the development of Systemic Granulomatosis in meagre	24	1	11.00	R	PU	20
D24.2	The effect of Ca/P ratio in the diet in the development of Systemic Granulomatosis in meagre	24	1	11.00	R	PU	24
D24.3	Cloning of key marker genes of innate and adaptive immune responses in meagre	24	5	12.00	R	PU	26
D24.4	Efforts towards the isolation and characterization of Nocardia from infected meagre	24	1	6.00	R	PU	36

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Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D24.5	The effect of high plant protein diets in the development of Systemic Granulomatosis in meagre	24	1	13.00	R	PU	36
D24.6	Testing of commercial Vibrio vaccine	24	1	5.00	R	PU	42
D24.7	Diagnostics protocol for Chronic Ulcerative Dermatopathy in meagre, aetiological factors and solutions	24	1	11.00	R	PU	44
D24.8	Report on the prevention/ treatment of Chronic Ulcerative Dermatopathy in meagre	24	1	4.00	R	PU	44
D24.9	Determination of effective treatments for common monogenean parasites in meagre	24	3	10.00	R	PU	48
D24.10	Kinetics of antibody and cytokine production established post-pathogen exposure or stimulation with PAMPs	24	5	12.00	R	PU	48
D24.11	Recommended levels of pro- and anti-oxidant nutrients to prevent Systemic	24	2	6.00	R	PU	54

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	Granulomatosis in meagre						
D24.12	Determination of the efficacy of vaccination of meagre against Vibriosis	24	3	5.00	R	PU	54
D24.13	Description of immune gene expression post-immunisation and challenge of meagre with a Vibrio vaccine	24	3	6.00	R	PU	54
D24.14	Diagnostics protocol for Systemic Granulomatosis, causes and solutions in meagre	24	1	14.40	R	PU	54
D24.15	Report on the prevention/ treatment of Systemic Granulomatosis in meagre	24	1	10.00	R	PU	54
D24.16	Report of the major bacterial and viral diseases found in meagre, and where useful treatments have been developed, complete protocols for their implementation by the industry will be provided	24	2	6.00	R	PU	57
D24.17	Diagnostic-recommendation manual for meagre fish health	24	1	6.00	R	PU	57
D25.1	Marker genes of mucosal immunity	25	5	16.00	R	PU	39



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	in greater amberjack cloned and ways to increase their expression level determined						
D25.2	Mucus defences of greater amberjack analysed and immune potential characterised	25	2	7.00	R	PU	39
D25.3	Impact of dietary regime on parasite resistance and mucosal defences of greater amberjack juveniles	25	5	20.00	R	PU	42
D25.4	Protocol for early diagnosis of epitheliocystis during early stages of greater amberjack culture	25	1	8.00	R	PU	44
D25.5	Impact of oral administration of greater amberjack with mucus stimulation products on immune resistance to parasitic infections and development of molecular markers for its evaluation	25	2	28.00	R	PU	57
D25.6	Rearing protocol against monogenean parasites	25	8	6.10	R	PU	57
D25.7	Report on the major	25	2	5.00	R	PU	57

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	bacterial and viral diseases found in greater amberjack, and where useful treatments have been developed, complete protocols for their implementation by the industry will be provided						
D25.8	Diagnostic-recommendation manual for greater amberjack fish health	25	1	5.00	R	PU	57
D26.1	Assess the use of two eukaryotic expression systems; microalgae and a protozoa ( <i>Leishmania tarentolae</i> ) for production of nodavirus capsid protein	26	7	1.02	R	PU	24
D26.2	Testing of the delivery of vaccine candidates through <i>Artemia</i> to Atlantic halibut larvae	26	7	1.40	R	PU	36
D26.3	Determine immune response and effectiveness of orally delivered VNN capsid protein on protection of Atlantic halibut larvae	26	7	1.74	R	PU	40
D27.1	Report on external environmental	27	6	1.00	R	PP	3

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	factors that affect or will affect the production chains of meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet						
D27.2	Report on current certification schemes and standards and their business dynamics in the fish supply chain	27	6	5.00	R	PP	3
D27.3	Report on competitive analysis for the supply chains of meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet	27	6	2.00	R	PU	12
D27.4	Report on trend mapping for the European aquaculture, seafood sector and protein market in the (near) future	27	6	5.00	R	PU	12
D27.5	Report with results of international survey on industrial buyers' attitudes and perceptions regarding cultured fish	27	6	4.00	R	PU	12
D27.6	List of critical success factors for market acceptance	27	6	3.00	R	PU	12

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D27.7	Report on the analysis of the business models and supply chains of the participating SME's	27	6	3.65	R	PU	12
D28.1	Report with results of focus groups with consumers and experts regarding ideas for new products	28	11	4.00	R	PU	14
D28.2	List of ideas for new product development	28	1	8.75	R	PU	16
D28.3	Report on product and process solutions for each species based on technological, physical and sensory characteristics	28	1	20.00	R	PU	18
D28.4	Physical prototypes of new products from the selected species meagre, greater amberjack, wreckfish, pikeperch and grey mullet	28	3	8.00	R	PU	26
D28.5	Report on results of quality evaluation study on basic quality characteristics of the developed products	28	1	12.00	R	PU	54
D28.6	Report on results of sensory descriptive analysis of	28	1	12.00	R	PU	54

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	the developed products						
D28.7	Report on correlation of technical quality with nutritional - rearing history	28	15	6.00	R	PU	54
D28.8	Technical assessment of selected species	28	1	7.10	R	PP	58
D29.1	Dataset of consumers' perceptions, attitudes, buying intentions, consumption, willingness to buy and pay, and value perceptions towards the selected species in the five	29	6	7.38	R	PU	9
D29.2	Report on the segmentation analysis based on consumer value perceptions about the selected species in the five countries investigated (value-based segmentation task)	29	11	7.00	R	PU	24
D29.3	Development of the actual product samples from the selected species for the sensory testing with consumers in the five countries investigated	29	3	7.50	R	PU	28
D29.4	Report on the actual products' sensory profiling	29	3	14.20	R	PU	29

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	in the five countries investigated						
D29.5	Development of the product mock-ups for use in the experimentation with consumers in the five countries investigated	29	11	9.20	R	PP	30
D29.6	Report on the experimentation with product mock-ups in the five countries investigated and identification of the optimal intrinsic-extrinsic product quality profiles for targeted segments	29	11	11.20	R	PU	36
D29.7	Development of the stimulus (i.e. written and broadcasted information material) that will be used in the communication experiments in the five countries investigated	29	11	9.50	R	PP	42
D29.8	Report on the experimentation with the communication stimulus and evaluation of their effectiveness in changing consumers attitudes and behaviour towards the products	29	11	9.50	R	PU	44

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	coming from the selected						
D30.1	Report on value propositions for the producers and Partners	30	10	5.00	R	PU	46
D30.2	Report on indications of resources for creating customer value for the specific products	30	10	8.00	R	PU	46
D30.3	Guidelines to cultivate buyer-supplier relationships per species	30	10	5.32	R	PU	48
D30.4	Revenue (pricing & costs structures) model per species	30	10	6.00	R	PU	48
D30.5	New product marketing strategies per species and product	30	10	6.40	R	PU	52
D30.6	Report on results of test markets per species	30	10	9.16	R	PU	54
D30.7	Feasibility study	30	6	9.25	R	PU	60
D30.8	Report on EU and international market development plans and recommendations	30	10	6.06	R	PU	58
D31.1	Establishment of web site (fishDIVERSIFY.eu)	31	18	1.20	R	PU	4
D31.2	Project logo and brochure	31	18	1.00	R	PU	6
D31.3	Publication of the first of two articles in Food Today	31	37	0.50	R	PU	6

# WT2: List of Deliverables

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D31.4	Production and release of audiovisual material	31	18	0.25	R	PU	6
D31.5	Collaboration agreement with food industry and consumer organization; linkage of websites	31	18	0.27	R	PU	9
D31.6	Annual presentation of DIVERSIFY (Y1) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator	31	1	0.30	R	PU	9
D31.7	Production and release of audiovisual material	31	18	0.50	R	PU	12
D31.8	Production and release of audiovisual material	31	18	0.50	R	PU	18
D31.9	Annual presentation of DIVERSIFY (Y2) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator	31	1	0.50	R	PU	21
D31.10	Presentations of DIVERSIFY at the Aqua Europe meetings (Diversification Sessions) by the Species leaders (Y2)	31	1	2.50	R	PU	21



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D31.11	Scientific publications in relevant journals	31	1	1.00	R	PU	60
D31.12	Production and release of audiovisual material	31	18	0.50	R	PU	24
D31.13	Production and release of audiovisual material	31	18	0.50	R	PU	30
D31.14	Annual presentation of DIVERSIFY (Y3) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator	31	1	0.50	R	PU	33
D31.15	Production and release of audiovisual material	31	18	0.50	R	PU	36
D31.16	Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK or Italy) (1st workshop)	31	18	1.50	R	PU	37
D31.17	Production and release of audiovisual material	31	18	0.50	R	PU	42
D31.18	Promotional workshops (2nd) for specialized audience in fish market sector (Spain, UK, Italy or Greece)	31	18	1.50	R	PU	43
D31.19	Annual presentation of DIVERSIFY (Y4)	31	1	0.50	R	PU	44

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator						
D31.20	Presentations of DIVERSIFY at the Aqua Europe meetings (Diversification Sessions) by the Species leaders (Y4)	31	1	2.85	R	PU	44
D31.21	Presentation of DIVERSIFY at the European SEAFOOD Expo	31	18	0.50	R	PU	44
D31.22	Production and release of audiovisual material	31	18	0.50	R	PU	48
D31.23	Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK or Italy) (3rd workshop)	31	18	1.00	R	PU	49
D31.24	Technical leaflets	31	18	5.24	R	PU	54
D31.25	Audio-visual document with the project's activities and main achievements	31	1	0.50	R	PU	54
D31.26	Audio-visual popularization document and publication of the second article in Food Today, electronic journal of EUFIC	31	37	0.50	R	PU	54

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D31.27	Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK or Italy) (4th workshop)	31	18	1.50	R	PU	55
D31.28	Annual presentations of DIVERSIFY at the Aqua Europe meetings (EU Forum) by the Project Coordinator (Y5)	31	1	0.50	R	PU	57
D31.29	“Know-how Transfer” seminar for the aquaculture industry (Spain), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet	31	3	1.80	R	PU	57
D31.30	“Know-how Transfer” seminar for the aquaculture industry (Greece), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet	31	1	2.00	R	PU	57
D31.31	Pikeperch “Know-how Transfer”	31	9	2.00	R	PU	58

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	seminar for the aquaculture industry (potential location: France, Belgium, Denmark), presenting the progress achieved through DIVERSIFY in the production technology						
D31.32	Atlantic halibut “Know-how Transfer” seminar for the aquaculture industry (potential location: Norway), presenting the progress achieved through DIVERSIFY in the production technology	31	7	2.00	R	PU	58
D31.33	“Know-how Transfer” seminar for the aquaculture industry ( Spain), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet	31	8	2.00	R	PU	59
D31.34	“Know-how Transfer” seminar for the aquaculture industry (Italy), presenting the progress	31	13	2.00	R	PU	59

# WT2: List of Deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	WP number <sup>53</sup>	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet						
D31.35	Production and release of audiovisual material	31	18	0.80	R	PU	60
Total				1,602.22			

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP1	Type of activity <sup>54</sup>	MGT
Work package title	Project management		
Start month	1		
End month	60		
Lead beneficiary number <sup>55</sup>	1		

## Objectives

1. Coordinate and implement the Technical Annex and Grant Agreement in a timely, efficient and successful manner,
2. Provide the periodic reporting to the EU for the evaluation of the implementation of the programme, ensuring that correct and consistent financial and technical progress reports are submitted by participants and presented to the coordinator and submitted to the European Commission on time and in accordance with relevant guidelines,
3. Organize and coordinate the work and exchange of information, samples and protocols among Partners involved in the same or different WPs,
4. Organize and coordinate the work and exchange of information among Partners involved in work with the same species, but different work packages.

## Description of work and role of partners

Task 1.1 Establishment of management bodies (led by HCMR). Given the nature of this large-scale project – such as long duration, involvement of many Partners, work on various species and activities in different disciplines - particular attention needs to be given to project management and coordination. This effort has been initiated from the very early stage of forming the consortium and preparing the proposal.

Coordination and management of DIVERSIFY will be achieved through the following bodies:

1. Project coordinator (PC): responsible for the overall management of the project and communications with the EC, and will be assisted by the Management Office and an Executive Secretary.

2. Species Leaders (SL): these are Partners with expertise in the selected species (one per species), who at the phase of the proposal preparation were responsible for identifying and prioritizing the main bottlenecks for the aquaculture production of each species. The SLs were selected from among the consortium based on their involvement, expertise and excellence in research with the particular species. The SLs are:

Dr. Alicia Estevez (IRTA, Spain) for meagre,  
 Dr. Nikos Papandroulakis (HCMR, Greece) for greater amberjack,  
 Dr. Pascal Fontaine (UL, France) for pikeperch,  
 Dr. Birgitta Norberg (IMR, Norway) for Atlantic halibut,  
 Dr. Blanca Alvarez (IEO, Spain) for wreckfish, and  
 Dr. Bill Koven (IOLR, Israel) for grey mullet.

During the implementation of the programme, the SLs will be involved in overseeing, compiling and disseminating the work done in the various RTD WPs of the project.

3. Group Work package Leaders (GWPL): The people leading the groups of related RTD WPs were selected, again given their expertise and excellence in research in the scientific discipline. The GWPLs and identified scientific disciplines are:

Dr. Neil Duncan (IRTA, Spain) for all Reproduction & Genetics WPs (GWP2),  
 Dr. Daniel Montero (FCPCT, Spain) for all Nutrition WPs (GWP3),

# WT3: Work package description

Dr. Bill Koven (IOLR, Israel) for all Larval husbandry WPs (GWP4),  
Dr. Nikos Papandroulakis (HCMR, Greece) for all Grow out husbandry WPs (GWP5),  
Dr. Chris Secombes (UNIABDN, UK) for all Fish health WPs (GWP6), and  
Drs. Gemma Tacken (LEI, the Netherlands) for all Socioeconomics WPs (GWP7).

During the implementation of DIVERSIFY, GWPLs will be responsible for coordination with each WP Lead Beneficiary (LB) for (a) the timely execution of all planned research activities in their specific WPs following the projects time schedule (Table 1.3ii Gantt chart), (b) the compilation of all results and preparation of the periodic reports and (c) liaising with the PC, WP8 Dissemination leader and the SLs (see below and Section Section 3.2 Dissemination) for the preparation of the dissemination material for the project (e.g., web information, brochures, presentations and articles). Due to the size and complexity of the work in DIVERSIFY, for each scientific discipline a number of WPs have been planned, according to the scientific discipline and species studied. For each WP, the LB and will have similar responsibilities to those of the GWPL, but limited to a smaller part of the work. Similarly, due to the participation of many Partners in most research activities, for each Task and Action in the WPs, a Partner has been identified who will be responsible for the implementation of the planned work (See Annex I-DOW, WT 3).

4. Steering Committee (SC): consists of the PC, the GWPLs, two SME representatives (F2B, ARGO) and one representative from the professional associations (APROMAR). The SC will be involved with the overall decision making of the project in matters of Technical Annex implementation and modification, evaluation of project progress, knowledge management and any conflict resolutions.

Task 1.2 Kick off meeting (led by HCMR). In the first month of the project, a meeting of the Scientific Responsible of all Partners will be held at the PC's facilities. The objectives include (a) review of the signed contract with the European Commission, (b) review of the Consortium Agreement (deliverable D.1.2 Consortium Agreement), (c) set up of administration and financial management procedures, and (d) planning and organizing the research activities for the project, with emphasis on those to be initiated during the 1st year, establishing, when necessary, procedures for sampling acquisition and sharing among Partners, and for exchange of scientific information. This task will produce deliverable D.1.1 Kick-off meeting.

Task 1.3 Annual Coordination Meeting (led by HCMR, assisted by GWPLs). At 12-month intervals, coordination meetings will be held at different Partner's facilities (FCPCT, IMR, IRTA, UL, HCMR). Due to logistical issues, the original plan of having meetings organized by FCPCT and IMR was not implemented, and instead UNIBA and IEO/ULL (jointly) organized annual coordination meetings. In these meetings, the GWPLs will (a) present a summary of the major achievements during the past year, (b) plan and coordinate the activities for the following year and (c) coordinate the preparation of dissemination material (scientific articles, conference presentations, etc.). In addition, selected tasks from each WP will be presented by the responsible scientists, based on the current progress and importance of the findings.

These annual coordination meetings (ACM) will last 3 days and will serve also a dissemination purpose (See details in WP31 Dissemination). During the first two days of the ACM, the GWPLs will (a) present a summary of the major achievements during the past year, (b) plan and coordinate the activities for the following year and (c) coordinate the preparation of dissemination material (scientific articles, conference presentations, etc.). The first two days of the meetings will be open to the public and will be attended by invited guests (both from Europe and world-wide). Such interactions will ensure the most rapid progress of the project by providing useful advice from the experience of people from outside the consortium. Also, the invited scientists will act in a way as a Project Advisory Board for the proposal (See also Part B, Section 2.1 Management of the consortium), providing critical assessments of the results and planned tasks for the following period.

The third day of the meeting will be restricted to DIVERSIFY Partners. In the morning, a join meeting of the SC and SLs will discuss the progress of the research, develop a general outline of the next year, and address problems that may arise. In the afternoon, the GWPLs will have separate WP sessions in order to (a) further discuss the results obtained in their WPs, (b) plan and organize in detail the work to be carried out during the following year and (c) coordinate the preparation of the interim report and dissemination material. This task will produce deliverables D.1.3, 1.6, 1.9, 1.10, 1.12 Annual Coordination Meeting.

Task 1.4 Communication with the European Commission (EC) and project Periodic Reporting (led by HCMR, assisted by GWPLs). Periodic progress reports will be compiled by the PC and submitted 1 month after the Annual Coordination Meetings, according to the guidelines provided by the EC (DG RTD). The progress reports will be prepared by the GWPLs, with material provided by each scientist responsible for the specific Tasks, which is clearly identified in the description of the WPs (Annex 1-DOW, WT 3). The necessary administrative and financial information (Financial Statements and Use of Resources) will be provided by the Scientific Responsible

# WT3: Work package description

from each Partner, directly to the PC. This task will produce deliverables D.1.4, 1.8, 1.11 and 1.13 Periodic Report, including financial and administrative reports., as well as Deliverable D.1.14 Final Report.

The EC's project scientific officer will be invited to all the project meetings and will receive the detailed minutes as soon as they are released. The consortium will keep her/him informed, timely, about any dissemination/communication activity and WS related to the project's implementation and results. In addition the consortium will inform (and when relevant will send a copy to) her/him about any publication, leaflet, and other dissemination/communication outcome as soon as it is produced, during and after the implementation of the project. All the activities, deliverables, publications and project outcomes will clearly indicate the EU financial support and when applicable will display the appropriate EU logos.

Task 1.5 Mid-term evaluation of progress (led by HCMR). A mid-term progress evaluation will be undertaken at the completion of the mid-term evaluation by the EC. The achieved work will be evaluated vis-à-vis the Technical Annex and any deviations will be addressed. We will examine if there is a need to modify the planned work and take any corrective measures. This task will produce deliverable D.1.7 Mid-term evaluation by Steering Committee, GWPL and Species Leaders.

Task 1.6 Interactions with other projects

To stimulate synergies and complementarities, links will be established between the DIVERSIFY project and other relevant national and EU ongoing projects should appropriate opportunities occur. The consortium will keep informed, timely, the EC's scientific officer of the project about interactions with other projects and research programmes. This task will result in Deliverable D1.5 Interactions with other projects.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	58.00
2	FCPCT	5.00
3	IRTA	1.35
4	IOLR	2.00
5	UNIABDN	1.00
6	DLO	2.00
7	IMR	0.50
8	IEO	0.50
9	UL	1.00
10	TU/e	0.50
11	AU	0.50
12	APROMAR	0.61
13	UNIBA	0.50
14	IFREMER	0.25
15	ULL	0.50
16	FUNDP	0.50
17	IMR/NIFES	0.50
18	CTAQUA	0.50
19	CMRM	0.50
20	SARC	0.50



# WT3: Work package description

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
21	DTU	0.50
22	SWH	0.50
23	ARGO	0.50
24	ITTICAL	0.29
25	DOR	0.10
26	GEI	0.50
27	FORKYS	0.50
28	CANEXMAR	0.50
29	ASIALOR	0.25
30	CULMAREX	0.10
31	IRIDA	0.25
32	MC2	0.25
33	FGM	0.25
34	BVFi	0.16
35	MAHAL	0.25
36	ANF	0.75
37	EUFIC	0.25
38	HRH	0.60
39	F2B	0.30
40	GMF	0.50
	Total	84.01

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D1.1	Kick-off meeting	1	3.10	O	RE	2
D1.2	Consortium Agreement	1	3.00	O	CO	3
D1.3	Annual Coordination Meeting for Y2	1	4.00	O	RE	13
D1.4	Periodic Report, including financial and administrative reports for Mo 1-12	1	7.00	R	CO	14
D1.5	Interactions with other projects	1	0.25	R	RE	18
D1.6	Annual Coordination Meeting for Y3	1	4.00	O	RE	25
D1.7	Mid-term evaluation of progress	1	3.00	R	PU	30

# WT3: Work package description

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D1.8	Periodic Report, including financial and administrative reports for Mo 13-30	1	12.00	R	CO	32
D1.9	Annual Coordination Meeting for Y4	1	4.00	R	RE	37
D1.10	Annual Coordination Meeting for Y5	1	4.00	R	RE	49
D1.11	Periodic Report, including financial and administrative reports for Mo 31-48	1	10.27	R	RE	50
D1.12	Annual Coordination Meeting (Final)	1	4.00	R	RE	60
D1.13	Periodic Report, including financial and administrative reports for Mo 49-60	1	11.00	R	PU	60
D1.14	Final Report	1	14.39	R	RE	60
			Total			84.01

## Description of deliverables

D1.1) Kick-off meeting: A meeting will be held in the premises of the coordinator at the start of the project to (a) review of the signed contract with the European Commission, (b) review of the Consortium Agreement, (c) set up of administration and financial management procedures, and (d) planning and organizing the research activities for the project, with emphasis on those to be initiated during Y1. [month 2]

D1.2) Consortium Agreement: A consortium agreement will be agreed upon by all Partners, according to the guidelines of the EU. [month 3]

D1.3) Annual Coordination Meeting for Y2: A meeting will be held at the facilities of FCPCT at the end of the Y1, in order to (a) present a summary of the major achievements during the past year, (b) plan and coordinate the activities for Y2 and (c) coordinate the preparation of dissemination material (scientific articles, conference presentations, etc.). In addition, selected tasks from each WP will be presented by the responsible scientists, based on the current progress and importance of the findings. [month 13]

D1.4) Periodic Report, including financial and administrative reports for Mo 1-12: A Scientific periodic report will be prepared according to the guidelines of DG RTD, including all deliverables until this stage. The necessary administrative and financial information (Financial Statements and Use of Resources) from each Partner will also be submitted at this time. [month 14]

D1.5) Interactions with other projects: A report of links established between the DIVERSIFY project and other relevant national and EU ongoing projects. The consortium will keep informed, timely, the EC's scientific officer of the project about interactions with other projects and research programmes [month 18]

D1.6) Annual Coordination Meeting for Y3: A meeting will be held at the facilities of IMR at the end of the Y2, in order to (a) present a summary of the major achievements during the past year, (b) plan and coordinate the activities for Y3 and (c) coordinate the preparation of dissemination material (scientific articles, conference presentations, etc.). In addition, selected tasks from each WP will be presented by the responsible scientists, based on the current progress and importance of the findings. [month 25]

D1.7) Mid-term evaluation of progress: A mid-term progress evaluation will be undertaken at Y3 in a joined meeting of the SC and SLs. The achieved work will be evaluated vis-à-vis the Technical Annex and any deviations will be addressed. We will examine if there is a need to modify the planned work and take any corrective measures. A report will be produced and be available to the EU. This task will produce deliverable

# WT3: Work package description

D.1.5 Mid-term evaluation by Steering Committee, Group Work Package Leaders (GWPL) and Species Leaders (SL). [month 30]

D1.8) Periodic Report, including financial and administrative reports for Mo 13-30: A Scientific periodic report will be prepared according to the guidelines of DG RTD, including all deliverables until this stage. The necessary administrative and financial information (Financial Statements and Use of Resources) from each Partner will also be submitted at this time. [month 32]

D1.9) Annual Coordination Meeting for Y4: A meeting will be held at the facilities of UL at the end of the Y3, in order to (a) present a summary of the major achievements during the past year, (b) plan and coordinate the activities for Y4 and (c) coordinate the preparation of dissemination material (scientific articles, conference presentations, etc.). In addition, selected tasks from each WP will be presented by the responsible scientists, based on the current progress and importance of the findings. [month 37]

D1.10) Annual Coordination Meeting for Y5: A meeting will be held at the facilities of IRTA at the end of the Y4, in order to (a) present a summary of the major achievements during the past year, (b) plan and coordinate the activities for Y5 and (c) coordinate the preparation of dissemination material (scientific articles, conference presentations, etc.). In addition, selected tasks from each WP will be presented by the responsible scientists, based on the current progress and importance of the findings. [month 49]

D1.11) Periodic Report, including financial and administrative reports for Mo 31-48: A Scientific periodic report will be prepared according to the guidelines of DG RTD, including all deliverables until this stage. The necessary administrative and financial information (Financial Statements and Use of Resources) from each Partner will also be submitted at this time. [month 50]

D1.12) Annual Coordination Meeting (Final): A final meeting will be held at the facilities of HCMR just before the end of the Y5, in order to (a) present a summary of the major achievements during the past year, (b) coordinate the preparation of dissemination material (scientific articles, conference presentations, etc.), and (c) coordinate the preparation of the final report. In addition, selected tasks from each WP will be presented by the responsible scientists, based on the current progress and importance of the findings. [month 60]

D1.13) Periodic Report, including financial and administrative reports for Mo 49-60: A Scientific periodic report will be prepared according to the guidelines of DG RTD, including all deliverables until this stage. The necessary administrative and financial information (Financial Statements and Use of Resources) from each Partner will also be submitted at this time. [month 60]

D1.14) Final Report: A final Scientific report will be prepared according to the guidelines provided by the DG RTD, and will include a summation of all the work and accomplishment achieved during the project. [month 60]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS1	Kick-off and annual coordination meeting (for Y1)	1	1	
MS2	Consortium Agreement	1	3	
MS3	Annual coordination meeting (for Y2)	1	14	
MS4	Periodic Report (Mo1-12) to DG RTD, including financial and administrative reports	1	18	
MS5	Annual coordination meeting (for Y3)	1	26	
MS6	Periodic Report (Mo13-30) to DG RTD, including financial and administrative reports	1	36	
MS7	Annual coordination meeting (for Y4)	1	38	
MS8	Annual coordination meeting (for Y5)	1	50	

# WT3: Work package description

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS9	Final coordination meeting	1	58	
MS10	Periodic Report (Mo30-48) to DG RTD, including financial and administrative reports	1	60	
MS11	Periodic Report (Mo4-608) to DG RTD, including financial and administrative reports	1	60	
MS12	Final Report to DG RTD	1	60	

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP2	Type of activity <sup>54</sup>	RTD
Work package title	Reproduction and Genetic-meagre		
Start month	1		
End month	36		
Lead beneficiary number <sup>55</sup>	3		

## Objectives

1. Evaluate the genetic variation in the available captive broodstocks of meagre,
2. Genetic characterization of fast and slow growers,
3. Development of tools that facilitate the implementation of genetic selection programs,
  - a. Develop protocols for the paired crossing of breeders with spontaneous spawning,
  - b. Describe sperm quality and cryopreservation techniques,
  - c. Develop in vitro fertilization protocols to provide planned genetic crosses,
  - d. Develop a set of SNP markers for genetic selection and stock characterisation.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

The tasks will (a) describe the status of the identified bottlenecks through the genetic characterisation of captive broodstocks, and slow and fast growers, and (b) provide the necessary tools for the implementation of selective breeding, which will solve the identified bottlenecks.

EU Budget allocation: 61,924€ (HCMR), 19,160€ (FCPCT), 104,850€ (IRTA) and 29,872€ (IFREMER)

Task 2.1 Evaluation of the genetic variation in captive meagre broodstocks (led by FCPCT). Preliminary studies have indicated that meagre broodstock in Europe, which were formed from juveniles obtained from mainly a single commercial hatchery, have reduced genetic variability as the juveniles originated from a small group of parents (Soula et al., 2011; Duncan et al., 2013a). Fin clips will be taken from individuals in broodstock groups held for aquaculture purposes (FCPCT, IRTA, HCMR, ARGO, FORKYS); a minimum of 30 fish will be sampled from each of the 10 different broodstock groups from the participating RTD centres and SMEs. The collection of finclips will be undertaken also in commercial hatcheries that are not participating in DIVERSIFY and the following partners will coordinate the collection in different regions of Europe: FCPCT, IRTA (Spain and Portugal), IFREMER (France), HCMR (Greece, Cyprus and Turkey), UNIBA (Italy and Malta) and IOLR (Israel). Already defined genetic markers (Andree et al., 2010; Soula et al., 2011) will be applied and existing multiplex optimised and basic population genetics parameters (allelic richness, heterozygosity indices and inbreeding coefficients) will be estimated in order to describe the genetic status of captive broodstock and identify future needs. These needs will probably be that the genetic pool must be enlarged with the incorporation of wild stock or the mixing of existing captive stocks to ensure that the genetic variability of the base populations used in European aquaculture will enable the genetic selection of desirable production traits whilst avoiding problems associated with inbreeding. This task will result in deliverable D2.2 Genetic characterisation of different meagre captive broodstocks and evaluation of available variability.

Task 2.2 Development of protocols for paired crossing in spontaneous spawning (led by IRTA). Previous studies (IRTA) examining parentage contribution, indicated that 86% of spawns in communal spawning tanks were from only two parents, indicating that planned single paired crosses could be achieved by isolated pairs. A total of six pairs will be selected that are at the right post vitellogenic stage or have flowing milt (Duncan et al., 2012) and placed in spawning tanks (IRTA). At weekly intervals (total of 6 weeks), the pairs will be induced to spawn with an injection of 15 µg GnRH $\alpha$  kg-1. From the second week onwards when fish are manipulated to inject the females, the males will be transferred to a different tank in order to be crossed with a different female, producing a total of 36 families at the end of the experiment. Egg fecundity and quality (i.e., morphology, fertilisation,

# WT3: Work package description

hatching and larval survival) will be assessed using 96-well microtiter plates (Panini et al., 2001; Duncan et al., 2012). Microtiter plates are loaded individually with fertilized eggs and are maintained in a controlled temperature incubator until yolk-sack absorption. The plates are examined under a stereoscope every day, and embryonic development, hatching and larval survival are monitored.

Two experiments were added to the planned work of Task 2.2, in order to obtain more data on the potential of repeated injections to induce spawning in meagre. The objective of this additional work is to determine how many successful spawns individual females can produce in response to consecutive weekly injections and to replicate the paired-spawning experiments of IRTA with work with another broodstock in a different facility (HCMR). Single pairs of fish (one male and one female) will be transferred to 5,000-l tanks under simulated natural photoperiod, but controlled temperature ranging between 19 and 20°C. Females will be considered eligible for spawning induction if they contain oocytes in full vitellogenesis with a diameter of >550 µm. Male fish will be considered eligible for spawning induction, if they were releasing substantial amounts of sperm upon application of gentle abdominal pressure. Injections of GnRH $\alpha$  will be administered once a week (every Monday) between May and August 2014 using four pairs of fish per treatment (n=4). Females will be treated with a GnRH $\alpha$  injection of 15 µg kg<sup>-1</sup>. Four males will be treated at the start of the experiment with 50 µg kg<sup>-1</sup> using a GnRH $\alpha$  implant, in order to enhance spermiation. After treatment with GnRH $\alpha$ , fish will be placed in tanks connected to overflow egg collectors and allowed to spawn. If a female fails to spawn in response to 2 consecutive injections, it will be removed and not considered for the remaining experiment. When a cumulative total of two females (i.e., 50%) fail to spawn in response to 2 consecutive injections, the experiment will be concluded, and no further injections will be given. Eggs will be collected after every spawn and evaluated using microtiter plates, as described above. (Panini et al., 2001).

The second experiment will be run in parallel with a similar one by partner P3. IRTA in (Y2), in order to develop a method that optimizes the number of families produced by a given number of individual breeders. Four pairs of fish (one male and one female) will be transferred to 5,000-l tanks under simulated natural photoperiod and constant temperature (19 and 20°C). Fish will be considered eligible for spawning induction using the same criteria as in the previous experiment. Injections of GnRH $\alpha$  (15 µg kg<sup>-1</sup>) will be administered once a week (every Monday) in May 2015 to the four females and the four males. Every week, the males will be paired with a different female and after treatment with GnRH $\alpha$  they will be placed in the separate spawning tanks and allowed to spawn for a week. Eggs will be collected and evaluated as described in the previous section for fecundity and fertilization success, and then for embryo survival 24 h after egg collection, hatching success and larval survival on day 5 after egg collection.

This task will result in deliverable D2.3 Protocol for paired spontaneous tank spawning of meagre.

Task 2.3 Description of sperm characteristics and cryopreservation methods (led by IFREMER). Part of the state-of-the-art of in vitro fertilization protocols is to have good quality sperm available when the females ovulate and ova are stripped. At three time-points in the spawning season, sperm quality of 10 males held in IRTA will be assessed (IFREMER, IRTA) by (a) sperm concentration through image analysis, (b) sperm motility (% mobile cell, velocity, linearity of tracks) using the CASA plugin of ImageJ software (Wilson-Leedy & Ingermann, 2007; Fauvel et al., 2010), (c) sperm membrane integrity by eosin/nigrosin sperm viability test (Björndahl et al., 2003) and (d) sperm energetic status using ATP measurement kits (Boryshpolets et al., 2009). Then chilled storage will be studied according to Fauvel et al., (2012) and cryopreservation will be adapted using a commercial diluent (Cryofish from Cryobiosystem, France). The conditions of sperm dilution in terms of volume, osmolality, pH and cryoprotectant type will be tested by comparison of sperm motility performance based on CASA analysis. This task will result in deliverable D2.6 Description of sperm characteristics and cryopreservation protocol of meagre sperm.

Task 2.4 Development of in vitro fertilization methods for planned crosses (led by IRTA). The state-of-the-art method to obtain planned crosses to produce desired families is strip spawning of eggs and in vitro fertilisation. Ten females (five wild and five cultured fish) at the right post vitellogenic stage (Duncan et al., 2012) will be induced to ovulate at weekly intervals with a single injection of 15 µg GnRH $\alpha$  kg<sup>-1</sup> (IRTA). The females will be examined for ovulation at predetermined hours post injection, based on preliminary data from HCMR indicating that ovulation occurs after 32-36 h at 19°C. This work will be lead by IRTA with participation from HCMR and IFREMER, who will send their scientists to the IRTA facilities at the time of the experiment. When obtained, ovulated eggs will be fertilised with fresh or stored sperm and egg quality parameters determined as above (Task 2.2). A data set will be built up that will determine the interaction between, female stage at stimulation, time of response to hormone injection (ovulation) and egg quality to identify optimal stripping times after

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hormone injection. This task will result in deliverable D2.7 Protocol for the strip spawning of meagre females and in vitro fertilization.

Task 2.5 Development of Single Nucleotide Polymorphisms (SNP) marker tools for the genetic characterization of fast and slow growers (led by HCMR). One of the principal bottlenecks to meagre production is variable growth rates, causing uncertainty in the prediction of total yield from each on-growing cycle. Fast and predictable growth is an important and highly desired trait, which affects the profitability of food animal production, since feed costs account for the largest proportion of production costs. SNPs explain the greatest part of the genetic differences between individuals and are suitable for genetic evaluation and strategies that employ molecular genetics for selective breeding. Therefore, this task aims at using SNPs to identify markers and genes associated with genetic variation in growth through Next Generation Sequencing (NGS) of the whole transcriptome of 10 fish from different families and phenotypic size (of the same age) that will provide a data-set of over 100,000 SNPs. The chosen SNP discovery strategy will be two-fold. First, the whole transcriptome of muscle and liver of more than 10 fish from different families and phenotypic size will be sequenced to gain information regarding the gene sequence of meagre and to identify thousands of SNPs that can have impact on the functional role of protein-coding genes. This step will lead to a catalogue of polymorphic loci at the expressed part of the genome and will set the ground for understanding growth and other traits of interest in meagre. Second, to identify SNPs that will have significant association with growth, we will conduct a preliminary QTL mapping experiment using ddRAD (double-digest Restriction site Associated DNA) Sequencing. Fin clip samples and phenotypic growth data (weight and morphometric measures) will be obtained from more than 250 individual meagre from tasks in WP20 Grow out husbandry – meagre. These fish are going to be genotyped in order to infer parentage allocations and the progeny from one or two large families (approx. 130 fish), which exhibit the greatest phenotypic variation will be used for RADSeq library construction and sequencing on an Illumina HiSeq platform. Following sequencing, SNP discovery pipelines will be run to identify common SNPs among all progeny that will provide the basis for QTL mapping. Discovered SNPs will also be used in a genetic linkage mapping analysis to build the first linkage map of the species. Finally, a second RADseq library will be constructed following the same protocol for some 140 breeders from SMEs and based on the results obtained from the QTL analysis a primary selection programme will be initiated based on a sub-group of fish. This task will result in deliverables D2.1 SNP library and chip to genetically characterize meagre or to use in marker assisted breeding programs, D2.4 Construction of a genetic linkage map in meagre (Mo36), and D2.5. Identification of genetic markers related to growth for use in marker assisted breeding programs for meagre through QTL mapping (Mo 36).

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	15.00
2	FCPCT	7.00
3	IRTA	15.90
14	IFREMER	2.75
	Total	40.65

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D2.1	SNP library and chip to genetically characterise meagre or to use in marker assisted breeding programs (M18)	1	8.00	R	PU	18

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D2.2	Genetic characterisation of different meagre captive broodstocks and evaluation of available variability (M12)	2	7.00	R	PU	12
D2.3	Protocol for paired spontaneous tank spawning of meagre	3	8.65	R	PU	21
D2.4	Construction of a genetic linkage map in meagre	1	5.00	R	PU	36
D2.5	Identification of genetic markers related to growth for use in marker assisted breeding programs for meagre through QTL mapping	1	5.00	R	PU	36
D2.6	Description of sperm characteristics and cryopreservation protocol of meagre sperm	14	3.75	R	PU	36
D2.7	Protocol for the strip spawning of meagre females and in vitro fertilization	3	3.25	R	PU	36
Total			40.65			

## Description of deliverables

D2.1) SNP library and chip to genetically characterise meagre or to use in marker assisted breeding programs (M18): The deliverable will present a database containing the DNA sequences of over 100,000 SNPs that have been identified as useful markers for QTL analysis and marker assisted breeding programs and include results to establish genetic influence in growth rate, fast or slow growers. [month 18]

D2.2) Genetic characterisation of different meagre captive broodstocks and evaluation of available variability (M12): The deliverable will present the genetic characterisation of breeders from populations held in research centres and SMEs implied in DIVERSIFY project and stocks held across the aquaculture industry. The results presented will be obtained using a microsatellite multiplex and will describe the genetic structure within and between meagre populations (number of alleles, heterozygosity observed and expected, allele range, exclusion probability, Hardy-Weinberg equilibrium test, consanguinity, etc.). The genetic characterisation of all these stocks will be used to propose strategies to improve the genetic basis for the domestication of meagre through the selection for sustainable optimal culture performance. [month 12]

D2.3) Protocol for paired spontaneous tank spawning of meagre: A protocol will be developed for the induction of spontaneous tank spawning of pairs of meagre broodstock (one male and one female). The deliverable will present the procedures, description of holding environment, stage of maturity required of breeders, dosage and timing for hormone application and egg collection. In addition, the deliverable will include the results from repeated trials including the following data: success rate of paired spawning, relative fecundity (number of total eggs obtained per kilo of female), latency period (timing of application of hormones in relation to egg collection), and egg quality parameters. [month 21]

D2.4) Construction of a genetic linkage map in meagre: SNPs that will have significant association with growth, will be identified using a preliminary QTL mapping experiment using ddRAD (double-digest Restriction site Associated DNA) Sequencing. Discovered SNPs will also be used in a genetic linkage mapping analysis to build the first linkage map of the species. [month 36]



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D2.5) Identification of genetic markers related to growth for use in marker assisted breeding programs for meagre through QTL mapping: A report that genetically characterises more than 250 fish from Tasks in WP20. The report will present the results to establish a genetic bases of growth in meagre and the differences in genetic variation between fast and slow growing meagre. [month 36]

D2.6) Description of sperm characteristics and cryopreservation protocol of meagre sperm: A report will provide 1) motility features of a high quality sperm based on the analyses of artificial fertilization results; 2) the characteristics of cryopreservation media and improved freezing protocols (dilution rate, type of straw, cooling rate); 3) the concentration of thawed sperm maximizing fertilization success. The report will include results from experiments that determine and validate the protocols. [month 36]

D2.7) Protocol for the strip spawning of meagre females and in vitro fertilization: A protocol will be developed for the strip spawning of meagre females and in vitro fertilization. The deliverable will present the procedures, for the extensive handling and manipulation of large meagre breeders, stage of maturity required of breeders, dosage of hormone, timing for hormone application, timing of ovulation, timing of stripping eggs and sperm and methods for in vitro fertilisation. In addition, the deliverable will include the results from repeated trails that determine and validate the procedures and include the following data: relative fecundity (number of total eggs obtained per kilo of female), latency period (timing of application of hormones in relation to egg collection), and egg quality parameters. [month 36]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS16	SNP library with candidate SNPs potentially associated to growth of meagre	3	18	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP3	Type of activity <sup>54</sup>	RTD
Work package title	Reproduction and Genetics - greater amberjack		
Start month	1		
End month	54		
Lead beneficiary number <sup>55</sup>	13		

## Objectives

1. Describe the endocrine control of reproduction in captive broodstocks, and the nutritional status of fish during the reproductive season,
2. Assess reproductive potential of wild vs. captive amberjack broodstocks and identify possible reproductive/metabolic dysfunctions during gametogenesis,
3. Develop spawning induction methods for captive-reared and F1 broodstocks of both the Mediterranean and Atlantic stocks,
4. Apply the developed spawning induction methods for broodstocks maintained in cages, and examine the efficiency of an egg collector to obtain fertilized eggs,
5. Develop a Computer Assisted Sperm Analysis method (CASA) for the evaluation of greater amberjack sperm during the reproductive season, and evaluate the possible effects of captivity.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

This WP includes tasks for (a) the description of the reproductive cycle of greater amberjack in captivity in the Mediterranean, where the season is shorter and well defined by temperature limits, (b) the development of effective and efficient spawning induction methods for broodstocks maintained in captivity, and (c) the collection of eggs from fish maintained in sea cages.

EU Budget allocation: 121,336€ (HCMR), 50,000€ (FCPCT), 111,200€ (IOLR) 50,000€ (IEO), 125,800€ (UNIBA), 29,970€ (IFREMER), 17,190€ (ULL), 209,660€ (ARGO) and 61,376€ (ITICAL, exited the consortium on 31/5/2016) and 31,800€ (GMF, joined the consortium in Oct 2016)

Task 3.1 Description of the reproductive cycle of greater amberjack (led by UNIBA). Wild-caught broodstocks will be maintained in captivity by one of the SMEs (ARGO). ARGO will arrange for the capture of broodstock from the wild, their transfer to appropriate facilities near shore and their maintenance in captivity for the duration of the experiment (1+ year). Broodstock will be sacrificed (n=8) at three different times during the reproductive season. Blood, brains, pituitaries, gonads, muscle and liver will be sampled by scientist from different Partners (UNIBA, HCMR, IOLR, IFREMER) to study the reproductive cycle, and spines will be collected for age determination (UNIBA). Wild fish (n=12) will be sampled at the same time (UNIBA) as a reference for evaluating reproductive function of captive fish. Proliferation and/or apoptosis of germ cells during spermatogenesis will be examined using Proliferating Cell Nuclear Antigen (PCNA) and the TUNEL method. Characterization of amberjack sperm will be done using CASA (IFREMER), looking at % spermatozoa motility, velocity, linearity of tracks, sperm membrane integrity by eosin/nigrosin, sperm viability and sperm energetic status using ATP measurement (same procedure as 2.1.3). A comparison of liver vitellogenin (Vg) and ovary Vg receptor (VgR) gene expression between captive and wild females will be assessed by cDNA sequencing and real time-PCR (qPCR); an analysis of oocyte yolk accumulation will be performed on histological sections using image analysis (UNIBA). Measurement of the sex steroid hormones T, E2, 11-KT and 17,20β DHP will be done using ELISAs (HCMR) and the expression of LH and FSH, as well as their plasma protein levels will be measured (IOLR). Utilizing a yeast (*Pichia pastoris*) expression sequence, recombinant LH and FSH proteins will be produced and used to develop the respective hormone-specific ELISAs (IOLR). Similar methodology will be applied to develop an ELISA for measuring leptin (IOLR), a key metabolic hormone known to interact with the endocrine system to provide critical information about the nutritional status. The nutritional status of the captive and

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wild fish will be compared (ULL). The muscle, liver, and gonads samples will be analysed (as described in Rodríguez-Barreto et al., 2012) to determine: (a) proximate and fatty acid composition, including specific lipid classes (i.e. phosphatidyl serine, inositol, choline and ethanolamine), (b) vitamin C and E and (c) carotenoids (ULL). These nutrients are important in sperm function and egg, embryo and larval development (Izquierdo et al., 2001; Tocher et al., 2008; Rodríguez-Barreto et al., 2012). This task will result in deliverables D3.1 Establishment of quantitative PCR assays to measure transcript levels of target genes in greater amberjack (i.e., LH $\beta$ , FSH $\beta$ , leptin, Vg and Vg receptor), D3.2 Establishment of hormone specific ELISAs for measuring LH, FSH and leptin in greater amberjack, D3.3 Identification of possible reproductive dysfunction of gametogenesis of greater amberjack reared in captivity based on the comparative evaluation of fish sampled in the wild, in terms of proliferating and apoptotic germ cells, vitellogenin accumulation, yolk content in the oocytes and nutritional status, D3.4 Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of greater amberjack sperm, D3.5 Description of the process of oogenesis in captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of ovarian development, (c) pituitary levels of FSH and LH, (d) plasma levels of FSH, LH, leptin, sex steroid hormones and Vg, and (e) nutritional status and D3.6 Description of the process of spermatogenesis in captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of testicular development, (c) pituitary levels of FSH and LH, (d) plasma levels of FSH, LH, leptin, sex steroid hormones, (e) proliferation and apoptosis of germ cells, (f) sperm quality, (g) fish nutritional status and (h) egg biochemical composition.

Task 3.2 Development of an optimized spawning induction protocol for captive greater amberjack in the Mediterranean (led by HCMR). Mature wild fish will be captured using a purse seine by one of the SMEs (ARGO). Using these fish, captive broodstocks (n=10 males and females) will be established in HCMR, ARGO and ITICAL and will be maintained in tanks (HCMR, ITICAL) and cages (HCMR, ARGO and GMF). After 1 year in captivity (Y2), two different methods will be examined using both fish maintained in sea cages during the year (ARGO, GMF) and fish maintained in tanks (HCMR), either multiple GnRHa injections given every 7 days or implants for sustained release of GnRHa spanning 3-weeks (HCMR). HCMR will prepare the GnRHa loaded implants that will be used for the experiments. In Y3, two different doses of the most effective treatment from Y2 will be evaluated, in the broodstock that gave the best results in Y2. In Y4, the timing of application (June - July) of the most effective treatment/dose will be optimized, based on the data obtained from the previous years. In the other SME Partners (GMF), spawning induction on a single broodstock will be undertaken to validate protocols and to ensure egg production for the WP4 Larval husbandry. For this reason, HCMR will send a scientist to these facilities to train ARGO and GMF personnel in evaluating the reproductive stage of the fish, and administering the hormonal treatments. HCMR will also provide them with GnRHa implants. Evaluation of spawning quality will be carried, using individual egg incubations in 96-well microtiter plates (same procedure as 2.2, Mylonas et al., 2004). This task will result in deliverable D3.9 Development of a spawning induction therapy for captive reared broodstock in the Mediterranean Sea, based on the use of GnRHa in the correct mode of administration (hormone/implant), dose and timing of application.

Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT). Mature wild-caught fish adapted to culture conditions will be divided into 3 groups of 6 males and 6 females. At the onset of the spawning season in the eastern Atlantic (April-May), after evaluation of reproductive status (same procedures as Task 2.3; Mylonas et al., 2004) fish will be treated with either single GnRHa injections every 7-10 days or GnRHa implants (that will be produced by HCMR) every 3 weeks, and one group will be left untreated as control. Evaluation of the spawning quality will be carried out for all spawns (same procedure as Task 2.2; Mylonas et al., 2004). A scientist from HCMR will also participate on site on the first year of the experiments, in order to provide expertise with the hormonal administration method. This task will result in deliverable D3.7 Comparative effectiveness of a GnRHa injection vs GnRHa implant treatment for the induction of spawning of greater amberjack in the eastern Atlantic.

Task 3.4 Development of an optimized spawning induction protocol for F1 greater amberjack in the eastern Atlantic (led by IEO). At the beginning of the spawning season the reproductive status of the broodstock will be evaluated (same procedures as 2.1.3; Mylonas et al., 2004) and fish treated with GnRHa implants (HCMR). Each year, females will be given GnRHa implants of different doses (25, 50, 75  $\mu\text{g kg}^{-1}$ ) while males will be implanted with 30  $\mu\text{g kg}^{-1}$ . A scientist from HCMR will also participate on site on the first year of the experiments, in order to provide expertise with the hormonal administration method. The evaluation of the spawning quality will be carried out for all spawns (same procedure as 2.1.2; Mylonas et al., 2004). GnRHa implant treatments will be repeated when spawning stops. At the time of each GnRHa implantation and the end of the spawning season the following parameters will be determined: (a) plasma levels of the sex steroid hormones (HCMR); (b) plasma levels of triglycerides, cholesterol, protein and enzymes (GPT, GOT, alkaline

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phosphatase, cholinesterase and amylase); (c) cortisol, glucose and lactate; and (d) electrolytes, in order to determine hematological and biochemical indicators of health and welfare. These analyses will be undertaken by IEO. This task will result in deliverable D3.8 Dose response of GnRHa implant therapy for the induction of spawning in F1 generation broodstock of greater amberjack in the eastern Atlantic.

Task 3.5 Spawning induction of greater amberjack and egg collection in cages (led by HCMR). Cage spawning is expected to facilitate the management of greater amberjack broodstocks that may exceed 30 kg in commercial operations. Therefore, DIVERSIFY will employ methods used in a previous EU 7th FP project (SELFDOTT) for the collection of fertilized eggs from Atlantic bluefin tuna broodstocks maintained and induced to spawn in cages (Mylonas et al., 2010a). The methods will be applied to a spawning cage in HCMR and ARGO. An egg collection device will be used that includes a two-piece curtain deployed around the perimeter of the cage from 20 cm above to 3.5 m below the water level. Spawning induction (HCMR) will be undertaken during the peak of the spawning season (see Task 3.2). Eggs will be skimmed from the water surface every day at sunrise using fine mesh dip nets. The evaluation of the spawning quality will be carried out for all spawns (same procedure as Task 2.2; Mylonas et al., 2004). This task will result in deliverable D3.10 Method for inducing spawning and collecting greater amberjack eggs in sea cages.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	24.00
2	FCPCT	19.00
4	IOLR	14.00
8	IEO	5.60
13	UNIBA	30.00
14	IFREMER	2.50
15	ULL	3.80
23	ARGO	80.00
24	ITTICAL	9.00
40	GMF	9.50
Total		197.40

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D3.1	Establishment of quantitative PCR assays to measure transcript levels of target genes in greater amberjack (i.e., LH $\beta$ , FSH $\beta$ , leptin, Vg and Vg receptor)	4	6.00	R	PU	12
D3.2	Establishment of hormone specific ELISAs for measuring LH, FSH and leptin in greater amberjack	4	6.00	R	PU	18
D3.3	Identification of possible reproductive dysfunction of gametogenesis of	13	20.50	R	PU	24

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	greater amberjack reared in captivity based on the comparative evaluation of fish sampled in the wild, in terms of proliferating					
D3.4	Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of greater amberjack sperm	14	5.50	R	PU	32
D3.5	Description of the process of oogenesis in captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of ovarian development, (c) pituitary levels of FSH	13	35.00	R	PU	46
D3.6	Description of the process of spermatogenesis in captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of testicular development, (c) pituitary level	13	35.00	R	PU	46
D3.7	Comparative effectiveness of a GnRH $\alpha$ injection vs GnRH $\alpha$ implant treatment for the induction of spawning of greater amberjack in the eastern Atlantic	2	18.00	R	PU	48
D3.8	Dose response of GnRH $\alpha$ implant therapy for the induction of spawning in F1 generation broodstock of greater amberjack in the eastern Atlantic	8	10.50	R	PU	54
D3.9	Development of a spawning induction therapy for captive reared broodstock in the Mediterranean Sea based on the use of GnRH $\alpha$ in the correct mode of administration (hormone/implant), dose and timing of	1	44.90	R	PU	54
D3.10	Method for inducing spawning and collecting greater amberjack eggs in sea cages	1	16.00	R	PU	54
<b>Total</b>			<b>197.40</b>			

## Description of deliverables

D3.1) Establishment of quantitative PCR assays to measure transcript levels of target genes in greater amberjack (i.e., LH $\beta$ , FSH $\beta$ , leptin, Vg and Vg receptor): Molecular biology methodologies will be set up in order to measure expression levels of target genes in greater amberjack. The deliverable will present: (a) methods to

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estimate egg quality by measuring liver vitellogenin (Vg) (the yolk precursor protein) and its ovary receptor (VgR) gene expression levels through a quantitative PCR (qPCR); (b) Calibrated real-time PCR assays developed and validated for the reliable mRNA quantification of target genes. This method will present: (a) sequences encoding for greater amberjack LH $\beta$ , FSH $\beta$ , leptin, Vg and Vg receptor, and (b) gene specific primer sets facilitating optimal assay sensitivity and specificity. [month 12]

D3.2) Establishment of hormone specific ELISAs for measuring LH, FSH and leptin in greater amberjack: Calibrated immuno-assays will be developed and validated for a sensitive and reliable hormone measurement. The deliverable will present: (a) yeast expression system, a means for efficient production of recombinant greater amberjack LH $\beta$ , FSH $\beta$ , leptin, (b) recombinant hormone/hormone-subunits to be used as antigen/standard, and (c) polyclonal antibodies, including anti- LH $\beta$ , anti-FSH $\beta$ , and anti-leptin, allowing for optimal immuno-assay sensitivity and specificity. [month 18]

D3.3) Identification of possible reproductive dysfunction of gametogenesis of greater amberjack reared in captivity based on the comparative evaluation of fish sampled in the wild, in terms of proliferating: Reproductive maturation has been reported to be unreliable in greater amberjack reared in captivity. This deliverable will characterize the reproductive dysfunction by comparing gametogenesis between wild and captive amberjack using methodologies to (a) assess the influence of captivity on spermatogenesis by looking at germ cell proliferation and apoptosis; and (b) evaluate the effects of captivity on vitellogenesis by monitoring liver Vg and VgR genes expression as well as oocyte yolk accumulation. Moreover the deliverable will report the role of a key hormone related to nutritional status (e.g. leptin) in order to get information on captive fish nutritional status and, as a consequence, to get gametes, embryo and larvae of very high quality. [month 24]

D3.4) Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of greater amberjack sperm: The deliverable will be a report containing the original characteristics of motility and concentration of amberjack sperm at different periods of the reproductive season. The analyses based on the opensource image J will be detailed in a user guide so as to allow end users develop by themselves future sperm quality assessment. [month 32]

D3.5) Description of the process of oogenesis in captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of ovarian development, (c) pituitary levels of FSH: The endocrine control of the reproductive cycle in female greater amberjack has not been described yet. This deliverable will provide an evaluation of the brain-pituitary-gonad axis during oogenesis in captive greater amberjack, as a way of assessing reproductive function and predicting spawning performance. The deliverable will (a) assess the size and the age at first sexual maturity of greater amberjack females; (b) evaluate captive fish body condition by using different parameters such as the condition index (CI) and the gonadosomatic index (GSI); (c) describe the reproductive cycle of captive fish in order to identify the optimal time for the administration of hormonal treatments for the induction of spawning; (d) identify the possible effects of captivity on the reproductive axis by measuring the pituitary and plasma levels of the two gonadotropins (FSH and LH), as well as the sex steroid (17 $\beta$ -estradiol, Testosterone, 17,20b-DHP) plasma concentration; (e) provide information on the nutritional status of captive females by measuring a key metabolic hormone (e.g. leptin) as well as Vg plasma level and oocyte yolk accumulation; and (f) assess egg composition by determining fatty acid, vitamin and carotenoid content. [month 46]

D3.6) Description of the process of spermatogenesis in captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of testicular development, (c) pituitary level: The endocrine control of the reproductive cycle in male greater amberjack has not been described yet. This deliverable will provide an evaluation of the brain-pituitary-gonad axis during spermatogenesis in captive greater amberjack, as a way of assessing reproductive function and predicting spawning performance. The deliverable will (a) assess the size and age at first sexual maturity of greater amberjack males; (b) evaluate captive fish body condition by using parameters such as the condition index (CI); (c) describe the reproductive cycle of captive fish in order to get a synchronized effect of the hormonal treatment in both sexes; (d) identify the possible effects of captivity on reproductive axis by measuring the pituitary and plasma levels of the two gonadotropins (FSH and LH), as well as sex steroid (Testosterone, 11-ketotestosterone, 17b-DHP) plasma concentration; (e) identify the possible effects of captivity on spermatogenesis in terms of proliferation and apoptosis of germ cells as well as on sperm quality (motility, velocity, ATP content, etc); (f) provide information on the nutritional status of captive males by measuring a key metabolic hormone (e.g. leptin). [month 46]

D3.7) Comparative effectiveness of a GnRH $\alpha$  injection vs GnRH $\alpha$  implant treatment for the induction of spawning of greater amberjack in the eastern Atlantic: The deliverable will be a scientific report that compares effectiveness of a GnRH $\alpha$  injection vs GnRH $\alpha$  implant treatment for the induction of spawning of greater

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amberjack in the eastern Atlantic. The deliverable will present the results of the two hormonal induction therapies applied in replicated experiments, including egg release frequency, fecundity and egg quality parameters (i.e. morphology, fertilization, hatching and larval survival). Finally a series of conclusions and protocol recommendations for final users will be provided. [month 48]

D3.8) Dose response of GnRHa implant therapy for the induction of spawning in F1 generation broodstock of greater amberjack in the eastern Atlantic: A spawning induction protocol will be developed for greater amberjack broodstock born in captivity (F1 generation) in the eastern Atlantic. The deliverable will present the results of the hormonal induction therapy with implants of different doses of GnRHa, including egg release frequency, fecundity and egg quality parameters (i.e. morphology, fertilization, hatching and larval survival). In addition the deliverable will include data about the effect of the hormonal therapy on plasma levels of the sex steroid hormones and several hematological and biochemical parameters indicators of health and welfare (a) plasma levels of triglycerides, cholesterol, protein and enzymes (b) cortisol, glucose and lactate and (c) electrolytes. [month 54]

D3.9) Development of a spawning induction therapy for captive reared broodstock in the Mediterranean Sea based on the use of GnRHa in the correct mode of administration (hormone/implant), dose and timing of: An optimized spawning induction protocol for captive greater amberjack broodstock in the Mediterranean Sea will be produced. The deliverable will include the methodologies to (a) recruit wild fish and acclimate them in the rearing tanks and cages; (b) assess proper way (e.g. multiple injection or sustained delivery system) as well as proper dose for GnRHa treatment; (c) identify the right time to induce spawning based on histological observation of fish reproductive state as well as on previous years data. The spawning induction protocol will be able to be transferred directly to and be implemented by the industry, in order to establish captive broodstocks and induce them to spawn reliably. [month 54]

D3.10) Method for inducing spawning and collecting greater amberjack eggs in sea cages: A method will be developed for the induction of spawning and collection of eggs of greater amberjacks in sea cages. The deliverable will present the methodology to (a) manipulate the large broodstock in the sea cage to apply hormones, (b) procedures and doses for hormone application, (c) cage set up for egg collection, i.e. clear descriptions of curtain deployed around the perimeter of the cage and (d) methods to actually retrieve the eggs from the water surface of the cage. In addition the deliverable will include the results from repeated trails using and refining the methodologies including the following data: number of eggs obtained per kg female body weight, egg quality parameters, timing of application of hormones in relation to egg collection. [month 54]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP4	Type of activity <sup>54</sup>	RTD
Work package title	Reproduction and Genetics - pikeperch		
Start month		1	
End month		16	
Lead beneficiary number <sup>55</sup>		1	

## Objectives

1. Evaluate the genetic variability of captive broodstock in commercial RAS farms in Europe,
2. Compare this variability with the variability of wild individuals and define how a future genetic breeding program should be established for sustainable optimal performances through domestication of pikeperch.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

The activities of this WP deal mainly on Genetics issues and not reproduction per se, with the intention of developing the necessary knowledge for the industry implementation of breeding selection programs.

EU Budget allocations: 15,000€ (HCMR) and 5,000€ (UL)

Task 4.1 Evaluation of the genetic variation in available domesticated broodstocks of pikeperch (led by UL). Up to now, there has been no evaluation of the current genetic diversity of captive pikeperch. Yet, because there are only a few commercial hatcheries that produce pikeperch (around 10 farms), it is likely that the genetic diversity might be very low compared to the genetic variability of natural populations (Saisa et al., 2010). Each pikeperch farm uses its own isolated stock captured either from the wild or supplied by another farmer. Therefore, pikeperch populations differ from one farm to another depending upon the geographical origin of the wild populations from which the captive stocks derived.

Several studies have been published on the variability of wild pikeperch. For instance, Saisa et al. (2010) have studied the genetic variability of three coastal and five freshwater populations in the northern part of the Baltic sea by using 12 microsatellite loci previously developed for the walleye *Sander vitreum* (Borer et al., 1999; Wirth et al., 1999) and the yellow perch *Perca flavescens* (Leclerc et al., 2000). They found that the coastal populations differ genetically from the lake populations, which present a higher genetic diversity. Salminen et al., (2012) have further studied the genetic consequences and gene flow of pikeperch stocking in three lakes by comparing the pre- and post-release patterns using a subset of 9 microsatellites loci from Saisa et al. (2010). The genetic structures of populations were disrupted by the releases of fish. Two other studies have analysed the genetic structure and dynamics of pikeperch at a regional scale (Björklund et al., 2007; Poulet et al., 2009) using some of the above cited microsatellites loci.

Our first objective (HCMR) is to develop for the species a highly informative and efficient microsatellite multiplex consisted of more than 10 markers, which will allow the adequate genotyping of the fish sampled. Microsatellite loci will be first ordered by increasing size in base pairs (bp) and size range, and in each range one of the primers for each microsatellite locus, e.g. the reverse, will be fluorescently labelled with the dyes conformed to the ABI technology systems (FAM, NED, VIC and PET) and using the Qiagen multiplex PCR kit. The PCR conditions will be optimized in order to finally have a powerful molecular tool for genotyping. The above-mentioned commercial kit gives the advantage of maximal transferability of molecular protocols between labs.

Few microsatellites have already been developed in pikeperch (Kohlman and Kersten, 2008), but numerous have proved to cross-amplify from phylogenetically close Percid species (i.e., walleye *Sander vitreum*, Eurasian perch *Perca fluviatilis*, Yellow perch *Perca flavescens*) (e.g. Leclerc et al., 2000; Gerlach et al., 2001; Björklund et al., 2007; Li et al., 2007; Zhan et al., 2009; Dubut et al., 2010; Douxfils et al., 2011). Among these genetic markers, microsatellite loci showing a high level of polymorphism will be used to characterize the genetic diversity of the available captive pikeperch populations in parallel to wild stocks from which these stocks were



# WT3: Work package description

founded and across European geographical regions (from North to South and from fresh and brackish water). Overall, there are some 65 microsatellites that have been described in the literature for these species. Sampled populations will be from commercial farms, and at least eight populations with more than 50 fish each will be sampled and analyzed through genotyping with microsatellite markers. The collection of finclips will be undertaken in commercial hatcheries that are not participating in DIVERSIFY and the following partners will coordinate the collection over Europe: UL, FUNDP, DTU and ASIALOR. The "European Percid Fish Culture (EPFC) Network (a EAS thematic group on) will facilitate the harvest of fin samples. Basic population genetics parameters (allelic richness, heterozygosity indices, inbreeding coefficients) are going to be estimated with open access software, and also whether or not there is substantial genetic structure will be investigated since it is of particular importance not only for the management but also for the traceability of the species products. This task will result in deliverable D4.1 Genetic analysis of domesticated pikeperch broodstocks.

Task 4.2 Evaluation of the genetic variation in non-domesticated broodstocks of pikeperch (led by HCMR). Since differences in biological characteristics may be related to genetic background, it can be expected that captive populations founded from wild populations of different geographical regions may display different zootechnical performances, as already shown in a preliminary study on Eurasian perch (Mandiki et al., 2004). Moreover, during the first period of habituation to captivity conditions, the rearing process is often conducted empirically (i.e., selecting fish on the basis of reproductive and growth performances and using a founding population of small size), without any real management of the genetic variability as for Eurasian perch culture (Teletchea et al., 2009b). In other fish species it has been shown that such rearing practices result rapidly in a decrease in genetic variability and/or in a genetic drift of captive stocks. In the Eurasian perch, a 2-3fold decrease in allelic diversity is already observed after 4-5 generations reared under captive conditions (Doux fils et al., 2011). Ensuring sufficient genetic variation within populations is fundamental, because it determines the potential of adaptation to hostile changes in environmental/rearing conditions. Moreover, loss of genetic variability within the first generations of breeding practices will limit the potential for future genetic improvement from artificial selection in that cultured stock.

For this purpose, more than five wild pikeperch populations (at least 50 fish from each population) will be sampled from natural fisheries that have been identified by the consortium participants (UL). Wild fish will be caught, anesthetised, a fin clip taken and released. The fin clips will be analyzed through genotyping (HCMR) with the same set of microsatellite markers as above. Genetic differentiation between wild samples and between wild and domesticated will be finally estimated following standard methodologies. Basic population genetics parameters (allelic richness, heterozygosity and inbreeding coefficients in each stock and phylogeographic relationships between populations) will be estimated in order to describe the genetic status of wild broodstock and for comparison with captive broodstocks. The genetic characterisation of all these stocks will be used to propose strategies to establish founding broodstocks that provide the genetic basis for the domestication of pikeperch through the selection for sustainable optimal culture performance. This task will result in deliverable D4.2 Population genetic analysis of wild and comparison with domesticated pikeperch populations to be applied in future breeding programs of the species.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	4.00
9	UL	1.00
	Total	5.00

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D4.1	Genetic analysis of domesticated pikeperch broodstocks	1	3.00	R	PU	12
D4.2	Population genetic analysis of wild and comparison with domesticated pikeperch populations to be applied in future breeding programs of the species	1	2.00	R	PU	16
Total			5.00			

## Description of deliverables

D4.1) Genetic analysis of domesticated pikeperch broodstocks: A highly informative and efficient microsatellite multiplex with more than 10 markers will be described for pikeperch based on loci showing a high level of polymorphism and that were identified in pikeperch and other phylogenetically close Percid species. In addition results from the use of the multiplex will be presented that characterize the genetic diversity of the available captive pikeperch populations from commercial farms with the analysis of more than 50 fish from each of at least eight populations. Genetic characterization will include basic population genetics parameters (allelic richness, heterozygosity indices, inbreeding coefficients) to identify whether or not there is substantial genetic structure, which is of particular importance not only for the management but also for the traceability of the species products. [month 12]

D4.2) Population genetic analysis of wild and comparison with domesticated pikeperch populations to be applied in future breeding programs of the species: The deliverable will present the genetic characterisation (analysed with the same microsatellite multiplex that will be described in D4.1) of wild stocks from which the captive stocks were founded and from across European geographical regions (from North to South and from fresh and brackish water). Results will be included from more than five wild pikeperch populations (at least 50 fish from each population). Genetic differentiation between wild samples and between wild and domesticated will be finally estimated following standard methodologies. Basic population genetics parameters (allelic richness, heterozygosity and inbreeding coefficients in each stock and phylogeographic relationships between populations) will be estimated in order to describe the genetic status of wild broodstock and for comparison with captive counterparts. The genetic characterisation of all these stocks will be used to propose strategies to establish founding broodstocks that provide the genetic basis for the domestication of pikeperch through the selection for sustainable optimal culture performance. [month 16]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS17	Database of genetic variability of pikeperch	1	12	

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP5	Type of activity <sup>54</sup>	RTD
Work package title	Reproduction and Genetics - Atlantic halibut		
Start month	1		
End month	48		
Lead beneficiary number <sup>55</sup>	7		

## Objectives

1. Improve fecundity and gamete quality in F1/F2 broodstock.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

The activities of this WP deal with the enhancement of reproductive performance of hatchery produced Atlantic halibut broodstocks, in order to optimize egg collection by the industry.

EU Budget allocations: 5,040€ (HCMR), 141,000€ (IMR), 11,025€ (NIFES) and 77,636€ (SWH)

Task 5.1 Documentation of reproductive performance in wild-captured vs cultured female Atlantic halibut (led by IMR). Even though empirical data suggest a significant difference in spawning performance between wild-captured and farmed Atlantic halibut females, there currently is a lack of systematic documentation. The Atlantic halibut is a group-synchronous, periodic spawner and in captivity wild-captured females will release 6-12 batches of eggs during a period of 2-4 weeks in the spawning season, which lasts from late February to late April. In order to obtain eggs with high viability, females have to be stripped according to their individual ovulatory rhythms, to prevent over-ripening and deterioration of the eggs (Norberg et al., 1991). While wild-captured females generally adapt well in captivity, displaying high fecundity with egg batches spawned at regular intervals, hatchery-produced F1/F2 females appear to suffer from a reproductive dysfunction, releasing small batches of eggs at irregular intervals. Consequently, individual spawning cycles will be documented in cultured and wild-captured females (IMR).. At IMR, 4-6 individual females will be tagged and followed. Fertilized eggs will be photographed using a using a dissecting microscope (113.23 pixels/mm), for measurements of egg diameter, blastomere symmetry and fertilisation rate. Diameters of spawned eggs will be measured automatically in ImageJ (<http://rsb.info.nih.gov/ij/>) using custom made plug-in and macros (<http://simon.bio.uva.nl/ObjectJ/objectj.html>) (IMR). The following parameters will be registered: Length of spawning period; number of batches; ovulatory interval; volume and number of eggs per batch; egg diameter; fertilisation and hatching rate; cell symmetry, egg steroid concentration (E2, T, cortisol). This task will result in deliverable D5.1 Documentation of reproductive performance in wild-captured vs cultured female Atlantic halibut.

Task 5.2 GnRH implant therapy as a means to improve spawning performance (led by HCMR). Long-term release implants for gonadotropin releasing hormone agonist (GnRHa) will be produced (HCMR) using a non-degradable Ethylene-Vinyl Acetate copolymer (EVAc) using established procedures (Mylonas & Zohar, 2001). The GnRHa implants are expected to release the loaded hormone for a period of 4 weeks, with 20% of the hormone released during the first 24h. The release kinetics of the implants will be confirmed by HCMR using an in vitro release assay, with GnRHa implants embedded in low-melting point agarose and maintained at 6°C. Mature farmed, F1/F2, female Atlantic halibut will be implanted either with GnRHa or a sham implant (IMR, SWH), and spawning performance compared between the implanted and controls. The broodstock will be maintained by SWH and IMR will lead the study assisted by the staff of SWH. Prior to implantation, appearance and degree of swelling of the ovipore will be registered and ovarian biopsies taken, in order to estimate stage of maturation. GnRHa doses will be based on previously established optimum treatment of male Atlantic halibut (Vermeirssen et al., 2004). Differences in registered spawning parameters as described above (Task 5.1) will be compared by appropriate statistical methods to determine optimum dose and timing of GnRH treatment. An optimized GnRHa therapy protocol may be efficient to improve spawning performance of F1/F2 Atlantic halibut,

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and to increase availability of eggs of stable and predictable quality. This task will result in deliverable D5.2 An optimised GnRHa therapy protocol to improve spawning performance of F1/F2 Atlantic halibut, and to increase availability of eggs of stable and predictable quality.

Task 5.3 Fecundity regulation (led by IMR). Potential disturbances in reproductive development in F1/F2 females will be assessed by fecundity analyses, ovarian histology, gene transcript levels of fshr and lhcg (Kobayashi et al., 2008; Mittelholzer et al., 2009) and analysis of plasma steroid and vitellogenin profiles (Norberg, 1995; Norberg et al., 2001) through gametogenesis in F1/F2 females and wild-captured fish (IMR). Ovarian samples will be taken by biopsy of anesthetized fish (maintained by SWH) at the following stages: late vitellogenesis, prespawning/hyaline, one week into spawning and two weeks into spawning. Potential and realized fecundity, and size-frequency distribution of oocytes in spawning females will be determined in wild-captured and F1 spawners (IMR). Analyses will be performed using the Auto-diametric fecundity method (IMR; Thorsen and Kjesbu, 2001) in NIFES: Fixed samples are stored at least 14 days before analysis. A subsample is spread on a Petri dish and photographed. The sizes of at least 200 maturing (vitellogenic) oocytes are then analysed from the pictures by automatic particle analysis using the freeware image analysis program Image J (<http://imagej.nih.gov/ij/>). From the mean oocyte diameter, oocyte density (n/g ovary) is estimated using a packing density formula given by Thorsen and Kjesbu (2001). Potential fecundity is then estimated by multiplication of oocyte density and ovary weight. Differences between F1 and wild-captured fish will be analysed using appropriate statistical methods (IMR, NIFES). This task will result in deliverable D5.3 Identification of potential disturbances in reproductive development in F1/F2 Atlantic halibut females.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	0.50
7	IMR	8.00
17	IMR/NIFES	1.00
22	SWH	11.00
	Total	20.50

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D5.1	Documentation of reproductive performance in wild-captured vs cultured female Atlantic halibut	7	7.00	R	PU	30
D5.2	An optimised GnRHa therapy protocol to improve spawning performance of F1/F2 Atlantic halibut, and to increase availability of eggs of stable and predictable quality	7	6.50	R	PU	30
D5.3	Identification of potential disturbances in reproductive development in F1/F2 Atlantic halibut females	7	7.00	R	PU	48
		Total	20.50			

## Description of deliverables

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D5.1) Documentation of reproductive performance in wild-captured vs cultured female Atlantic halibut: This deliverable will be a systematic documentation of reproductive performance of wild-captured and cultured female Atlantic halibut. Empirical data suggest a significant difference in spawning performance between wild-captured and farmed Atlantic halibut females, but there currently is a lack of systematic documentation. Consequently, individual spawning cycles in cultured and wild-captured females and reproductive performance will be documented. The following parameters will be registered: Length of spawning period; number of batches; ovulatory interval; volume and number of eggs per batch; egg diameter; fertilisation and hatching rate; cell symmetry, egg steroid concentration (E2, T, cortisol). [month 30]

D5.2) An optimised GnRHa therapy protocol to improve spawning performance of F1/F2 Atlantic halibut, and to increase availability of eggs of stable and predictable quality: This deliverable will describe a protocol for efficient induction of ovulation in halibut. Long-term release implants for gonadotropin releasing hormone agonist (GnRHa) will be used. Experiments will be described where mature farmed, F1/F2, female Atlantic halibut will be implanted either with GnRHa or a sham implant, and spawning performance compared between the implanted and controls as in D5.1. The following data will be reported, appearance and degree of swelling of the ovipore in order to estimate stage of maturation prior to hormone application and spawning parameters (as described Task 5.1). These parameters will be compared by appropriate statistical methods to determine optimum dose and timing of GnRH treatment. [month 30]

D5.3) Identification of potential disturbances in reproductive development in F1/F2 Atlantic halibut females: This deliverable will be a documentation of potential physiological differences between wild-captured females and F1/F2 female halibut. The deliverable will describe an analysis of fecundity analyses, ovarian histology, gene transcript levels of fshr and lhcg, and analysis of plasma steroid and vitellogenin profiles through vitellogenesis and final maturation. Potential and realized fecundity, and size-frequency distribution of oocytes in spawning females will be presented in wild-captured and F1 spawners. Differences between F1 and wild-captured fish will be analysed using appropriate statistical methods and reported. [month 48]

### Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS18	Documentation of ovulatory cycles in wild and F1 halibut broodstock	7	30	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP6	Type of activity <sup>54</sup>	RTD
Work package title	Reproduction and Genetics – wreckfish		
Start month	1		
End month	54		
Lead beneficiary number <sup>55</sup>	8		

## Objectives

1. Increase the availability of wreckfish broodstocks in captivity,
2. Describe the reproductive cycle in captivity at the level of the pituitary and gonad,
3. Develop spawning induction procedures for in vitro fertilization, as well as spontaneous tank spawning,
4. Develop a CASA for evaluation of wreckfish sperm and establish cryopreservation protocols for use in in vitro fertilization applications.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

This WP includes tasks to (a) increase the availability of wreckfish broodstocks, (b) study the reproductive cycle of fish in captivity to understand the reproductive dysfunctions (c) develop spawning induction methods for the acquisition of eggs and (d) develop methods for the study of wreckfish sperm and cryopreservation for in vitro fertilization applications.

EU Budget allocations: 48,632€ (HCMR), 4,000€ (IRTA), 88,370€ (IEO), 28,600€ (IFREMER), 3,360€ (ULL), 70,203€ (CMRM) and 17,767€ (MC2)

Task 6.1 Collect wild fish to establish new broodstocks (led by CMRM). To increase the number of broodstock available wild fish, juveniles and adults will be collected from the wild (IEO, CMRM and MC2). The collection of adult fish is complicated as these fish leave in great depths (>400 m) and all available broodstock were obtained from the wild as juveniles. Collection will be coordinated (CMRM, IEO) with professionals that collect fish for large public aquariums i.e. Acuario del Grove (Grove Aquarium), MC2 and Flying Sharks (<http://www.flyingsharks.eu/>). The collected fish will be maintained under controlled conditions of temperature and photoperiod, and will be monitored every 4 months for growth and reproductive maturation, using gonadal biopsies. This task will both provide collection methods and availability of captive broodstock for SMEs and the aquaculture industry. This task will result in deliverable D6.4 Establish reliable collection methods and protocols to form new wreckfish broodstocks.

Task 6.2 Describe reproductive cycle (led by IEO). Due to the rarity of available wreckfish, in order to study its reproductive cycle in captivity, we will use all the available broodstocks in the EU, which are maintained in captivity at the facilities of HCMR (n=5), IEO (n=10), MC2 (n=24) and CMRM (n=12). Blood samples will be collected from the fish at different times during the annual reproductive cycle, and will be analyzed for the sex steroid hormones T, 11-KT, E2 and 17,20β-DHP using established ELISAs (HCMR) and gonadotropins (FSH/LH) using established non-homologous assays (IOLR). The assays of analysis of sex steroids (HCMR) and gonadotropins (IOLR) will be modified and validated for wreckfish and sample collection will be coordinated by the IEO from the different broodstocks (HCMR, IEO, MC2 and CMRM). Gonadal biopsies will be obtained at each sampling time and will be examined under the microscope to determine oocyte size and stage of development using wet mounts (IEO, IRTA) and histology (HCMR). An IRTA researcher will visit to participate in sample collection and samples will be sent to HCMR for histological analysis. Collected sperm will be evaluated using standard sperm analysis (density, motility % and duration) (same procedure as in Task 2.3). In addition, wild fish from the fishery will be obtained during the reproductive period (CMRM, IEO), and will be examined for gonadal development (HCMR) and nutritional status (CMRM, ULL) (same procedure as 3.1), in order to establish a base line in wild fish and compare with the data set obtained from captive broodstock. Sample collection from wild fish will be coordinated by the CMRM and IEO and samples will be sent to HCMR

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for histological analysis and ULL for nutritional analysis. This task will result in deliverable D6.5 Describe the reproductive cycle of wreckfish.

Task 6.3 Development of spawning induction procedures (led by IEO). Available information from Partners of DIVERSIFY (Mylonas et al., 2004; Fauvel et al., 2008; Peleteiro et al., 2010) indicates that GnRHa implants (HCMR) may be effective in inducing oocyte maturation and ovulation, and that stripping protocols may be needed. Therefore, GnRHa implants will be used in the available stocks (HCMR, CMRM, and MC2). All broodstocks will be monitored for reproductive function (see task 6.2). When fish are at the correct maturation stage they will be induced to spawn using GnRHa implants, testing doses of 50-200 µg kg<sup>-1</sup>. Two approaches will be taken: (a) Fish will be placed in large tanks ≥40-m<sup>3</sup> under controlled photothermal conditions and allowed to spawn spontaneously (HCMR, MC2). Large tanks were shown in groupers to overcome the lack of spawning after ovulation (Marino et al., 2003). If fish fail to spawn, they will be sampled to identify failure of maturation or spawning, the latter being the experience in smaller tanks. Depending on the outcome, the approach will be adjusted with new doses (insufficient maturation) or strip spawning (lack of spawning). (b) Experiments will be conducted in smaller tanks (≤15 m<sup>3</sup>) and fish will be monitored for ovulation. Ovulated eggs will be inseminated in vitro using sperm from spermiating males and the eggs will be incubated (IEO, CMRM, HCMR). If fish fail to spawn, they will be sampled to confirm if the failure is related to lack of maturation and new implant doses will be tested. A researcher from IRTA will visit to participate in spawning induction procedures. Eggs will be monitored for quality (See Task 2.2). Nutritional quality (See WP3) of egg batches will also be determined (CMRM, ULL) to compare with nutritional status of wild fish (Task 6.2) and to identify nutrients that may be lacking from the broodstock diet. Samples will be sent to ULL for nutritional analysis. Between the different stocks, husbandry variables such as sampling procedures, disturbance due to sampling, photothermal regime and nutrition (raw fish supplemented with commercial broodstock diets) will be standardized and maintained as close to identical as possible. Standardization of these parameters will facilitate comparison of results from different stocks to determine optimal tank sizes, implant doses and spawning protocols. This task will contribute to deliverables D6.7 Develop a spawning induction method for spontaneous spawning of wreckfish in large tanks, and will also result in D6.3 Develop spawning induction methods with in vitro fertilization of wreckfish and D6.6 Define an in vitro fertilization protocol to be employed by the industry to spawn wreckfish.

Task 6.4 Evaluation of sperm characteristics and cryopreservation protocols (led by IFREMER). As indicated above, spawning induction methods for wreckfish should include in vitro fertilization, as spontaneous spawning is not achieved reliably (i.e., only in an Aquarium condition so far), and is expected to be a problem in industrial conditions. This dysfunction is common for other benthic and cave dwelling “groupers”, such as the dusky grouper (*Epinephelus marginatus*) (Marino et al., 2003). Therefore, to enhance the operation of in vitro fertilization and dissociate sperm from egg collection, it is necessary to study sperm characteristics in wreckfish and develop cryopreservation methods (IFREMER). An IFREMER researcher will visit (IEO, CMRM and MC2) to participate in the development and modification of sperm analysis and cryopreservation procedures. Characterization of wreckfish sperm (IFREMER) will be done using CASA looking at % spermatozoa motility, velocity, linearity of tracks, sperm membrane integrity by eosin/nigrosin, sperm viability and sperm energetic status using ATP measurement (same procedure as 2.1.3). For this task, sperm will be obtained from broodstock maintained by IEO, CMRM and MC2. In order to increase the available volume, and perhaps the quality of the available sperm, GnRHa implants will be used to increase sperm availability (HCMR), as has been achieved in other species (Sorbera et al., 1996). Males at the beginning of the spawning period (end of April) will be treated (IEO) and sampled at 0, 3, 7 and 21 days afterwards. Sperm production (volume) and quality will be evaluated (IEO, IFREMER) and the sex steroids T, 11-KT and 17,20β-DHP will be monitored (HCMR) in the blood to correlate with milt production. General cryopreservation protocols of marine fish sperm will be adapted to the specific requirements of wreckfish sperm (IFREMER) (same procedure as 2.1.3). Furthermore, to test reliability of protocols, artificial insemination trials will be performed (IEO/IFREMER). This task will result in deliverables D6.1 Establish a Computer Assisted Sperm Analysis (CASA) for wreckfish sperm and D6.2 Develop a cryopreservation method for wreckfish.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	7.90
3	IRTA	2.00

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## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
8	IEO	6.60
14	IFREMER	3.00
15	ULL	0.90
19	CMRM	11.70
32	MC2	2.06
<b>Total</b>		<b>34.16</b>

## List of deliverables

Delive- rable Number <sup>61</sup>	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature <sup>62</sup>	Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D6.1	Computer Assisted Sperm Analysis (CASA) for wreckfish sperm	14	2.00	R	PU	24
D6.2	Cryopreservation method for wreckfish	8	3.50	R	PU	24
D6.3	Spawning induction methods with in vitro fertilization of wreckfish	8	8.76	R	PU	36
D6.4	Establish reliable collection methods and protocols to form new wreckfish broodstocks	19	3.10	R	PU	36
D6.5	Description of the reproductive cycle of wreckfish	8	10.40	R	PU	48
D6.6	An in vitro fertilization protocol to be employed by the industry to spawn wreckfish	8	3.00	R	PU	48
D6.7	Spawning induction method for spontaneous spawning of wreckfish in large tanks	1	3.40	R	PU	54
<b>Total</b>			<b>34.16</b>			

## Description of deliverables

D6.1) Computer Assisted Sperm Analysis (CASA) for wreckfish sperm: A report will provide motility features of wreckfish sperm and a movie explaining the general procedure of sperm activation video recording and Computer Assisted Sperm Analysis (CASA) use will be proposed on the website of the project. [month 24]

D6.2) Cryopreservation method for wreckfish: The characteristics of cryopreservation media and improved freezing protocols (dilution rate, type of straw, cooling rate) will be reported as well as the assessment of freezing consequence on sperm features [month 24]

D6.3) Spawning induction methods with in vitro fertilization of wreckfish: A method will be developed for the induction of spawning with in vitro fertilization of wreckfish. The deliverable will present the methodology to (a) manipulate the broodstocks in the tank to collect ovulated eggs and sperm, (b) procedures and doses for in vitro insemination, and (c) control the quality of eggs and sperm. In addition, the deliverable will include the results from repeated artificial insemination trails and refining methodologies including the following data: standard



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parameters maintained between the different stocks, number of eggs obtained per kilo of female, egg quality parameters. [month 36]

D6.4) Establish reliable collection methods and protocols to form new wreckfish broodstocks: A wreckfish capture system will be described. These animals will be used as future broodstocks. The deliverables will describe: 1) The less aggressive fishing arts used to capture the fish, 2) Ideal fish size for adaptation to captivity, considering type of food required and detection of possible parasites associated to this size/age and 3) sex determination by biopsy or echogram, in order to maintain ideal proportions of male/female. The deliverable will also include information regarding growth resulting from samplings every 4 months, and a protocol for the industry, concerning capture and acclimation of broodstock. [month 36]

D6.5) Description of the reproductive cycle of wreckfish: The reproductive cycle of the wreckfish has not yet been completely described and understood, and therefore this deliverable will describe, 1) reproductive season and behaviour, based on data from wild caught specimens as well as captive ones, for which research on gonad histology will be performed, 2) Blood samples of captive broodstock will be analyzed to determine sexual steroid levels, while ovary biopsies will determine the maturity stage of the oocytes, 3) Samples of sperm will be analyzed and characterized. This deliverable will include information on the reproductive behaviour of the wreckfish and definitions on the quality criteria of the sexual products (oocytes and sperm). [month 48]

D6.6) An in vitro fertilization protocol to be employed by the industry to spawn wreckfish: A method to perform in vitro fertilization for commercial use will be described. The deliverable will include a protocol on how to obtain fertilized eggs to produce larvae based on: 1) Determination of maturity stage of males and females based on the techniques described in D6.4, 2) utilization of oocytes and sperm fresh or cryopreserved (D 6.3), to perform artificial fertilization, relation sperm/oocyte, percentage of fecundation/hatching. This protocol will be published for its use by the sector. [month 48]

D6.7) Spawning induction method for spontaneous spawning of wreckfish in large tanks: A method for the induction of spawning of wreckfish in large tanks will be developed. The deliverable will present the methodology to (a) manipulate the broodstocks in the tank to apply GnRH $\alpha$  implants, (b) procedures and doses for hormone application, (c) control photothermal conditions in large tanks and (d) methods to actually retrieve the eggs from the water surface. In addition, the deliverable will include the results from repeated trials and refining methodologies including the following data: standard parameters maintained between the different stocks, number of eggs obtained per kilo of female, egg quality parameters, timing of application of hormones in relation to egg collection. [month 54]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS34	Successful maturation and spawning of wreckfish to produce good quality eggs	8	5	This will allow implementation of larval rearing experiments (WP 18)
MS35	Successful maturation and spawning of wreckfish to produce good quality eggs	8	17	This will allow implementation of larval rearing experiments (WP 18)
MS36	Successful maturation and spawning of wreckfish to produce good quality eggs	8	29	This will allow implementation of larval rearing experiments (WP 18)
MS37	Successful maturation and spawning of wreckfish to produce good quality eggs	8	41	This will allow implementation of larval

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## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
				rearing experiments (WP 18)

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP7	Type of activity <sup>54</sup>	RTD
Work package title	Reproduction and Genetics – grey mullet		
Start month	1		
End month	60		
Lead beneficiary number <sup>55</sup>	4		

## Objectives

1. Evaluate the effectiveness of hormone-based treatments on synchronizing gonadal development and improving gamete (eggs and sperm) quality in mature grey mullet,
2. Develop hormone-based treatments for induced spawning of grey mullet,
3. Optimize a scaled-up breeding of grey mullet in captivity under natural and manipulated photo-thermal regimes,
4. Assess the effects of captivity on first sexual maturity and reproductive potential of captive-reared and hatchery-produced grey mullet broodstocks.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

The activities of this WP deal with the development of reproductive control methods for grey mullet, both captive-reared and hatchery-produced.

EU Budget allocations: 4,965€ (HCMR), 20,000€ (IRTA), 49,000€ (IOLR), 6,558€ (UNIBA), 6,908€ (IFREMER), 12,500€ (ULL), 20,050€ (DOR), 10,942€ (ITTICAL, exited the consortium on 31/5/2016)

Task 7.1 Evaluation of the effectiveness of hormone-based treatments on synchronizing gonadal development (led by IOLR). Wild-caught fingerlings reared to maturity under captive conditions will be separated into ten broodstock groups and acclimated to ambient photo-thermal conditions (IOLR). Towards the onset of the reproductive cycle (mid-July in the Mediterranean Sea) females will be injected intramuscularly with either (i) GnRHa slow-release implants (HCMR) prepared by Ethylene-vinyl acetate (EVAc), (ii) the dopamine antagonist domperidone or (iii) a combination of the two. Males will be injected with either (i) recombinant FSH, (ii) 17 $\alpha$ -methyltestosterone (MT) loaded on EVAc slow-release implants (HCMR), or (iii) a combination of the two. The control fish will be injected with saline only. Recombinant bioactive gonadotropins (i.e., FSH and LH) will be produced in a yeast expression system (IOLR). Fish will be biopsied during the progression of gametogenesis (July-October), and their reproductive status will be evaluated using (a) wet mounts of ovarian biopsies, (b) histological evaluations of biopsies, and (c) analyses of reproductive hormones including sex steroids and LH (IOLR). In addition, characterization of grey mullet sperm (IFREMER) will be done using CASA, looking at % mobile spermatozoa, velocity, linearity of tracks, sperm membrane integrity by eosin/nigrosin, sperm viability test and sperm energetic status using ATP measurement. This task will result in deliverables D7.1 Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of grey mullet sperm, D7.2 Production of recombinant bioactive LH and FSH assay for grey mullet, and will contribute to D7.3 Comparative effectiveness of hormonal treatments for spawning induction in captive grey mullet and D7.5 Description of the process of oogenesis in captive-reared vs hatchery-produced grey mullet, including aspects of growth, body indices, and histological evaluation of ovarian development.

Task 7.2 Development of hormone-based treatments for inducing spawning (led by IOLR). Reproductively mature fish (as determined by gonadal biopsy; Task 7.1) will be selected and treated with either (i) GnRHa alone or in combination with a dopamine antagonist, metaclopramide, and/or recombinant LH (IOLR). At each spawn, eggs will be collected and evaluated for fecundity and fertilization. The average diameter of the spawned eggs, total egg volume and the volume of fertilized viable eggs (=floating eggs) will be recorded for each spawn, and

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correlated with larval quality (hatching, larval survival to yolk absorption). This task will contribute to deliverable D7.3 Comparative effectiveness of hormonal treatments for spawning induction in captive grey mullet.

Task 7.3 Optimization and scale-up of a breeding protocol for grey mullet in captivity (led by IOLR). The best performing hormonal therapy for accelerating gonadal development (based on results of Task 7.1) and spawning induction (based on Task 7.2) will be employed to test our ability to further extend the reproductive season (i.e., multiple spawns per individual, manipulated photo-thermal regimes), as well as improve reproductive productivity by mass-spawning (IOLR, DOR). For that purpose captive-reared sexually mature broodstocks will be acclimated to either ambient or shifted photo-thermal conditions. Spawning induction trials will be carried out using broodstocks differing in their size (2 to 20 individuals) and sex ratio (i.e., equal, skewed in favour of males, skewed in favour of females). Spawning females will be subjected again to the same hormonal treatment (as above), in order to obtain further spawns (IOLR).

At the same time, the selected hormonal therapy will be applied to induce spawning in wild-caught mature fish vs. wild fish that will be acclimated to and matured in captivity (IRTA). This step is expected to confirm the consistency and reliability of the spawning induction protocol. Furthermore, the biochemical composition of wild fish and the spawned eggs from both groups will be analysed (ULL) (same procedure as Task 3.1) to provide an important reference for identifying specific nutritional requirements for improved egg quality that will be correlated with larval quality (hatching, larval survival to yolk absorption). This task will contribute to deliverables D7.3 Comparative effectiveness of hormonal treatments for spawning induction in captive grey mullet and D7.7 Development of a breeding protocol for captive reared grey mullet broodstock based on optimized hormonal treatment, group structure and photo-thermal regime.

Task 7.4 Assessment of the effects of captivity on first sexual maturity of wild-caught and hatchery-produced fish (led by IOLR). Grey mullet fingerlings will be maintained under different stocking densities until sexual maturity (IOLR, GEI). Fish (n=20 of each group) of two age categories (2 and 3 year-old) will be sacrificed at two critical periods during the reproductive season (August at early vitellogenesis and October at late vitellogenesis). Morphometric parameters including the weight of the body and internal organs (i.e., gonads, liver and fat-body) will be recorded and the respective indices (i.e., GSI, HIS and FSI) will be calculated. In addition gonad samples will be subjected to histological analyses and oocyte yolk accumulation will be monitored using image analysis software (UNIBA). Advanced and spontaneous sexual maturity under captive conditions can facilitate grey mullet roe production (bottarga) as a high valued product from grey mullet. This task will contribute to deliverable D7.5 Description of the process of oogenesis in captive-reared vs hatchery-produced grey mullet, including aspects of growth, body indices, and histological evaluation of ovarian development and D7.6 Culture procedure that identifies the on-growing period for the production of grey mullet roe (bottarga) from wild and hatchery juveniles.

Task 7.5 Establish a shipping protocol for grey mullet eggs (led by DOR). Using surplus eggs from the spawning experiments (Task 7.3) DAG will carry out in-house simulation. These trials will be based on protocols developed in a previous FP7 project (SELDOTT) to transport Atlantic bluefin tuna eggs to different locations in the Mediterranean. In this protocol special attention was paid to the plastic transport container (20 l cubitainers used in the wine industry), type of disinfection agent and concentration, egg density (10-15,000 eggs/l), volume of seawater (10-15 l), size and placement of ice packs, insulating material surrounding the cubitainer and the use of pure oxygen in the cubitainer. In these trials temperature will be constantly monitored by the use of a data logger. This task will result in deliverable D7.4 Develop protocol for shipping grey mullet eggs.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	0.50
3	IRTA	2.00
4	IOLR	7.00
13	UNIBA	3.70
14	IFREMER	0.50
15	ULL	3.40

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## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
24	ITTICAL	3.20
25	DOR	3.00
26	GEI	0.00
Total		23.30

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D7.1	Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of grey mullet sperm	14	0.50	R	PU	12
D7.2	Production of recombinant bioactive LH and FSH assay for grey mullet	4	2.00	R	PU	18
D7.3	Comparative effectiveness of hormonal treatments for spawning induction in captive grey mullet	4	2.00	R	PU	24
D7.4	Protocol for shipping grey mullet eggs	4	3.00	R	PU	24
D7.5	Description of the process of oogenesis in captive-reared vs hatchery-produced grey mullet, including aspects of growth, body indices, and histological evaluation of ovarian development	13	6.70	R	PU	48
D7.6	Culture procedure that identifies the on-growing period for the production of grey mullet roe (bottarga) from wild and hatchery juveniles	4	6.60	R	PU	54
D7.7	Development of a breeding protocol for captive reared grey mullet broodstock based on optimized hormonal treatment, group structure and photo-thermal regime	4	2.50	R	PU	60
Total			23.30			

## Description of deliverables

D7.1) Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of grey mullet sperm: The best adapted CASA parameters for mullet sperm analyses will be determined and reported to end users to optimize their abilities to check the fertility of the semen in the course of their future experiments. A movie explaining the general procedure of sperm activation video recording and CASA use will be proposed on the website of the project. [month 12]

D7.2) Production of recombinant bioactive LH and FSH assay for grey mullet: Production of recombinant grey mullet gonadotropins (LH and FSH) for therapeutic use. The deliverable will present: (a) sequences encoding for grey mullet gonadotropin subunits, including GP $\alpha$ , LH $\beta$ , and FSH $\beta$ , (b) yeast expression system, a means for the

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rapid production of recombinant grey mullet gonadotropins, (c) recombinant LH and FSH holo-hormones, each arranged as a yoked  $\alpha+\beta$  protein, demonstrating a proven bioactivity. [month 18]

D7.3) Comparative effectiveness of hormonal treatments for spawning induction in captive grey mullet:

A protocol will be developed for the induction of spawning of captive grey mullet. The deliverable will present the procedures, dosage and timing for hormone application, which enhance synchronized gonadal development/gamete maturation within and between genders, as well as induce spawning. In addition, the deliverable will include the results from repeated trails including the following data: relative fecundity (number of total eggs obtained per kilo of female), latency period (timing of application of hormones in relation to egg collection), and egg quality parameters. [month 24]

D7.4) Protocol for shipping grey mullet eggs: Establishment of procedures for handling grey mullet eggs in order to allow their transport to various larval rearing facilities. The deliverable will present procedures for the optimal (a) egg disinfection, (b) shipment conditions including: egg density, duration, temperature and pH. The deliverable will summarize the results from repeated trails addressing different environmental parameters in order to optimize the procedure and maximize larval survival. [month 24]

D7.5) Description of the process of oogenesis in captive-reared vs hatchery-produced grey mullet, including aspects of growth, body indices, and histological evaluation of ovarian development: Account of captive effects on pubertal development in grey mullet populations. With the aim of highlighting potential correlations between domestication and improved growth and maturational processes, the deliverable will include a comparative documentation of first sexual maturity and growth performance in wild and hatchery-produced grey mullet specimens reared in captivity. [month 48]

D7.6) Culture procedure that identifies the ongrowing period for the production of grey mullet roe (bottarga) from wild and hatchery juveniles: A protocol for grey mullet culture will be developed giving rise to optimal production of high quality roe (bottarga). The deliverable will present effects of the age, stocking density and feeding management of the fish, on the size, texture and colour of the roe. In addition, the deliverable will pinpoint the pre-eminent timing for harvesting the roe, which yields maximal roe size, yet, preserves its firm texture. [month 54]

D7.7) Development of a breeding protocol for captive reared grey mullet broodstock based on optimized hormonal treatment, group structure and photo-thermal regime: Recommendation for a grey mullet breeding management protocol for commercial use. The deliverable will present the (a) hormone-based therapeutic (b) group structure, and (c) photo-thermal regime, that enhance synchronized gonadal development/gamete maturation as well as induce spawning. The deliverable will provide results from repeated trails using and refining the methodologies including the following data: relative fecundity (number of total eggs obtained per kilo of female), latency period (timing of application of hormones in relation to egg collection), and egg quality parameters. [month 60]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP8	Type of activity <sup>54</sup>	RTD
Work package title	Nutrition – meagre		
Start month	1		
End month	48		
Lead beneficiary number <sup>55</sup>	2		

## Objectives

1. Improve current larval weaning feeds for meagre,
2. Determine nutritional requirements to promote feed utilization, consistent growth rates and fish welfare to reduce size variation.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

The objective of this WP will be to better define the nutritional needs of meagre during both pre-growing and on-growing phases to improve growth consistency and fish welfare and health.

EU Budget allocation: 46,300€ (FCPCT), 19,000€ (ULL), 8,000€ (SARC) and 8,000€ (DTU)

Task 8.1 Improvement of larval weaning feeds (led by FCPCT). In other species, essential fatty acids (EFA) and certain micronutrients (Vits A, K and D) are known to be indispensable during early weaning (Izquierdo & Koven, 2011; Hamre et al., 2013). The optimum levels of essential fatty acids and related micronutrients will be determined in weaning diets for meagre: the effect of nutrients on culture performance, morphometric parameters, gut occupancy, larval organ and skeleton development, and biochemical composition (FCPCT). Besides, meagre welfare status in terms of resistance to handling stress, stress bio-markers such as gene expression of HSPs (FCPCT), specific fish behaviour (see below), evaluation of metabolic cost after sub-lethal stress, video analysis of activity, escape responses and sensory acuity (DTU) will be studied. Learning ability will be explored by a maze test and video recording (DTU). The maze consists of a 40 x 40 cm square with four potential exits in each corner. One of the exits lead juvenile fish out of the maze to a darker area with a cover. Initial freezing time before searching, time spent searching and number of visits to exits before leaving the maze are recorded and compared for a number of individuals for each dietary treatment. Studies will also include total digestive enzyme (protease, amylase and lipase) and gut ATPase activities related to these dietary treatments (ULL). This Task will result in deliverable D8.1 Improvement of larval weaning diets.

Task 8.2 Determination of nutritional requirements to promote feed utilization, consistent growth rates and fish welfare (Led by FCPCT) The essential fatty acid requirements will be examined in grow out diets for meagre (SARC) by feeding six levels of docosahexaenoic, eicosapentaenoic and araquidonic acids. The effect of these fatty acids on meagre growth, welfare and health status will be studied. Feed intake, growth parameters (total length, body weight, SGR, K index), feed utilization, whole fish and muscle lipid and fatty acid composition and morphology of liver as a main organ related to feed utilization and fish metabolism, will be studied by FCPCT to determine the effects on growth. Survival, gut as the main absorption organ and principal entrance of pathogens, kidney as an important organ related to fish welfare and heart as an organ sensitive to essential fatty acids deficiency, will be studied by FCPCT to assess the potential importance of these fatty acids for meagre health. Besides, meagre welfare status in terms of resistance to handling stress, stress bio-markers (gene expression of HSPs) will be also studied by FCPCT. Total digestive enzymes (protease, amylase and lipase) and gut ATPase activities will be studied by ULL. This Task will result in deliverable D8.2 Recommended essential fatty acids contents in diets to promote meagre growth, welfare and health, based on the effects of the diets on growth; stress resistance and biomarkers; and survival, gut integrity and morphology of selected tissues such as kidney and heart.

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## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
2	FCPCT	20.00
15	ULL	4.50
20	SARC	1.50
21	DTU	1.00
	Total	27.00

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D8.1	Improvement of larval weaning diets	2	11.00	R	PU	24
D8.2	Recommended essential fatty acids contents in diets to promote meagre growth, welfare and health	2	16.00	R	PU	48
		Total	27.00			

## Description of deliverables

D8.1) Improvement of larval weaning diets: A report with a recommendation of the optimum levels of Lys in on-growing diets for meagre based mainly on plant ingredients (low fish meal inclusion) will be presented in this deliverable. The deliverable will describe the effects of diets containing six levels of lysine and will include: a) the main methodology employed, b) the effect on fish performance c) feed utilization and d) the requirements of this aminoacid to improve growth, survival and stress responses. [month 24]

D8.2) Recommended essential fatty acids contents in diets to promote meagre growth, welfare and health: The deliverable will be in the form of a report to present the recommendation of the optimum levels of essential fatty acids in on-growing diets for meagre. The deliverable will include the main methodology employed, followed by the results that led to the recommendation of the essential fatty acid levels. The deliverable will describe the effects of essential fatty acids and will include: a) growth performance, b) feed utilization, c) welfare, d) health status, e) fish behavior and f) digestion processes. [month 48]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS19	Basic diet formulation for meagre grow-out studies	2	12	
MS20	Digestive utilization of experimental weaning diets for meagre	2	24	



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## One form per Work Package

Work package number <sup>53</sup>	WP9	Type of activity <sup>54</sup>	RTD
Work package title	Nutrition – greater amberjack		
Start month	1		
End month	58		
Lead beneficiary number <sup>55</sup>	2		

## Objectives

1. Improve of larval enrichment products to enhance production of larvae and juvenile,
2. Develop diets for grow-out in order to maximize growth potential,
3. Development of an appropriate broodstock diet to improve unreliable reproduction in amberjack.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

This WP will address three of the main bottlenecks identified in greater amberjack: Limited production of larvae and juvenile, scarce information on nutritional requirements during grow-out and the lack of reliable reproduction and egg availability by focussing three main objectives: improvement of larval development and survival by adequate nutrients levels in enrichment products, effect of nutritionally enhanced grow out diets on juvenile performance and effective broodstock feeding regimes to boost reproduction.

EU Budget allocation: 24,072€ (HCMR), 45,413 € (FCPCT), 19,500€ (IEO), 9,500€ (ULL), 18,875€ (SARC) and 34,400€ (CANEXMAR)

Task 9.1 Improve larval enrichment products to enhance production of larvae and juveniles (led by FCPCT). To determine the optimum levels of essential fatty acids in enrichment products, fat-soluble vitamins, antioxidants, carotenoids and Tau, specific nutrients that have been suggested to be particularly important for the larvae of this and other *Seriola* species, the following feeding trials will be conducted:

Sub-task 9.1.1 (FCPCT) The optimum essential fatty acid will be determined in enrichment products for live preys. Greater amberjack larvae will be fed live preys enriched with different levels of essential fatty acids and ratios prepared by FCPCT in order to determine the requirements for these nutrients during early larval development. Larval performance in terms of survival, growth and welfare (survival to handling stress test) will be studied. Proximate and fatty acid composition of enrichment products, live preys and larvae will be analysed. Morphogenesis of digestive system and bone, as well as morphogenetic biomarkers (IGF, BMP, ALP) and related parameters (bone mineralization and deformities) will be studied by FCPCT. This Sub-task will contribute to Deliverable D9.1 Optimum levels and ratios of essential fatty acids in relation to Tau and combined PUFA-carotenoids in greater amberjack enrichment products.

Sub-task 9.1.2 (IEO, ULL) will examine the combined effect of PUFA-rich lipids and carotenoids. Triplicate trials will be carry out with amberjack larvae fed live preys enriched using three different levels of PUFA-rich lipids combined with two carotenoids levels. Larval performance (survival and growth parameters) and indicators of welfare (survival to handling stress test and cortisol, glucose, osmolality and total protein in whole final larvae body homogenates) will be studied (IEO). Biochemical composition including lipid classes, fatty acid and carotenoid profiles of enrichment products, live preys and larvae will be analysed (ULL). This Sub-task will contribute to deliverable D9.1 Optimum levels and ratios of essential fatty acids in relation to Tau and combined PUFA-carotenoids in greater amberjack enrichment products.

Task 9.2 Development of diets for grow-out of amberjack to maximize growth (led by HCMR).

Sub-task 9.2.1 (HCMR) Lysine requirements will be determined in small amberjack (HCMR). Diets based mainly on plant ingredients (low fish meal inclusion) will be formulated and produced containing six levels of lysine in order to determine the optimum concentration in favor of health and welfare status of fish, by monitoring growth,

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survival and stress response (gene expression of HSPs). This Sub-tasks will result in deliverable D9.2 Lys requirements of greater amberjack juveniles.

Sub-task 9.2.2 (CANEXMAR) The grow-out diet developed with the above information will be tested at an SME level, in order to assay its efficiency to maximize growth potential and enhance fillet quality. Survival, growth feed utilization and fillet quality will be determined (CANEXMAR). This Sub-task will result in deliverable D9.3 Performance of grow-out diets developed in order to maximize growth potential.

Task 9.3 Design adequate feeding regimes for broodstock to optimize reproduction (led by IEO). To overcome the unreliable reproduction of greater amberjack by addition of specific nutrients that have been shown to be important for gonad maturation and fecundity, the following trials will be conducted:

Sub-task 9.3.1 (FCPCT) The optimum ARA, EPA and DHA levels as essential fatty acids for reproductive success of greater amberjack will be studied. Groups of greater amberjack broodstock will be fed diets containing different essential fatty acids levels (SARC) in order to determine the effect on reproduction reliability. The effects on gonad maturation, frequency of spawns, fecundity, fertilization rates, hatching rates and larval survival rates will be determined. Proximate composition of diets and eggs will be analysed. Incidence of pathological episodes during and after the spawning season will be also considered. This Sub-task will contribute to deliverable D9.4 Recommended protein, carotenoids, Tau and EFA dietary levels in greater amberjack broodstocks. This Task will be complementary to Task 3.3 in WP 3 Reproduction and Genetics – greater amberjack, in order to optimized spawning protocols for F1 greater amberjack.

Sub-task 9.3.2 (IEO, ULL) Previous studies carried out by IEO and ULL showed relevant differences in the lipid profile and fatty acid content of muscle, liver and ovary between wild and born in captivity greater amberjack broodstock (Rodriguez-Barreto et al., 2012). With the aim of approach the lipid and carotenoids eggs profile of cultured females to their wild counterparts, experimental diets with optimized EFA and carotenoid contents will be tested in groups of amberjack broodstock. Fecundity, egg quality and haematological and biochemical indicators of fish health will be studied (IEO). Sperm, eggs and larvae will be analyzed for lipid contents and lipid classes, EFA and carotenoids profiles (ULL). This Sub-task will contribute to deliverable D9.4 Recommended protein, carotenoids, Tau and EFA dietary levels in greater amberjack broodstocks. This Task will be complementary to Task 3.4 in the WP 3 Reproduction and Genetics – greater amberjack, in order to optimized spawning protocols for greater amberjack.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	6.00
2	FCPCT	18.00
8	IEO	2.80
15	ULL	5.50
20	SARC	2.50
28	CANEXMAR	12.00
	Total	46.80

## List of deliverables

Delive- rable Number <sup>61</sup>	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature <sup>62</sup>	Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D9.1	Optimum levels and ratios of essential fatty acids in relation to Tau and combined PUFA-carotenoids	2	20.00	R	PU	24

# WT3: Work package description

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	in greater amberjack enrichment products					
D9.2	Lys requirements of greater amberjack juveniles	1	6.00	R	PU	36
D9.3	Performance of grow-out diets for greater amberjack developed in order to maximize growth potential	28	14.50	R	PU	58
D9.4	Recommended protein, carotenoids, Tau and EFA levels in greater amberjack broodstocks	8	6.30	R	PU	58
			Total			46.80

## Description of deliverables

D9.1) Optimum levels and ratios of essential fatty acids in relation to Tau and combined PUFA-carotenoids in greater amberjack enrichment products: This deliverable will present a list of the optimum levels and ratios of essential fatty acids and carotenoids that should be included in enrichment products for rotifers to be fed to greater amber jack larvae. The deliverable will present the methodology employed and the main results that led to the recommended improvements including the consequences of the improvement in the larval production. The deliverable will describe the effects of essential fatty acids, Tau and carotenoids and will include: a) the effects in larval performance b) welfare, c) fatty acid analysis, lipid classes, and carotenoid profiles of enrichment products, live preys and larvae. [month 24]

D9.2) Lys requirements of greater amberjack juveniles: The deliverable will recommend the optimum levels of Lys in on-growing diets for amberjack based mainly on plant ingredients (low fish meal inclusion) will be presented in this deliverable. The deliverable will describe the main methodology employed, followed by the effects of diets containing six levels of lysine on fish performance and feed utilization. The requirements of this aminoacid to improve growth, survival and stress responses will be also included. [month 36]

D9.3) Performance of grow-out diets for greater amberjack developed in order to maximize growth potential: A report with the effects of the improved diet on the grow-out of greater amberjack will be provided in this deliverable. The deliverable will describe the effects of the improved diet and will include: a) detailed information about the on-growing rearing conditions, b) growthperformance, c) survival, d) feed efficiency and e) significance for the industry. The efficiency of the developed grow-out diet on fillet quality will be also determined. [month 58]

D9.4) Recommended protein, carotenoids, Tau and EFA levels in greater amberjack broodstocks: An improved diet to overcome the unreliable reproduction of greater amberjack will be developed, including recommended levels for protein, carotenoids, taurine and essential fatty acids for its formulation. The deliverable will present the results from the performed trials including the effect of the different experimental diets on gonad maturation, frequency of spawns, fecundity, fertilization rates, hatching rates, larval survival and proximate composition of eggs and sperm. In addition the deliverable will include the diet effect on (a) incidence of pathological episodes during and after the spawning season and (b) hematological and biochemical parameters indicators of health and welfare. [month 58]

# WT3: Work package description

Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS21	Basic formulation for amberjack grow-out studies	2	12	
MS22	Definition of reproductive quality parameters to be studied in amberjack	2	12	

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP10	Type of activity <sup>54</sup>	RTD
Work package title	Nutrition – pikeperch		
Start month	4		
End month	48		
Lead beneficiary number <sup>55</sup>	21		

## Objectives

1. Increase knowledge on the effect of nutrients essential for first feeding of pikeperch,
2. Develop specific enrichment products and formulated diets to improve pikeperch larval performance.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

Overall, we aim to obtain information on the dietary requirements of pikeperch for phospholipids, selected micronutrients and EFAs using a multifactorial approach. Further we aim to examine the influence of enrichment of live feeds with EFAs at various dietary levels on larvae and juvenile physiology. All gained results will allow improving enrichment products and formulated diets for pikeperch larvae and juveniles.

EU Budget allocation: 10,000€ (FCPCT), 20,000€ (ULL), 57,500€ (FUNDP), 44,000€ (DTU) and 28,268€ (ASIALOR, exited the consortium on 31/5/2016) and 39,320 € (F2B, joined the consortium in Aug 2016)

Task 10.1 Effect of selected dietary nutrients on pikeperch larval development and performance (led by DTU). Initially a multifactorial approach will be used to investigate the impact of nutritional quality on growth rate, stress and disease resistance as well as on deformity occurrence of developing pikeperch larvae, in order to increase juvenile yield. A possible explanation is likely to be related to the dietary ratios of Ca/P. At present formulated feeds developed for marine fish larvae are used for weaning pike perch, with Ca/P ratios much higher than those observed in common freshwater fish diets (Kestemont et al., 2007). The multifactorial approach will be conducted in DTU facilities, based on a frSub-taskal and factorial experimental design generated by Planor software, and fish will be fed diets differing by their levels of CA/P, vit A, D and C, and level of ARA, EPA and DHA, with two modalities per conditions (high and low levels). Developing pikeperch larvae of 3-4 dph will be fed for 28 days specific diets. Based on the results and gathered information from the above mentioned experiment, a confirmatory study will be performed to establish adequate levels of those selected nutrients in order to develop specific enrichment products and formulated diets, to increase pike perch survival during weaning and increase early juvenile welfare. On year 4, the developed products and protocols will be tested under commercial farm conditions (ASIALOR/F2B).

The influence of the selected nutrients will be studied on several parameters including: husbandry variables and fish response to stress tests (FUNDP, DTU), organ development and tissue morphology of the digestive tract, liver, etc. (FUNDP), digestive enzymes activities in the stomach, pancreas and intestine (FUNDP), liver proteomics and in situ hybridization techniques (FUNDP), analyses of candidate genes expression (FUNDP), and lipid and fatty acid analyses (DTU). Specifically, skeleton morphogenesis will be studied (FCPCT) by a radiographic method and specific staining of bone and cartilage (Izquierdo et al., 2012), in combination with complementary approaches such as real time quantitative PCR in order to quantify expression of some relevant genes involved in the skeletal system (Sox9, Osterix, Runx, Mef2c, Twist, ALP, BMP, OC, ON, OP) (FCPCT). In addition, standardized methodologies agreed upon by Partners involved in both WP10 and 16 will be used in order to be able to directly compare and integrate results from both WPs, and more rapidly advance knowledge in this field. This Task will result in deliverable D10.1 Recommended Ca/P, vitamins and phospholipids to improve larval development and reduce skeleton alterations in pikeperch.

# WT3: Work package description

Task 10.2 Effects of pikeperch early fatty acid nutrition on long-term stress sensitivity (led by DTU). In this task, the long term influence of early enrichment (i.e., fish marine phospholipids, DHA concentrations and n-6/n-3 ratios) on pikeperch physiology and welfare indicators (i.e. blood or whole bodies will be assayed for cortisol, and brains for 5-HTergic activity (the ratio brain of concentrations of serotonin and its major metabolite 5-HT and hydroxyl-indoleacetic acid), and gene expression of target genes involved in regulation of the stress response, including; glucocorticoid receptors (GR, MR) corticotrophin releasing factor receptors, (CRFR1-3), serotonergic receptors 5HTR1A, using qPCR) will be examined on both larvae (where possible) and juvenile fish. The various dietary nutritional treatments (i.e., high and low dietary levels of DHA and phospholipids as well as 3 different ratios of n-6/n-3 by inclusion of vegetable oils) will be linked to tolerance of sub-lethal physiological stresses of larvae / post larvae (responses to temperature change or hypoxia) and compromised behaviors (i.e. video analysis of activity levels, risk taking, escape responses) (DTU). Using an approach where oxygen consumption is measured in larvae/post larvae following a stressful event, also known as post stress oxygen consumption, this provides valuable insight on the metabolic cost of recovering from different stressors, and the duration recovery based on dietary treatment (DTU). The approach allows for assessing different severities or durations or repetitions of a stress event (DTU). Neural development is investigated using visual and mechano-sensory acuity during avoidance responses as proxies, and verified from ultra structural examination by light and electron microscopy (DTU).

Studies of pathways of fish tissue lipid / fatty acid catabolism / resynthesis, fatty acid composition and influence on eicosanoid activity in relation to early feeding and dietary lipid composition will be performed (DTU). To further investigate on lipid metabolism on stress related tolerance, salinity will be used as an environmental cue. Experiments will be conducted with up to 10 ppt salinity on pikeperch post larvae and larger juveniles. Studies on salinity will be combined with effects of feeding n-6 /n-3 ratios (vegetable oils vs. fish oils) as on the enzymatic activity of desaturase and elongase pathways. The tissue capability to synthesize LC-PUFAs in larval/juveniles will be studied (i.e. by radiotracing of <sup>14</sup>C fatty acid metabolism (ULL), analyses of specific tissues and by in situ hybridization techniques (FUNDP) and examine effects on long term physiology as described above (FUNDP). Skeleton morphogenesis and mineralization by staining and RT-PCR methods will be also studied (FCPCT). Larvae/juvenile will be incubated with <sup>14</sup>C fatty acid LC-PUFA precursors (18:3n-3 and 18:2n-6) as well as <sup>14</sup>C EPA, ARA and DHA after rearing under different salinities (ULL). According to previous results, whole individuals or isolated cells can be used for research on dynamic aspects of lipid nutrition by performing metabolism assays with <sup>14</sup>C radio-labelled fatty acids. These assays will allow checking fatty acid assimilation by the cells or the whole organisms, and follow it through processes of esterification into a specific lipid class, or even transformation into longer and more unsaturated fatty acids. Total fatty acid incorporation rates will be measured in scintillation vials and Beta-counting, whereas the amount of fatty acid esterified in a specific lipid class or transformed by desaturation- elongation steps, will be picked up by using TLC auto-radiography (ULL) (Díaz López et al., 2010; Fonseca et al., 2012). All these findings may display novel and valuable information concerning fundamental aspects of lipid metabolism of pikeperch. Furthermore based on results in Task 10.1. studying the influence of selected nutrients (i.e. CA/P ) levels (FUNDP, DTU and Task 4.3.1., studying effects of nutritional related issues (i.e. feeding frequency, meal timing, food composition) these methods will be adopted and be implemented in the trials for stress resistance of the species to nutritional challenges. All these findings may display novel and valuable information concerning fundamental aspects of lipid metabolism of pikeperch. Total fatty acid incorporation rates will be measured in scintillation vials and Beta-counting, whereas the amount of fatty acid esterified in a specific lipid class or transformed by desaturation-elongation steps, will be picked up by using TLC auto-radiography (ULL) (Díaz López et al., 2010; Fonseca et al., 2012). This Task will result in deliverable D10.2 Protocol for optimal early fatty acid enrichment to reduce stress sensitivity in pikeperch.

Guideline protocols for optimal early enrichment will be evaluated under commercial farm conditions (F2B). This Task will result in deliverable D10.3 Formulation for a diet better adapted to pikeperch requirements.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
2	FCPCT	5.00
15	ULL	4.00
16	FUNDP	11.00

# WT3: Work package description

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
21	DTU	6.50
29	ASIALOR	4.50
39	F2B	7.00
Total		38.00

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D10.1	Recommended Ca/P, vitamins and phospholipids to improve larval development and reduce skeleton alterations in pikeperch	21	17.00	R	PU	36
D10.2	Protocol for optimal early fatty acid enrichment to reduce stress sensitivity in pikeperch	21	11.50	R	PU	36
D10.3	Formulation for a diet better adapted to pikeperch requirements	39	9.50	R	PU	48
Total			38.00			

## Description of deliverables

D10.1) Recommended Ca/P, vitamins and phospholipids to improve larval development and reduce skeleton alterations in pikeperch: This deliverable will recommend the optimum levels of vit A, D, and C and phospholipids that should be included in enrichment products for pike perch larvae. The deliverable will present the methodology employed and the main results that led to the recommended improvements including the consequences in the larval production. The deliverable will describe the effects of vitamins and phospholipids and will include: a) husbandry variables and fish response to stress tests, organ development and digestive enzymes activities, b) liver proteomics, in situ hybridization and selected genes expression, c) lipid and fatty acid analyses and d) skeleton morphogenesis. [month 36]

D10.2) Protocol for optimal early fatty acid enrichment to reduce stress sensitivity in pikeperch: This deliverable will present a protocol to reduce stress sensitivity in pike perch by the balance in fatty acids contained in enrichment products for pike perch larvae. The deliverable will present the methodology employed and the main results that allowed to develop this protocol including the effects of essential fatty acids and phospholipids on: a) on pikeperch physiology, b) welfare indicators including stressors responses, compromised behaviors and post stress oxygen consumption, c) neural development, d) fish tissue lipid / fatty acid catabolism / resynthesis, fatty acid composition and eicosanoid activity, d) the tissue capability to synthesize, e) in situ hybridization and f) skeleton morphogenesis. [month 36]

D10.3) Formulation for a diet better adapted to pikeperch requirements: Based on the trials and conclusions of the previous deliverables, this report will provide a formulation adapted to pike perch requirements for a better fish performance and higher stress resistance in this species. [month 48]

# WT3: Work package description

Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS23	Definition of parameters for skeleton studies in pike perch	21	12	
MS24	Influence of salinity or temperature on LC-PUFAs synthesis in pike perch	21	36	



# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP11	Type of activity <sup>54</sup>	RTD
Work package title	Nutrition – Atlantic halibut		
Start month	1		
End month	48		
Lead beneficiary number <sup>55</sup>	17		

## Objectives

1. Develop a protocol for early weaning,
2. Develop a production strategy for on-grown Artemia,
3. Improve growth in late larval stages, and juvenile quality, through feeding with on-grown Artemia,
4. Better understand the effects of RAS vs FTS on Atlantic halibut larval nutrient utilization,
5. Investigate how dietary phospholipids after weaning affects growth and lipid metabolism.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

Atlantic halibut larvae are approximately 12 mm in standard length (SL) at first-feeding and, because of their relatively large larval size, they are first-fed on Artemia. The main constraints for Atlantic halibut hatcheries are (1) slow growth during the late larval stages and (2) high mortalities caused by opportunistic bacteria and (3) slow growth after weaning.

The slow growth in late larval stages could be overcome by early weaning. Most often, weaning of Atlantic halibut occurs only at 70 days post first-feeding (dpff), but attempts have been made to introduce formulated diets from 20 and 50 dpff, with varying results. The first problem arising is that the larvae refuse to eat formulated feed (Harboe, Hamre and Erstad, unpublished results). It has frequently been observed, however, that they ingest inert particles such as Artemia cysts and pollen from pinewood, the main similarity being that both particles have neutral buoyancy and a bright color. Previous experiments have also shown better feed ingestion with floating compared to sinking feed particles. Furthermore, the structure of the visual system of halibut larvae indicates that they hunt prey in the horizontal plane (Helvik pers. com.), favoring feed intake when particles stay in the same position in the water column for some time. Additionally the type of feed could also affect digestive capacity determined as proteases, carbohydrases and lipases activities (Caruso et al., 2009) or even ATPase activity, which in gut is essential to ensure the ion gradient necessary for nutrient uptake.

Another strategy to alleviate the slow growth in the later larval stages is to use on-grown Artemia. Ongrown Artemia are larger, contain more protein and phospholipids and have a different micronutrient status from Artemia nauplii (Hamre and Harboe, NIFES, preliminary results). They also have a lower shell to nutrient content. This may explain why Atlantic halibut larvae fed on-grown Artemia develop into juveniles with better pigmentation and eye migration than Atlantic halibut fed Artemia nauplii (Olsen et al., 1999; Hamre and Harboe, NIFES, preliminary results). The industry is considering implementing this knowledge in the production line, but will need further documentation.

Atlantic halibut larvae kept in a RAS system will encounter matured water, which will affect their gut flora (Nayak, 2010) in a way that probably has a positive effect on intestinal health. Gnotobiotic and conventional studies indicate the involvement of gut microbiota in nutrition and epithelial development (Nayak, 2010). Gastrointestinal bacteria may also produce essential nutrients such as vitamins and polyunsaturated fatty acids, and enzymes that can aid digestion (Ray et al., 2012). These considerations favor the hypothesis that the general nutrient absorption and retention in the fish is affected by RAS. Iodine retention must have an extra focus, since NO<sub>3</sub>-at levels found commonly in recirculation systems block iodide uptake by the sodium iodide symporter and may cause goiter in the fish (Morris et al., 2011; Ribeiro et al., 2011).

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The third important bottleneck in halibut production is slow growth after weaning. One possible reason for this is a suboptimal diet. We have shown that juvenile Ballan wrasse increase the growth rate by up to 40% when lipids are added as phospholipids (PL) instead of triacylglycerols (TAG, Sæle et al., unpublished), while requirements for PL in *A. halibut* juveniles are not known.

EU Budget allocation: 43,159€ (IMR), 20,000€ (ULL), 111,000€ (NIFES), 8,000€ (SARC)

**Task 11.1 Early Weaning of Atlantic halibut (led by IMR)** The activity in the present task will focus on manipulating the conditions of the larval environment in order to optimize feed intake, using *Artemia* cysts that we know are eaten by the larvae, as a reference particle. The experimental diets, one floating and one sinking, will be produced using the latest knowledge in diet formulation (SARC), including the best quality feed ingredients, a high level of phospholipids, easily digestible protein and attractants. We will produce turbidity in the rearing water by addition of clay particles. Reflection and contrast of the feed particles will be varied by varying light intensity. This task will result in deliverable D11.2 Report on optimal characteristics of feed particles and feeding environment for early weaning of Atlantic halibut larvae.

**Task 11.2 Development of a production strategy for on-grown *Artemia* (led by IMR).** In the experiments on production of ongrown *Artemia* in Task 4.4.3, the nutrient profile (macro and micro nutrients, NIFES) of on-grown *Artemia* compared to *Artemia* nauplii will be characterized, both at the research facility (IMR) and in the Atlantic halibut hatchery (SWH). This task will result in deliverable D11.1 Report on nutrient profile of *Artemia* nauplii and ongrown *Artemia*.

**Task 11.3 Nutrient retention and digestive physiology of Atlantic halibut juveniles fed *Artemia* nauplii or on-grown *Artemia* (led by NIFES).** In the experiments in task 4.4.4, Atlantic halibut larvae will be fed *Artemia* nauplii from first-feeding (IMR). At 20 dpff one group of larvae will be transferred to on-grown *Artemia* whereas the other group will be continued on nauplii. The experiment will last until 70 dpff. In the present task, Atlantic halibut larvae from the experiment will be characterized with respect to digestive enzymes and ATPase activities (ULL). The on-grown *Artemia* will be nutritionally characterized (NIFES) and Atlantic halibut nutritional status (macro and micro nutrients) at different stages in development will be determined (NIFES). This task will result in deliverable D11.3 Report on the nutrient retention and digestive physiology in Atlantic halibut larvae fed *Artemia* nauplii and on-grown *Artemia*.

**Task 11.4 Comparison of nutrient retention in Atlantic halibut larvae reared in RAS vs FTS (led by NIFES).** The study in task 4.4.1 aims to determine the potential effects changes of RAS vs FTS on gut flora and fish performance. One group of Atlantic halibut will be held in a flow through system while another group will be held in a RAS system. In the present task, analyses of digestive physiology (digestive enzymes and ATPase) will be performed (ULL) and the nutritional profile of the larvae at 30 and 60 dpff will be measured in order to compare nutrient retention between the groups (NIFES). This task will result in deliverable D11.4 Report on the nutrient retention and digestive physiology in Atlantic halibut larvae reared in RAS vs FTS.

**Task 11.5 Effect of dietary PL on digestion, absorption and metabolism of lipids in Atlantic halibut juveniles (led by NIFES)** Atlantic halibut juveniles (start weight approximately 1g) will be fed diets with increasing levels of PL (10-50% of total lipid) according to a regression design with 3 replicates and 5 levels, for two months. Growth, body indices, proximate composition of whole body and tissues will be monitored. Given that enough feces may be recovered from the halibut intestines, lipid digestion and absorption as well as the general uptake of nutrients will be described. Genetic markers will be used to describe how dietary PL influences the lipid metabolism, with focus on the intestine, including lipid digestive enzymes, enzymes involved in resynthesis of TAG and PL, remodelling of PL, synthesis of chylomicrons and export of lipids from the intestine. This task will result in deliverable D11.5 Report on the effect of dietary phospholipids on Atlantic halibut juveniles.

### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
7	IMR	2.00
15	ULL	4.50

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## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
17	IMR/NIFES	6.00
20	SARC	1.70
Total		14.20

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D11.1	Report on nutrient profile of Artemia nauplii and ongrown Artemia from IMR and SWH	7	1.00	R	PU	24
D11.2	Report on optimal characteristics of feed particles and feeding environment for early weaning of Atlantic halibut larvae	7	2.70	R	PU	36
D11.3	Report on the nutrient retention and digestive physiology in Atlantic halibut larvae fed Artemia nauplii and on-grown Artemia	17	4.00	R	PU	36
D11.4	Report on the nutrient retention and digestive physiology in Atlantic halibut larvae reared in RAS vs FTS	17	4.50	R	PU	36
D11.5	Report on the effect of dietary phospholipids on Atlantic halibut juveniles	17	2.00	R	PU	48
Total			14.20			

## Description of deliverables

D11.1) Report on nutrient profile of Artemia nauplii and ongrown Artemia from IMR and SWH: This deliverable will report the nutrient profile of Artemia, both at nauplii and ongrown stages from samples taken from the experiment described in WP4.4.1. Nutrient levels in Artemia nauplii and ongrown Artemia will be described e.g. macronutrients, free and total amino acids, soluble protein, fatty acids, lipid classes, vitamins and minerals. [month 24]

D11.2) Report on optimal characteristics of feed particles and feeding environment for early weaning of Atlantic halibut larvae: This deliverable will relate the optimal characteristics of feed particles and feeding environment for early weaning of Atlantic halibut larvae. Artemia cysts (which are eaten by halibut larvae) will be used as a reference diet and compared to one sinking and one floating formulated diet with yttrium. The other variables are light intensity and contrast. It will include a methodology description as well as the results on short term feed intake monitored by counting Artemia cysts in the gut of the larvae or by analysing larval yttrium. [month 36]

D11.3) Report on the nutrient retention and digestive physiology in Atlantic halibut larvae fed Artemia nauplii and on-grown Artemia: The deliverable will include a report on the nutrient retention and digestive physiology in Atlantic halibut larvae fed Artemia nauplii and on-grown. Artemia Samples will be taken from the experiment described in WP4.4.4. The report will contain the methodology and the results of the nutrient profiles described in D3.4.1 characterized in larvae. Digestive enzyme activities and ATPase will be analyzed. [month 36]

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D11.4) Report on the nutrient retention and digestive physiology in Atlantic halibut larvae reared in RAS vs FTS: The results of nutrient retention and digestive physiology in Atlantic halibut larvae reared in RAS vs FTS will be presented in this delivery. Samples will be taken from the experiment described in WP4.4.2. It will include the methodology description as well as the characterization of development nutrient profiles, as described in D3.4.1, in larvae reared in the two different systems. Digestive enzyme activities and ATPase will be analyzed. [month 36]

D11.5) Report on the effect of dietary phospholipids on Atlantic halibut juveniles: This deliverable will report the effect of dietary phospholipids on Atlantic halibut juveniles. It will include the methodology and results of the feeding experiment with five levels of dietary PL up to 50% of total lipid. It will also include the analysis of the intestinal contents to quantify digestion and absorption of lipid classes and fatty acids. Expression analyses of genes connected to lipid digestion, absorption and metabolism will be also included. [month 48]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS25	Ranges of digestive enzymes activities in Atlantic halibut	7	32	

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP12	Type of activity <sup>54</sup>	RTD
Work package title	Nutrition - wreckfish		
Start month	1		
End month	57		
Lead beneficiary number <sup>55</sup>	19		

## Objectives

1. Test the effectiveness of live prey and influence of enrichment on wreckfish larvae,
2. Determine the influence of broodstock feeds on fecundity and spawning quality.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

Studies on wreckfish nutritional requirements and optimum diets are missing. There are only a few references related to feeding habitats from commercial caught (Brick Peres & Haimovi, 2003) and feeding rates in captivity (Papandroulakis et al., 2004), but some information can be obtained from studies or results obtained in other species of groupers (Liao & Leano, 2008).

EU Budget allocation: 2,000€ (FCPCT), 15,000€ (IEO), 37,680€ (CMRM)

Task 12.1 Live preys and enrichments for wreckfish larvae (led by CMRM). To determine the quality of enrichment products and the effect on wreckfish larvae quality, the following feeding trials will be conducted:

Sub-task 12.1.1 Some enrichment products will be developed for live food for larval wreckfish (FCPCT). The first study will focus on EFA content in live preys, since this is one of the main factors affecting marine fish larval survival and growth (IEO). The influence of the different live food enrichments on the nutritional composition of the larvae will be studied. Biochemical analysis of samples of live food as well as larvae from the different trials will be done in triplicates (CMRM).

Sub-task 12.1.2 This Sub-task will depend on the results obtained above and will focus on either fat-soluble vitamins or antioxidants in different enrichment products (FCPCT). The effect of enrichments nutritional quality on nutritional quality on larvae composition will be studied, as well as the biochemical analysis of samples from the former trials (CMRM). All the biochemical analysis will be done in triplicates.

This task will result in deliverable D.12.1. Determine the effect of live prey enrichment products on wreckfish larval performance

Task 12.2 Influence of broodstock feeding regimes for fecundity and spawn quality (led by IEO). The effect of feeding regimes (FCPCT) based on fresh and compound feeds will be studied on wreckfish reproductive performance (IEO, CMRM). Based on wild brood stock feeding habits. The studies will focus particularly on protein/energy ratios and essential fatty acid levels. The best feeding regime will be combined with the best induction protocol in Task 6.3. to optimize the effectiveness of the spawning induction protocols. Effects on fecundity and egg and sperm quality will be determined (IEO, CMRM). Effect of dietary nutrients levels on embryogenesis and biochemical composition of eggs from the former trials will also be examined (CMRM). This task will result in deliverable D12.2 Recommendations for wreckfish broodstock feeds.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
2	FCPCT	2.00

# WT3: Work package description

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
8	IEO	1.10
19	CMRM	3.50
	Total	6.60

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D12.1	Effect of live prey enrichment products on wreckfish larval performance	19	3.30	R	PU	54
D12.2	Recommendations for wreckfish broodstock feeds	19	3.30	R	PU	57
	Total		6.60			

## Description of deliverables

D12.1) Effect of live prey enrichment products on wreckfish larval performance: The quality of live preys and enrichments and its effects on wreckfish larvae quality will be presented in this deliverable. The deliverable will include: (a) some enrichment products for live food developed for larval wreckfish, (b) establish application protocols of enrichment products, (c) the EFA content in enriched live preys (d) the EFA content in wreckfish larvae, (e) the effects of the live food enrichment on the survival, growth and biochemical composition of wreckfish larvae. In addition and depending of the results obtained with the former studies (f) fat-soluble vitamins or antioxidants in different live food will be determined and (e) studies about its influence in larval quality will be done. Reports about the effect of enrichments nutritional quality on the survival, growth and quality on larvae composition will be prepared [month 54]

D12.2) Recommendations for wreckfish broodstock feeds: This deliverable will report the influence of broodstock feeding regimes on fecundity and spawn quality. The deliverable present: (a) the effect of two feeding regimes based on fresh and commercial dry feeds on gonadal development, (b) the protein/energy ratios and EFA levels of feeding regimes and eggs, (c) the best feeding regime combined with the best induction protocol (WP.2.5), (d) the effect of feeding regime on fecundity and egg and sperm quality, (e) the effect of dietary nutrients level on embryogenesis and biochemical composition of eggs. Reports will be prepared with recommendations for wreckfish broodstock feeds. [month 57]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS26	Obtain viable gametes (oocytes and sperm) for larvae production in wreckfish	19	36	

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP13	Type of activity <sup>54</sup>	RTD
Work package title	Nutrition – grey mullet		
Start month	1		
End month	55		
Lead beneficiary number <sup>55</sup>	4		

## Objectives

1. Improve enrichment products, weaning, grow out and broodstock diets,
2. Comparing the effect of two types of potential soybean meals to be used in the IOLR grow out diet for grey mullet on growth, intestinal morphology and inflammation, peroxidation and antioxidant mechanisms and intestinal pathology.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

Preliminary studies have indicated that the essential fatty acids DHA, EPA and ArA improved survival, growth and stress resistance in grey mullet (Eda et al., 1990, Tamaru et al., 1992). Nevertheless, essential fatty acid (EFA) requirements need to be determined during larval and juvenile rearing. This is because requirements may change as a function of the fish's post metamorphic transition from being strict carnivores to detritus and plant omnivores (Zouiten et al., 2008) and as juveniles seek out less saline environments. Tau is a beta amino-sulfonic acid that is considered a growth promoter in many fish species possibly due to its conjugation with bile acids (Yokogoshi & Oda, 2002), leading to enhanced absorption of EFA. The rate-limiting enzyme in bile salt synthesis is cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) whose increased activity has been correlated with dietary Tau (Hagey et al., 2010; Bellentani et al., 1987; Kibe et al., 1980).

Taurine is also important in muscle function (Huxtable, 1992), modulating neural systems and preventing retinal degeneration (López-Escalera et al., 1988). Larvae of a number of marine fish lack cysteine sulfinatase decarboxylase, the key enzyme in Tau synthesis (Yokoyama et al., 2001; Goto et al., 2003; Chen et al., 2005; Takagi et al., 2008). This is not surprising as the marine environment is Tau rich (0.5-1.0%; Divakaran, 2006) including copepods (1-1.5% DW), the dominant, natural larval prey. In contrast rotifers used in hatcheries worldwide, have only trace levels of Tau (Conceicao et al., 1997; van der Meer et al., 2008) and when enriched improved larval performance (Koven et al., 2012; Matsunari et al., 2005; Chen et al., 2004) during metamorphosis ("silvering"), fish undergo an ontogenetic shift from strict carnivores to herbivores feeding on detritus and algae. In support of this, amylase production showed a continuous increase during larval and juvenile development in the thick lipped grey mullet (*Chelon labrosus*; Zouiten et al., 2008).

Therefore, grey mullet weaning diets must include inexpensive sources of low-cost amylolytic energy compounds. Complete replacement of fishmeal has been largely unsuccessful, especially for marine carnivores (Sales, 2009). This may be partially due to a Tau deficiency in plant protein sources. A number of recent studies have shown a clear benefit of Tau supplementation to fishmeal replacement diets such as in cobia (*Rachycentron canadum*; Lunger et al., 2007) and common dentex (*Chatzifotis* et al., 2008).

Soybean (SBM) meals have less of an impact on the environment, (low phosphorus levels comprised mostly of indigestible phytate phosphorus) and have relatively favorable protein and essential amino acid levels (Hardy 2002). On the other hand, soybean meal contains anti-nutritional factors (ANF) such as trypsin inhibitors which reduces protein digestibility by binding with trypsin. To a large degree these ANFs can be neutralized by heat processing. However, they might also reduce protein quality. Nevertheless, high inclusion levels of soybean protein in diets, such as the IOLR mullet diet, can result in blunting or flattening of intestinal mucosa leading to reduced absorptive potential (Rumsey et al. 1995) as well as causing inflammation of the distal intestine (Refstie et al. 2001). This condition is also associated with high moisture levels in feces, suggesting a more

rapid gut transit and a decrease in digestion and absorption. Other components of soybean products reported to affect growth, feed intake or metabolism are saponins, isoflavones, oligosaccharides and phytate (Hardy, 2002). The soy protein ANF  $\beta$ -conglycinin is an allergenic protein that has negative effects on growth, digestive and absorptive abilities. In a recent study (Zheng et al. 2013) dietary  $\beta$ -conglycinin significantly decreased the activities of trypsin, chymotrypsin, lipase, creatine kinase, Na<sup>+</sup>, K<sup>+</sup>-ATPase and alkaline phosphatase in the intestine and enterocytes of Jian carp as well as protein content of the hepatopancreas and decreased intestinal weight, length and fold height. Moreover  $\beta$ -conglycinin affected the endogenous antioxidant system by decreasing activities of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) and glutathione (GSH) content in the intestine and enterocytes. Taken together  $\beta$ -conglycinin is a major SBM factor inducing inflammation and oxidation leading to dysfunction of intestinal digestion and absorption in fish. However, today a number of non-GMO products (e.g. Navita Premium Feed Ingredients, USA), which use less harsh processing are available in the market. The producers claim that these genetically selected strains have much lower levels of these ANFs, without processing, and have successfully partially or completely replaced fish meal in a number of freshwater and marine species. The current IOLR/NCM mullet feed, which has showed promising results, has replaced fishmeal with a number of plant based meals including soybean protein (extract from SBM) as well as poultry meal (PM). Although the levels of soybean protein and poultry meals are relatively low 12 and 13%, respectively, it is preferable that animal products are reduced to a minimum. On the other hand, raising the SBM may result in problems of sub-optimal absorption and possible inflammation.

EU Budget allocation: 45,000€ (FCPCT), 20,000€ (IRTA), 182,509€ (IOLR), 25,220€ (UNIBA) and 29,976€ (CTAQUA)

Task 13.1 Improvement of larval performance (led by IOLR).

Sub-task 13.1.1 (IOLR) Improvement of larval performance through adequate first feeding regimes (led by IOLR). We will examine the effect of DHA/EPA/ArA ratio and Tau on larval and juvenile performance during rotifer and Artemia feeding prior to and following ("silvering") transition from carnivorous larva to herbivorous juvenile (IOLR). These studies will be carried out in a sixty 20 l aquarium system with full salinity and temperature control. A 3x3 factorial experiment (27 aquaria) will be carried out where three levels of DHA and three levels of taurine during rotifer feeding will be tested (3 replicate aquaria/level). At the end of rotifer feeding the larvae will be fed the Artemia control (low DHA, low taurine) to 40 dph. At the same time as the rotifer trial, larvae in another 27 aquaria will be fed the rotifer control (low DHA, low taurine) until 15 dph. After this point, a 3x3 factorial experiment (27 aquaria) will be carried out where three levels of DHA and three levels of taurine during rotifer Artemia feeding will be tested (3 replicate aquaria/level). Rotifer and Artemia enrichment samples will be taken at 4 times during the course of these studies. In addition, larval samples will be taken at 15 and 40 dph for growth, survival, taurine level, presence of urinary crystals and fatty acid profiles. A follow up trial will test the selected rotifer and Artemia DHA-aurine treatments as well as a combined rotifer and Artemia DHA-aurine treatment. Each treatment will be tested in replicates of 7 aquaria/treatment and evaluated in terms of growth, survival, presence of urinary crystals, silvering synchrony and fatty acid profile. These results will shed light on which developmental stage (rotifer or artemia feeding) DHA and taurine are the most effective and if there is an additive effect when the selected rotifer and Artemia DHA-aurine treatments are both fed. This Sub-task will result in deliverable D13.1 Determine changes in the essential fatty acid requirement as a function of developmental stage and in grey mullet.

Sub-task 13.1.2 (IOLR) Using the most effective DHA-aurine diet from (13.1) to investigate the effect of supplemental ArA on larval growth, survival, presence of urinary crystals, as well as synchrony in "silvering" during metamorphosis. In this trial, five rotifer enrichment treatments will be produced having the same DHA and taurine levels selected from the results in 13.1 but will differ in 4 different EPA/ArA ratios. Rotifers fed these treatment preparations will be fed to mullet larvae and tested in 5 replicate aquaria/treatment until 15 dph and then fed the selected DHA-aurine Artemia treatment from 13.1. At the same time mullet larvae from 2-15 dph will be fed the selected DHA-aurine rotifer diet from 13.1 in twenty-five aquaria. From 16-40 dph, the larvae will be fed four Artemia enrichment treatments having the same DHA and taurine levels but will differ in their EPA/ArA ratios. In both the rotifer and Artemia EPA/ArA trials, the fish will be sampled at 15 and 40 dph for growth, survival (40 dph), taurine levels, silvering synchrony and fatty acid profiles. Digestive system and bone development study will be conducted by determining biomarkers (IGF, BMP, ALP) and related parameters (bone mineralization and deformities) and Light Microscopy and Electronic Microscopy morphometric analysis (FCPCT). This Sub-task will result in deliverable D13.2 Determine a developmental stage ability to synthesize key enzymes in Tau and bile acid synthesis in grey mullet.



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## Work package description

Task 13.2 Determining mullet nutritional needs for improved weaning to a dry diet (led by IOLR).

Sub-task 13.2.1 (IOLR) Determine expression of Tau rate limiting enzyme; cysteine sulfinatase decarboxylase (CSD) at various stages (larval and grow out). Samples will be taken from studies in Task 13.1. Grey mullet larvae may not have Tau synthesis capability but develop it later on as juveniles.

Sub-task 13.2.2 (IOLR) Determine expression of rate limiting enzyme of bile salt synthesis, cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) at various stages (larval and grow-out). Samples will be taken from studies in Task 13.1. Taurine as a precursor for bile salts may be particularly important during larval rearing when the digestive tract is very short and undeveloped and bile salt resorption is reduced. However, as juveniles have a well-developed digestive tract and bile salt resorption capability, Tau synthesis may be less important. This Task will contribute to deliverable D13.3 Determine the effects of pigments, essential fatty acids and Tau in grey mullet brood stock diets on egg quality, fecundity, hatching success, larval first feeding and vitellogenin expression accumulation.

Task 13.3 Determining grey mullet nutritional needs for a more cost-effective production (led by IOLR).

Sub-task 13.3.1 (IOLR) Effect of DHA/EPA/ARA ratio in non-fish meal grow-out diets on fish performance. A 3x3 factorial study will be carried out in twenty-seven 200 l V-tanks where three levels of DHA and three EPA/ArA ratios will be tested in replicates of 3 tanks/treatment. The best performing diet will be evaluated in terms of growth, FCR, PER and survival as well as whole body proximate analysis and fatty acid analysis. All test diets will be produced at the IOLR. This Sub-task will contribute to deliverable D13.4 Determine the effects of essential fatty acids and Tau in non-fish meal feeds on flesh and bottarga quality in grey mullet.

Sub-task 13.3.2 (IOLR) Effect of four levels of Tau supplementation to best performing DHA/EPA/ArA non-fish meal grow out diet from 13.3.1 on fish performance This trial will be tested in six-teen 400 l tanks where each treatment will be tested in 4 replicate tanks/treatment. The best performing diet will be evaluated in terms of growth, FCR, PER and survival as well as whole body proximate analysis and fatty acid analysis. All test diets will be produced at the IOLR. This Sub-task will contribute to deliverable D13.4 Determine the effects of essential fatty acids and Tau in non-fish meal feeds on flesh and bottarga quality in grey mullet.

Sub-task 13.3.3 Comparing the effect of two types of potential soybean meals to be used in the IOLR grow out diet for grey mullet on growth, intestinal morphology and inflammation, peroxidation and antioxidant mechanisms and intestinal pathology. This study will compare the effect of two types of soybean protein meals to be used in the IOLR grow out diet for grey mullet. The first soybean protein meal is a heat treated product distributed in Israel by Ambar Ltd while the second is a non-GMO soybean meal (Navita Premium Feed Ingredients) that has naturally high protein density (>50%), low oligosaccharides (<1%) and low trypsin inhibitors (10,000 TIU). Six grow out diets will be prepared which will test three ratios of SBM%:PM% (12:13, 20:5 and 25:0) using each of the two sources of SBM proteins (Ambar and Navita). The study will run for 3 months using juvenile mullet stocked in twenty-four 200 l V-tanks where each treatment will be tested in replicates of 4 tanks. Fish will be weighed every two weeks to determine growth, FCR and examined for signs of pathology. Digestive tracts from samples of fish (3 fish/tank) will be taken after 6 and 12 weeks for analyses (IOLR, IRTA and FCPCT). IOLR will analyze for pancreatic (alkaline proteases, amylase, lipase, trypsin) stomach (pepsin), brush border (alkaline phosphatase), and cytosol (leucine- alanine peptidase) enzymes. FCPCT will carry out GALT and gut morphometric studies: Folds height, goblet cell density, lamina propria width (light microscopy studies) and microvilli height, microvilli density (Electron microscopy studies). Gut mucus composition and activity, markers as well as peroxidation (TBA-malondialdehyde) and Antioxidant systems enzyme (CAT, SOD, GPX) gene expressions. IRTA will concentrate on non-specific immunologic serum parameters markers of intestinal inflammation (molecular expression) and markers (immunohistochemistry) of cell proliferation and apoptosis in gut (PCNA and Tunnel assays) and analysis of microbiota. This Sub-task will result in deliverable D13.5 Evaluate and maximize the dietary incorporation of a non-GMO genetically selected soybean meal that will increase nutrient absorption and reduce DT inflammation.

Sub-task 13.3.4 (IOLR) The selected feed from Sub-tasks 13.3.1, 13.3.2, 13.3.3 will be compared to the current feed on the market used for mullet culture and fed to adult mullet until gonadal maturation. At the end of the study fish performance on these two feeds will be evaluated in terms of growth, FCR, PER as well as flesh and bottarga quality.

Sub-task 13.3.5 (CTAQUA) Comparison of vegetable oil-no fish meal grow out diet with a n-3 HUFA rich fish meal finishing diet on the nutritional and organoleptic values of fish flesh and bottarga quality.

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This Task will contribute to deliverable D13.4 Determine the effects of essential fatty acids and Tau in non-fish meal feeds on flesh and bottarga quality in grey mullet.

Task 13.4 Design adequate feeding regimes for brood stock to optimize reproduction success (led by UNIBA).

Sub-task 13.4.1 (IOLR) Broodstock dietary effects on mullet reproduction (e.g., natural pigments, DHA/EPA /ARA ratio, Tau) on egg quality, in terms of fecundity, hatching success, and larval first feeding.

Sub-task 13.4.2 (UNIBA) Definition of specific requirements of protein, TAU, ARA, DHA and carotenoid sources to optimize spawn quality in mullet. Analysis of liver Vtg gene expression, oocyte Vtg receptor gene expression and yolk accumulation under different dietary conditions.

This Task will contribute to deliverable D13.3. Determine the effects of pigments, essential fatty acids and Tau in grey mullet brood stock diets on egg quality, fecundity, hatching success, larval first feeding and vitellogenin expression accumulation.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
2	FCPCT	20.00
3	IRTA	1.10
4	IOLR	38.00
13	UNIBA	5.30
18	CTAQUA	3.75
	Total	68.15

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D13.1	Determine changes in the essential fatty acid requirement as a function of developmental stage and ambient salinity in grey mullet	4	11.00	R	PU	18
D13.2	Determine a developmental stage ability to synthesize key enzymes in Tau and bile acid synthesis in grey mullet	4	12.00	R	PU	18
D13.3	Determine the effects of pigments, essential fatty acids and Tau in grey mullet broodstock diets on egg quality, fecundity, hatching success, larval first feeding and vitellogenin expression accumulation	4	14.50	R	PU	36
D13.4	Determine the effects of essential fatty acids and Tau in non-fish meal feeds on flesh and bottarga quality in grey mullet	4	20.35	R	PU	48
D13.5	Evaluate and maximize the dietary incorporation of a non-GMO	13	10.30	R	PP	55

# WT3: Work package description

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	genetically selected soybean meal that will increase nutrient absorption and reduce DT inflammation					
	<b>Total</b>		68.15			

## Description of deliverables

D13.1) Determine changes in the essential fatty acid requirement as a function of developmental stage and ambient salinity in grey mullet: This deliverable will be based on knowledge from studies carried out on the effect of different essential fatty acid (EFA) ratios (DHA/EPA/ArA) and Tau levels on larval and juvenile performance during rotifer and Artemia feeding prior to and following ("silvering") transition from carnivorous larva to herbivorous juvenile. This information is important for protocol improvement as it is likely that the EFA requirements will change with age and mode of feeding. Moreover, the taurine requirement may be particularly critical as both rotifers and Artemia lack or have insufficient levels of this nutrient. The deliverable will describe the effects of these nutrients and will include: a) growth, b) survival, c) taurine level, d) presence of urinary crystals and e) fatty acid profiles. [month 18]

D13.2) Determine a developmental stage ability to synthesize key enzymes in Tau and bile acid synthesis in grey mullet: This deliverable will report the results of the studies conducted to determine a developmental stage ability to synthesize key enzymes in Tau and bile acid synthesis in grey mullet. Based on the work carried out at the IOLR a correlation between increased dietary taurine and the reduced presence of urinary crystals (calcium oxylate) in the urinary duct is expected. Urinary crystals are likely the result of inefficient production of bile salts, whose synthesis depends heavily on the presence of taurine. This deliverable will clarify if the gene expression for cholesterol 7-alpha hydroxylase, a key enzyme in bile salt synthesis, is up-regulated with increasing dietary taurin to facilitate diet development during larval rearing, juvenile feeding and grow out. The deliverable will describe the developmental stage ability to synthesize key enzymes in Tau and bile acid synthesis and will include the expression of rate limiting enzyme of bile salt synthesis, cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) at various stages (larval and grow-out). [month 18]

D13.3) Determine the effects of pigments, essential fatty acids and Tau in grey mullet broodstock diets on egg quality, fecundity, hatching success, larval first feeding and vitellogenin expression accumulats: This deliverable will determine the effects of pigments, essential fatty acids and taurine in grey mullet broodstock diets. In studies carried out at the IOLR, it was clear that high egg quality, in terms of colour and fatty acid content, frequently is associated with the success of larval rearing in the grey mullet. However, egg colour and quality varied greatly with subsequent failure to consistently rear larvae. The deliverable will describe the effect of dietary pigments (carotenoids and astaxanthin), essential fatty acids and taurine on: a) egg fecundity, hatching success and vitellogenin receptors, b) larval performance to design suitable brood stock diets that will give more consistent larval rearing success and c) vitellogenin expresssion accumulation. [month 36]

D13.4) Determine the effects of essential fatty acids and Tau in non-fish meal feeds on flesh and bottarga quality in grey mullet: The deliverable will present the effects of essential fatty acids and taurine in non-fish meal grow out diets for grey mullet developed by IOLR. In light of environmental considerations, economy, sustainability and the fact that mullet is an omnivore, the philosophy of mullet rearing in this project is to avoid the use of fish meal and in fact to minimize all animal products in the diet composition. On the other hand, the use of plant based meals can result in a reduction in growth performance due to sub-optimal levels of EFA and taurine which are found only in animal products and not in terrestrial plant based feeds. Apart from the potential adverse effect of reduced levels of EFA and taurine on fish flesh, there might also be an influence on bottarga quality. The deliverable will report the the effects of essential fatty acids and taurine in non-fish meal feeds on: a) growth, b) survival, c) feed utilization d) body composition and e) flesh and bottarga quality in grey mullet. [month 48]

D13.5) Evaluate and maximize the dietary incorporation of a non-GMO genetically selected soybean meal that will increase nutrient absorption and reduce DT inflammation: The report will evaluate the incorporation of a non-GMO genetically selected soybean meal low in anti-nutritional factors on diets for grey mullet. The inclusion of soybean meals in fish feeds can have adverse effects on fish performance due to the presence of

# WT3: Work package description

anti-nutritional factors and can result in blunting or flattening of intestinal mucosa leading to reduced absorptive potential as well as causing inflammation of the distal intestine. The current IOLR/NCM mullet feed contains a number of plant based meals including soybean as well as poultry meal (PM). In this deliverable a non-GMO genetically selected soybean meal will be evaluated as a replacement for the soybean meal. The deliverable will present the effects of these diets on a) fish growth, b) survival, c) feed utilization, d) pancreatic and brush border enzyme production, e) gut metamorphic characteristics, f) peroxide level, g) antioxidant enzyme gene expression and h) immunological serum markers for gut intestinal inflammation. [month 55]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS27	Definition of methodology to study cost-benefit of grey mullet weaning diets	4	12	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP14	Type of activity <sup>54</sup>	RTD
Work package title	Larval husbandry - meagre		
Start month	1		
End month	18		
Lead beneficiary number <sup>55</sup>	3		

## Objectives

1. To reduce costs by early weaning in meagre larvae and improve growth, survival and larval quality.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviation)

Larval rearing of meagre is relatively easy with a protocol generally based on the rotifer and Artemia feeding methodologies implemented in European sea bass and sea bream. However, meagre larvae are quite sensitive to high light intensity (more than 500 lux) or a long photoperiod (Roo et al., 2010; Vallés & Estévez, 2013). Although the precise requirements for essential amino and fatty acids are not completely known, the larvae show very good growth and survival rates using commercially available enrichment products for live prey. Meagre producers do not consider larval rearing to be a major stumbling block for meagre culture although cannibalism and variable size distribution in larvae and juveniles are the main concern as they would increase production costs and limit yields. Probiotic use at early stages of fish development may contribute to better face metamorphosis and weaning performance by stimulating growth, modulating digestive enzyme activity, improving intestinal epithelium integrity and altering biochemical composition (García de la Banda et al., 2012; Tapia-Paniagua et al., 2011, 2012). Therefore, advancing the early weaning of larvae from its dependence on Artemia on to a dry feed is a priority and the major focus of the larval work on meagre.

EU Budget allocation: 20,000€ (IRTA) and 6,000€ (ULL)

Task 14.1 Determining the earliest and most cost effective weaning period (led by IRTA).

Larvae will be cultured (IRTA) intensively following a standard technique (rotifers from 2 days post-hatch-dph, Artemia nauplii from 12 dph until 30 dph) at low larval density (50 larvae/l) and 12hL:12hD photoperiod with a light intensity of 500 lx (control group). In the experimental groups live prey supplemented or not with probiotics will be replaced at 7, 10 and 15 dph using commercially available weaning diets such as Gemma Micro (Skretting, Norway) or similar. Larval growth and size dispersion distribution, quality (typology and incidence of skeletal deformations), maturation of the digestive system in terms of activity of pancreatic and intestinal enzymes (ULL), as well as survival and larval biochemical composition (ULL) will be analysed at the end of the experiment and compared to control larvae. The experiment will be carried out at least in triplicates (a 4x3 or 4x4 design) in 100 L tanks connected to an IRTAMar™ recirculation unit (IRTA). This task will contribute to deliverable D14.1 Improved larval rearing protocol for meagre that includes weaning at an earlier age leading to reduced cost in live feed production and better quality juveniles.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
3	IRTA	2.40
15	ULL	1.30
	Total	3.70

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D14.1	Improved larval rearing protocol for meagre that includes weaning at an earlier age leading to reduced cost in live feed production and better quality juveniles	3	3.70	R	PU	18
		Total	3.70			

## Description of deliverables

D14.1) Improved larval rearing protocol for meagre that includes weaning at an earlier age leading to reduced cost in live feed production and better quality juveniles: In order to improve the larval rearing protocol for meagre, it is desirable to wean the fish onto a dry feed as soon as possible to improve juvenile quality and reduce costs. These studies will test the effect of advancing the weaning age by feeding available weaning diets such as Gemma Micro (Skretting, Norway) or similar at different larval ages. The most effective weaning age will be evaluated in terms of larval growth and size dispersion distribution, quality (typology and incidence of skeletal deformations) and maturation of the digestive tract through the activity of pancreatic and intestinal enzymes. In addition, survival and larval biochemical composition will be analysed at the end of the experiment and compared to control larvae. The deliverable will be an improved protocol that will include the most effective weaning age that results in better quality juveniles. [month 18]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS28	Protocol for weaning meagre larvae	3	18	

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP15	Type of activity <sup>54</sup>	RTD
Work package title	Larval husbandry - greater amberjack		
Start month	5		
End month	48		
Lead beneficiary number <sup>55</sup>	2		

## Objectives

1. Effects of different feeding strategies on larval performance in intensive systems,
2. Development of feeding protocol and rearing system in mesocosm semi-intensive systems,
3. Development of industrial protocol for larval rearing.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

The tasks will address (a) the development of appropriate feeding strategies (prey concentration, immune modulators), (b) the effect of the rearing environment (tank type, light) during larval rearing and (c) the development of an appropriate industrial protocol for the larval rearing of greater amberjack.

EU Budget allocation: 79,445€ (HCMR), 105,700€ (FCPCT), 94,931€ (IEO), 39,866€ (ULL) and 70,000€ (FORKYS)

Task 15.1 Effect of feeding regime and probiotics (led by IEO). The effect of different live prey protocols (concentration and supply frequency) together with probiotics and immunostimulants as health promoting/nutritional supplements will be evaluated. The stimulation of the larval immune system by using probiotics (Díaz-Rosales et al., 2009) and immunostimulants (Awad et al., 2013) is a promising tool to increase survival rates at early stages of fish. Probiotics such as *Shewanella* genus, increases in vivo pathogen resistance (García de la Banda et al., 2010) and promotes an advanced and synchronized metamorphosis and a significantly higher growth (Lobo et al., in press). In addition, various studies have shown that the inclusion of dietary Echiium oil modulates the stress response of species (Villalta et al., 2007; Díaz-López et al., 2009). Several of these probiotic-immunostimulat substances will be assayed using live preys as vector. Results will be evaluated in terms of survival, growth, development, skeletal deformities and larval nutritional condition (RNA/DNA ratio). Larvae will also be examined (by IEO) for oxidative stress in terms of production of reactive oxygen species (ROS), peroxidative status (measured as thiobarbituric acid reactive substances (TBARS)), antioxidant defense enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and humoral parameters of the immune system (cortisol, activity of lysozyme, peroxidase, proteases and anti-proteases, anti-bactericidal). The ontogeny of the digestive enzymes of amberjack larvae focusing on total protease, lipase, amylase and ATPase activities will be also performed (ULL). This task will contribute to D15.2 Efficient prey density and protocol of using immune modulators in greater amberjack larval rearing.

Task 15.2 Comparison of semi-intensive and intensive rearing (led by HCMR).

Sub-task 15.2.1 (HCMR) Comparison between intensive (in RAS with 500 l tanks) and semi-intensive (Mesocosm with 40,000 l) tanks in triplicate trials where each has a duration of 30 days, which is the period required to undergo metamorphosis. Each system will be evaluated in terms of

- (i) ontogeny of visual system (influenced by feeding) through histological procedures,
- (ii) larval oxidative stress through the activity of specific enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione S-transferase and the concentration of glutathione),
- (iii) investigating the larval somatotrophic axis (consisting of the growth hormone-releasing hormone (GHRH), growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factors (IGF-I and II), associated

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carrier proteins (IGFBPs) and receptors) that represents the endocrine and autocrine regulator for skeletal muscle growth.

This Sub-task will contribute to D15.4 Ontogeny of greater amberjack larval visual and digestive system.

Sub-task 15.2.2 (FCPCT) The effect of stocking density on larval performance in terms of growth, survival, skeletal deformities and gene expression of stress and skeleton related genes will be studied. Three different larval rearing densities will be evaluated: 25, 50 and 75 eggs/l in triplicate 2000 l tanks for a period of 30 days.

This Sub-task will contribute to D15.1 Effective greater amberjack larval stocking densities.

Sub-task 15.2.3 (ULL) Ontogeny of the digestive system of greater amberjack larvae (from Actions 15.2.1 and 15.2.2) focusing on proteases, lipases, amylases and ATPase. This Sub-task will contribute to D15.4 Ontogeny of greater amberjack larval visual and digestive system.

Task 15.3 Effect of environmental parameters during rearing (led by FCPCT).

Sub-task 15.3.1 (FCPCT) The effect of tanks hydrodynamics will be studied. Based on results of 15.2, two different tank types; 40,000 l cylindrical vs 2,000 l cylindro-conical will be tested in duplicates for a period of 30 days. The hydrodynamics of the tanks will be also considered after analyzing the current profile with a Vectrino (high-resolution acoustic velocimeter). The effect of tank type on larval performance in terms of growth, survival, histology, biochemical composition, skeletal deformities and gene expression of stress and skeleton related genes will be recorded. This Sub-task will contribute to D15.3 Optimum hydrodynamics and light conditions during greater amberjack larval rearing.

Sub-task 15.3.2 (HCMR) Effect of light (intensity and duration) on larval rearing. Two light intensity ranges (200-600 and 800-1200) and 2 photophases (18L:06D and 24L:00D) will be tested. Tanks of 500 l will be used in triplicate trials each having a duration of 30 days. The effect of light will be evaluated in terms of larval growth, survival, quality and size dispersion. In addition, the somatotrophic axis (consisting of the growth hormone-releasing hormone (GHRH), growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factors (IGF-I and II) and associated carrier proteins (IGFBPs) will be investigated together with the receptors that represent the endocrine and autocrine regulators for skeletal muscle growth and are known to play key roles in the regulation of metabolism and physiological processes. This Sub-task will contribute to D15.3 Optimum hydrodynamics and light conditions during greater amberjack larval rearing.

Task 15.4 Development of industrial protocol (led by IEO).

Sub-task 15.4.1 (IEO) Development of an industrial protocol for larval rearing based on the results of the previous tasks. In order to determine larva and fry quality, fish will be sampled periodically during rearing to evaluate growth parameters, development, skeletal deformities and larval nutritional condition (RNA/DNA ratio). In addition, other parameters will be measured such as reactive oxygen species (ROS), peroxidative status (measured as thiobarbituric acid reactive substances (TBARS)), antioxidant defence enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR)) and humoral parameters of the immune system (cortisol, activity of lysozyme, peroxidase, proteases and anti-proteases, anti-bactericidal). This Sub-task will contribute to D15.5 An industrial protocol for greater amberjack larval rearing.

Sub-task 15.4.2 (FCPCT) Ossification pattern and incidence of skeletal deformities for amberjack larvae will be evaluated under different levels of intensification. Samples of amberjack larvae from hatching to the end of metamorphosis will be collected at regular intervals, to evaluate ossification pattern. Staining protocols to evaluate these samples will be determined in the project. The ossification and deformity results will be expressed as a function of size and not age as abnormal skeletal development may vary with the growth rate of individuals. This Sub-task will contribute to D15.5 An industrial protocol for greater amberjack larval rearing.

Sub-task 15.4.3 (FORKYS) Validation of the developed protocol initially at FCPCT and over two successive years in an SME hatchery (FORKYS). This Sub-task will contribute to D15.5 An industrial protocol for greater amberjack larval rearing.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	10.50



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## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
2	FCPCT	40.00
8	IEO	5.50
15	ULL	8.50
27	FORKYS	15.00
	Total	79.50

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D15.1	Effective greater amberjack larval stocking densities	2	18.50	R	PU	16
D15.2	Efficient prey density and protocol of using immune modulators in greater amberjack larval rearing	8	10.00	R	PU	27
D15.3	Optimum hydrodynamics and light conditions during greater amberjack larval rearing	2	18.00	R	PU	27
D15.4	Ontogeny of greater amberjack larval visual and digestive system	1	13.00	R	PU	27
D15.5	An industrial protocol for greater amberjack larval rearing	8	20.00	R	PU	48
		Total	79.50			

## Description of deliverables

D15.1) Effective greater amberjack larval stocking densities: Based on the results from the trials conducted this deliverable will describe the most effective larval stocking density to obtain the best larval performance. The deliverable will include a) the rearing conditions and methodology used, as well as the effect of stocking density on b) larval growth, c) survival, d) skeletal deformities, e) stress related gene expression and f) skeleton related genes. [month 16]

D15.2) Efficient prey density and protocol of using immune modulators in greater amberjack larval rearing: A feeding strategy for the larval rearing of greater amberjack will be developed. This will include definition of (a) prey concentration and supply frequency and (b) use of immune modulators substances. The results of the performed trials will be evaluated in terms of survival, growth, larval nutritional condition (RNA/DNA ratio), physiological parameters (oxidative stress and immune system) and ontogeny of the digestive enzymes. The deliverable will improve the protocol by determining efficient prey density and knowledge of using immune modulators. [month 27]

D15.3) Optimum hydrodynamics and light conditions during greater amberjack larval rearing: The definition of some environmental parameters towards an optimum larval rearing methodology will be delivered. These are related to the hydrodynamics (i.e. type-shape) in the rearing tanks and also the lighting (intensity and duration) conditions. Furthermore the results from the relevant trials related to the performance of the larvae (growth, quality etc) will be presented. The somatotropic axes will be also studied. [month 27]

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D15.4) Ontogeny of greater amberjack larval visual and digestive system: The characterization and development of the digestive system of the greater amberjack larvae will be conducted. The deliverable will present the methodology and the results of (a) the ontogeny of the digestive system and eye, (b) the variations of lipid deposition in the liver correlated with prey items used and the rearing system, (c) the visual ability at different developmental stages, (d) the correlation of amberjack feeding preference with the feeding protocol that was used, (e) the identification of critical phases during rearing (malnutrition periods) in order to improve larval performance and (f) the levels of oxidative stress in larvae reared in the different rearing systems, by measuring specific biomarkers (SOD, GPx, GR, GST and GSH). [month 27]

D15.5) An industrial protocol for greater amberjack larval rearing: An industrial protocol for the larval rearing of greater amberjack will be developed. The deliverable will be an improved protocol incorporating the results from the performed trials on larvae and fry quality in terms of (a) survival, (b) growth, (c) larval nutritional condition (RNA/DNA ratio), (d) physiological parameters (oxidative stress and immune system) and (e) ossification pattern and incidence of skeletal deformities. In addition, results of the developed protocol will be validated in a SME hatchery. [month 48]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS29	Successful maturation and spawning of eastern Atlantic or Mediterranean Sea wild, F1 generation gre	2	6	
MS30	Successful maturation and spawning of eastern Atlantic or Mediterranean Sea wild, F1 generation gre	2	18	
MS31	Protocol for tank design, lighting and probiotics of larval rearing of amberjack	2	24	
MS32	Successful maturation and spawning of eastern Atlantic or Mediterranean Sea wild, F1 generation gre	2	30	
MS33	Successful maturation and spawning of eastern Atlantic or Mediterranean Sea wild, F1 generation gre	2	42	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP16	Type of activity <sup>54</sup>	RTD
Work package title	Larval husbandry – pikeperch		
Start month	1		
End month	57		
Lead beneficiary number <sup>55</sup>	9		

## Objectives

1. Improvement of pikeperch larval rearing protocols by using a multifactorial approach,
2. Reduction of cannibalism rate to increase survival,
3. Development of industrial protocol to improve larval performance during rearing.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

A larval rearing system is complex and numerous factors influence larval development as well as behaviour and survival (Kestemont et al., 2003). The tasks will address the (a) identification of optimal combinations of factors (environmental, population and nutritional factors) to increase larval survival (reduction of cannibalism) and growth, and (b) the development of an efficient industrial protocol (higher growth rate, lower mortality and deformity rates) for the larval rearing of pikeperch.

EU Budget allocation: 30,000€ (IRTA), 62,040€ (UL), 58,515€ (DTU) and 34,030€ (ASIALOR, exited the consortium on 31/5/2016) and 43,020€ (F2B, joined the consortium in Aug 2016)

Task 16.1 Optimal combinations of factors to improve larval rearing (led by UL). Using a pilot scale larval rearing system (RAS, ten 700 L tanks) and based on existing protocols used by the SME (ASIALOR/F2B), successive experiments will be conducted over four years (from larval stage to 5 g mean weight, 4 months per experiment) using factorial designs (4 factors tested with 8 experimental units) (UL). Multifactorial designs such as fractional or complete factorial designs are efficient methods to successfully optimize larval protocols. Such methodology allows (i) to integrate the effects of each simple factor tested and interactions between them, (ii) to rank and evaluate the effects induced by factors or interactions, (iii) to identify rapidly an optimal combination of factors that increase larval survival, and (iv) to establish a first modeling of the complex multifactorial determinism of output variables. This method has been applied successfully by UL in aquaculture (e.g., Teletchea et al., 2009; Trabelsi et al., 2011).

Experimentally, the effects of the environmental parameters such as photoperiod, light intensity, temperature and water current (resulting in deliverable D16.1); nutritional parameters such as feeding frequency, meal timing and food composition (resulting in deliverable D16.2); and population parameters such as larval density, strain and domestication level (resulting in deliverable D16.3) will be studied successively. Then, a final and integrated experiment will be done to identify optimal combinations of factors and will result in deliverable D16.4. Specific experiments will be carried out for the weaning step by DTU (using similar methodology, collaboration DTU-UL). Cannibalism in pikeperch larvae being the major reason for mortality observed in larval rearing especially from 18 to 39 dph (Szkudlarek and Zakes, 2007), a specific care will concern this target. Finally (year 4), selected combinations (3 or 4 combinations in triplicates) of factors will be applied on farm conditions at an SME (ASIALOR/F2B). That will concern protocols for larval rearing and weaning. Results will be discussed and presented in deliverable D.16.5 (collaboration UL-DTU-ASIALOR/F2B).

How the combination of different environmental factors affects cannibalistic behavior and larval morphogenesis will be also assessed by the description of different organ systems that are involved in prey location and capture (sensory and digestive systems), locomotion and predator avoidance (sensory and musculoskeletal systems) and maturation of the digestive capabilities. This approach is based on the hypothesis that fish displaying

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cannibalistic behavior may be more morphologically developed and fit that their conspecifics, which may enhance their chances of displaying such behavioral pattern. In particular, larvae will be sampled to describe the ontogeny of the skeleton and skeletal deformities (IRTA). Sampled larvae will be fixed in buffered formaldehyde and stained with standard alizarine red (bones) and alcian blue (cartilage) for radiographic analysis. In addition samples for histology will be examined to study the ontogeny and functionality of the digestive system and evaluate the level of maturation of the digestive function by enzyme quantification and spectrophotometric procedures including analyses of the development of the sensory system like eye. These analyses will be combined to complementary approaches including real time RT-PCR in order to quantify and locate gene expression of transcription factors (Sox9, Osterix, Runx, Mef2c, Twist), signaling molecules (Bmp4, Bmp2, Ihh, Shh, Pdgfrb) and protein of extracellular matrix (ECM), involved in the skeletal system, and molecular tools to study some digestive hormones, e.g., CCK, leptin, ghrelin, Y-peptide, Pep-T1. Concerning cannibalism, based on small scale rearing and on the tagging of some individuals (microsatellites) or sub-populations (otoliths), the onset of cannibalism will be analyzed by video-recording (DTU).

Task 16.2 Development of an industrial protocol (led by F2B). From the results obtained in Tasks 16.1 (especially over the year 4), an industrial protocol (larval rearing + weaning) will be proposed and tested by an SME (F2B) during year 5 to improve pikeperch larval growth and to reduce significantly cannibalism and larval mortality (D16.6). This final protocol will integrate SME constraints.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
3	IRTA	2.00
9	UL	13.00
21	DTU	4.50
29	ASIALOR	7.50
39	F2B	7.70
	Total	34.70

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D16.1	Determine effect of environmental factors on pike perch larval rearing	9	4.00	R	PU	12
D16.2	Determine effect of nutritional factors on pikeperch larval rearing	9	4.00	R	PU	24
D16.3	Determine effect of population factors on pikeperch larval rearing	9	4.00	R	PU	36
D16.4	Identification of optimal combinations of factors for pikeperch larval rearing	9	4.00	R	PU	48
D16.5	Evaluation of selected rearing combinations for pikeperch on farm condition	9	3.50	R	PU	57
D16.6	Proposition of an industrial protocol for pikeperch rearing	29	15.20	R	PU	57
	Total		34.70			

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## Description of deliverables

D16.1) Determine effect of environmental factors on pike perch larval rearing: The effects of environmental factors (photoperiod, light intensity, water temperature, water current) and their interactions on pikeperch larval rearing (survival, cannibalism, growth and deformities rates) will be studied using a multifactorial experimental design and a complex analytical approach (including zotechnical, behavioural, histological and molecular parameters). The deliverable will present (a) a hierarchization and modelling of the effects observed with an identification of the main influential factors or interactions, and (b) a selection of optimal combinations of environmental factors to improve pikeperch larval rearing. Two successive breeding phases will be distinguished : initial larval growth on live preys (*Artemia nauplii*) and weaning. [month 12]

D16.2) Determine effect of nutritional factors on pikeperch larval rearing: The effects of nutritional factors (food composition, feeding frequency, meal timing, co-feeding duration) and their interactions on pikeperch larval rearing (survival, cannibalism, growth and deformities rates) will be studied using a multifactorial experimental design and a complex analytical approach (including zotechnical, behavioural, histological and molecular parameters). The deliverable will present (a) a hierarchization and modelling of the effects observed with an identification of the main influential factors or interactions, and (b) a selection of optimal combinations of nutritional factors to improve pikeperch larval rearing. Two successive breeding phases will be distinguished : initial larval growth on live preys (*Artemia nauplii*) and weaning. [month 24]

D16.3) Determine effect of population factors on pikeperch larval rearing: The effects of population factors (larvae density, geographical origin, domestication level, hatching time) and their interactions on pikeperch larval rearing (survival, cannibalism, growth and deformities rates) will be studied using a multifactorial experimental design and a complex analytical approach (including zotechnical, behavioural, histological and molecular parameters). The deliverable will present (a) a hierarchization and modelling of the effects observed with an identification of the main influential factors or interactions, and (b) a selection of optimal combinations of population factors to improve pikeperch larval rearing. Two successive breeding phases will be distinguished : initial larval growth on live preys (*Artemia nauplii*) and weaning. [month 36]

D16.4) Identification of optimal combinations of factors for pikeperch larval rearing: The effects of main influential factors identified in previous deliverables (D16.1, D16.2 and D16.3) and their interactions on pikeperch larval rearing (survival, cannibalism, growth and deformities rates) will be studied using a multifactorial experimental design and a complex analytical approach (including zotechnical, behavioural, histological and molecular parameters). The deliverable will present (a) a hierarchization and modelling of the effects observed with an identification of the main significant factors or interactions, and (b) a selection of optimal combinations of husbandry factors to improve pikeperch larval rearing. Two successive breeding phases will be distinguished : initial larval growth on live preys (*Artemia nauplii*) and weaning. [month 48]

D16.5) Evaluation of selected rearing combinations for pikeperch on farm condition: Considering the two successive breeding phases (initial larval growth on live preys and weaning), some combinations of factors selected from deliverable D16.4 will be tested in triplicate under farm conditions using SME facilities (integration of farm constraints). The deliverable will present (a) a validation or not of the optimal combinations previously identified in experimental conditions and (b) a proposal for an integrated and complete protocol (integration of the two successive phases) for optimizing pikeperch larval rearing. [month 57]

D16.6) Proposition of an industrial protocol for pikeperch rearing: Using SME facilities, the integrated and complete protocol presented in the deliverable D16.5 will be tested in triplicate on farm conditions using SME facilities (integration of farm constraints). The deliverable will present (a) a final evaluation of the integrated and complete protocol for optimizing pikeperch larval rearing and (b) a list of recommendations to fish farmers in order to avoid or limit some major bottlenecks. [month 57]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP17	Type of activity <sup>54</sup>	RTD
Work package title	Larval husbandry - Atlantic halibut		
Start month	1		
End month	48		
Lead beneficiary number <sup>55</sup>	7		

## Objectives

1. Improve larval survival and quality during early development of Atlantic halibut.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

The tasks will (a) compare recirculation aquaculture (RAS) and flow through (FT) systems during yolk sac and first-feeding larval stages, (b) evaluate the effectiveness of probiotics during early larval development and develop a protocol for industrial use in larval rearing and (c) develop an optimal production protocol for on-grown Artemia compared with use of Artemia nauplii.

EU Budget allocation: 284,620€ (IMR), 22,000€ (NIFES) and 42,000€ (SWH)

Task 17.1 Recirculation (RAS) vs Flow through (FT) systems during yolk sac and first feeding stages and the effects on larval survival, quality and growth (led by IMR). The commercial production of halibut fry is currently carried out in flow through systems (FT), while there is a growing consensus that RAS would offer more stable environmental and chemical water parameters that would lead to improved larval performance. Yolk sac incubators normally used for halibut rearing are 5000 l tanks with a sea water FT system having a water temperature of about 6°C. A RAS system will be constructed (IMR) from three generally used first-feeding tanks (Tropical Marine Center) and compared to the classic FT system (IMR, SWH), both during the yolk sac stage and during first feeding. Larvae will be evaluated in terms of growth, survival and larval quality in terms of pigmentation and eye migration success (IMR, SWH). It is not clear whether the intestinal microflora of halibut larvae is determined by the feed or by water quality parameters (see Bergh et al., 1994; Attramadal, 2011). In order to elucidate this, samples will be taken for examination of gut morphology (NIFES) and bacterial flora (IMR). Retention of nutrients (NIFES), and digestive physiology (ULL) will be performed in Task 11.3 from WP11 Nutrition – Atlantic halibut. The results will give information on at what stage, and how the intestine is colonised, and will form the basis for the implementation of probiotics in the industrial protocol to be developed in Task 17.2. This task will result in deliverable D17.2 Determine if RAS is a more effective protocol than FT for Atlantic halibut larvae.

Task 17.2 The effect of probiotics on larval microbiota and survival and development of an industrial protocol (led by IMR). Addition of probiotics is a way of improving survival of fish larvae, which is presently gaining increased interest (Vadstein et al., 2013). In order to improve the microbial flora, probiotics will be added in larval trials (IMR). The aim of this task is to clarify which bacteria are capable of both controlling pathogens and improve survival of halibut larvae. These trials will be modeled from protocols already published with cod (*Gadus morhua*) larvae, but adapted to halibut. The exceptionally long yolk sac stage of halibut and the critical early feeding stages make the application of probiotics during the early life stages of halibut particularly important. Probiotic trials will be based on the multiwell trials with cod (*Gadus morhua*) yolk sac larvae described recently by D'Alvise et al. (2012, 2013) and on the gnotobiotic protocols for sea bass developed by Dierckens et al. (2009). These techniques will be adapted to the longer yolk sac stage and the larger size of Atlantic halibut yolk sac larvae, in accordance with the challenge experiments with cod, halibut and turbot (*Scophthalmus maximus*) described by Sandlund et al. (2010). Multiwell trials imply a high number of replicates (n=50-100) with one larva in each well, giving statistical robustness and high experimental control., and the well established early feeding

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experiments (Rekecki et al. 2009, 2012, 2013). The bacteria will primarily be from the Roseobacter/ Ruegeria/ Phaeobacter group, which have been shown to possess probiotic properties, including TDA production (D'Alvise et al., 2012, 2013). Based on the results obtained by the in vitro trials, and the information obtained in task 17.1, a full scale trial will be performed in triplicate tanks, comparing RAS and FT systems and where probiotics are distributed either through addition to the water or via the feed. A protocol for industrial use of probiotics in Atlantic halibut juvenile production will be developed based on the results of this full-scale experiment. This task will result in deliverables D17.3 The effect of probiotics on Atlantic halibut larval microbiota and survival and D17.5 Development of an industrial protocol for probiotic treatment of halibut larvae.

**Task 17.3 Production of on-grown Artemia (led by IMR).** At present, halibut larvae are fed Artemia nauplii through the whole first feeding period. An observed reduction in growth rate during the later phases of first feeding indicates that this feed is insufficient to maintain high growth. A larger prey size, with a higher nutrient content may be a more appropriate choice for those stages. Therefore, a production protocol for on-grown Artemia will be further developed, based on Olsen et al., 1999, where water renewal and quality are crucial parameters (IMR, SWH). This protocol includes feeding, washing and disinfection of the Artemia, and will be tested both in experimental (IMR) and commercial scale (SWH). These experiments will be followed by analyses of bacterial activity (IMR). The biochemical profile of macro and micro nutrients of the on-grown Artemia compared to Artemia nauplii at the IMR research facility and at the commercial hatchery of SWH will be analysed by NIFES in WP11 Nutrition – Atlantic halibut. This task will result in deliverable D17.1 Production protocol of on-grown Artemia.

**Task 17.4 Comparison of feeding on-grown Artemia versus Artemia nauplii on larval performance (led by IMR).** Atlantic halibut larvae will be fed Artemia nauplii from first feeding. At 20 days post first feeding (dpff) one group of larvae will be fed on-grown Artemia whereas the other group will continue to be fed nauplii. The experiment will last until 70 dpff, when the halibut larvae have completed metamorphosis. Growth, survival and juvenile quality will be measured, including GI microflora, behavior, pigmentation, eye migration (IMR) and histological characterization of selected organs, including the intestine (NIFES). Nutrient analyses of samples taken in this trial (NIFES) and characterization of the digestive physiology of the larvae (ULL) will be carried out within WP 11 Nutrition – Atlantic halibut. This task will result in deliverable D17.4 Comparison of feeding on-grown Artemia versus Artemia nauplii on Atlantic halibut larval performance.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
7	IMR	13.00
17	IMR/NIFES	1.00
22	SWH	6.00
	Total	20.00

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D17.1	Production protocol of on-grown Artemia	7	3.50	R	PU	24
D17.2	Determine if RAS is a more effective protocol than FT for Atlantic halibut larvae	7	6.50	R	PU	36
D17.3	The effect of probiotics on Atlantic halibut larval microbiota and survival	7	4.00	R	PU	36

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D17.4	Comparison of feeding on-grown Artemia versus Artemia nauplii on Atlantic halibut larval performance	7	4.00	R	PU	36
D17.5	Development of an industrial protocol for probiotic treatment of halibut larvae	7	2.00	R	PU	48
Total			20.00			

## Description of deliverables

D17.1) Production protocol of on-grown Artemia: This deliverable is a protocol for production of ongrown Artemia for use during the later phases of first feeding, when a reduction in growth rate is observed. At present, halibut larvae are fed Artemia nauplii through the whole first feeding period, while a larger prey size may be more appropriate for the later stages. The protocol will include feeding, washing and disinfection of the Artemia, and will be tested both in experimental and commercial scale. Water renewal and quality are crucial parameters and bacterial activity will be analysed in the ongrowing systems. [month 24]

D17.2) Determine if RAS is a more effective protocol than FT for Atlantic halibut larvae: The deliverable will be the result of studies comparing the performance of halibut larvae in recirculatory systems (RAS) with traditional flow-through systems (FT), both during the yolk sac stage and during first feeding. The RAS system is expected to give a more stable rearing environment and will be constructed from three generally used first-feeding tanks (Tropical Marine Center). Larvae will be evaluated in terms of growth, survival and larval quality (pigmentation and eye migration success). Examination of gut morphology and bacterial flora will elucidate whether the intestinal microflora of halibut larvae is determined by the feed or by water quality parameters. The results will give information on at what stage, and how the intestine is colonised, and will form the basis for the implementation of probiotics in the industrial protocol to be developed. [month 36]

D17.3) The effect of probiotics on Atlantic halibut larval microbiota and survival: The deliverable will provide important information in order to develop an industrial protocol for probiotic treatment of halibut larvae. It will clarify which bacteria are capable of both controlling pathogens and improve larval survival, and at what larval stage probiotic treatment is most effective. This will be achieved by in vitro challenge trials based on multiwell trials with cod (*Gadus morhua*) yolk sac larvae and on the gnotobiotic protocols for sea bass, but adapted to the longer yolk sac stage and the larger size of Atlantic halibut yolk sac larvae. The bacteria will primarily be from the Roseobacter/Ruegeria/Phaeobacter group, which have been shown to possess probiotic properties. [month 36]

D17.4) Comparison of feeding on-grown Artemia versus Artemia nauplii on Atlantic halibut larval performance: The deliverable will demonstrate whether the production protocol for ongrown Artemia (deliverable 4.4.1) results in a prey organism that gives improved growth, survival and development in halibut larvae and juveniles. Atlantic halibut larvae will be fed Artemia nauplii for 20 days from first feeding. One group of larvae will subsequently be fed on-grown Artemia whereas the other group will continue to be fed nauplii until completion of metamorphosis. Normal development will be characterized by gut and intestinal microflora, behavior, pigmentation, eye migration and histological characterization of selected organs, including the intestine. [month 36]

D17.5) Development of an industrial protocol for probiotic treatment of halibut larvae: The deliverable is a protocol for industrial use of probiotics in Atlantic halibut juvenile production. The protocol will be developed based on the results of a full-scale experiment where probiotics are added to RAS and FT systems as a means to improve rearing environment and larval survival. The experiment will be based on a) information obtained in in vitro trials (D17.3) and b) results from the experiment on RAS and FT (D17.2). RAS and FT systems will be compared and probiotics will be distributed either through addition to the water or via the feed. [month 48]



# WT3: Work package description

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
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# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP18	Type of activity <sup>54</sup>	RTD
Work package title	Larval husbandry - wreckfish		
Start month	1		
End month	48		
Lead beneficiary number <sup>55</sup>	8		

## Objectives

1. Development of larval rearing protocol based on the most effective prey density, succession of prey type, light regime (intensity and duration), temperature and culture system,
2. Description of ontogeny of digestive system, vision, taste and smell organs in response to larval rearing methods.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

This WP will define appropriate feeding regimes, rearing systems and optimum environmental parameters (temperature) for the rearing of early life stages of wreckfish.

EU Budget allocation: 41,900 € (HCMR), 55,900 € (IEO), 30,916€ (CMRM) and 14,537€ (MC2)

Task 18.1 Development of feeding methodology (lead by HCMR)

Different feeding regimes (prey densities and succession of prey type) will be tested to develop a feeding protocol and avoid periods of food deprivation. Eggs for the trials will be available either from HCMR broodstock or by transferring the required amounts from IEO. Testing semi-intensive culture system (Mesocosm with 40,000 l) tanks (Papandroulakis et al., 2002), in triplicate trials from the end of endogenous feeding to the change to inert feeding (weaning phase). The culture system will be evaluated in terms of (1) ontogeny of larval digestive and visual system (influenced by feeding) through histological and image analysis procedures. In addition, the ontogeny of the digestive enzymes for wreckfish larvae will be studied under the different rearing regimes. This task will contribute to deliverables D18.1 Determine optimum temperature conditions for rearing and D18.3 Develop a feeding protocol for wreckfish larvae.

Task 18.2 Defining optimum conditions for larval rearing (lead by IEO)

Sub-task 18.2.1 Testing (IEO, MC2) the effect of two temperature ranges (14-17 and 19-22°C) in triplicate trials in 2000 l tanks in flow-through systems and using the same photoperiod regime from the end of endogenous feeding to the change to inert feeding (weaning phase). These studies will be evaluated in terms of growth, survival, larval quality and size. This task will contribute to D18.2 Determine optimum temperature conditions for rearing.

Sub-task 18.2.2 Test of two culture systems RAS (CMRM) and flow-through (IEO), (Rodriguez J.L. et al., 2010, Rodriguez J.L. et al., 2011) in terms of larval culture conditions and feeding protocols. Trials will be evaluated in terms of biochemical profile (proteins, lipids and EFA content), and biometric analyses and survival. This task will contribute to D18.4 Determine the most effective culture system (RAS vs flow-through) for wreckfish larvae.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	6.50

# WT3: Work package description

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
8	IEO	4.20
19	CMRM	3.10
32	MC2	1.01
Total		14.81

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D18.1	Development of the digestive system of wreckfish	1	3.00	R	PU	36
D18.2	Determine optimum temperature conditions for rearing wreckfish larvae	8	3.40	R	PU	36
D18.3	Develop a feeding protocol for wreckfish larvae	1	3.21	R	PU	36
D18.4	Determine the most effective culture system (RAS vs flow-through) for wreckfish larvae	8	5.20	R	PU	48
Total			14.81			

## Description of deliverables

D18.1) Development of the digestive system of wreckfish: The characterization and development of the digestive system of wreckfish larvae will be conducted, during the first 30 DAH. The deliverable will present the methodology and the results of (a) the ontogeny of digestive system and eye, (b) the variations of lipid deposition at the liver in correlation with the feeding items used, (c) the visual ability in the different developmental stages, (d) the identification of critical phases during larval rearing (malnutrition periods) in order to improve the rearing conditions and (e) the changes of main digestive enzyme activities (proteases, carbohydrases and lipases) during the larval to juvenile digestive system ontogeny [month 36]

D18.2) Determine optimum temperature conditions for rearing wreckfish larvae: An optimal temperature range for incubation and larval culture of the wreckfish until approximately 60 DAH will be developed. This protocol will be developed for each phase (embryonic development and larval culture) and its optimization will be evaluated from results obtained in larval survival, growth, deformities, pigmentation and vertical migration of the larvae. [month 36]

D18.3) Develop a feeding protocol for wreckfish larvae: Develop a feeding protocol for wreckfish larvae. A Feeding protocol will be developed based on studies of the different stages of the larval development after hatching; the yolk sac consumption, acceptance of exogenous food and duration of larval development and growth until the acceptance of inert food (weaning). The sequence of live food consumption will be determined, as a function of age, and the time of weaning. Results on survival and viability will also be provided, as well as morphometric determinations during larval culture. The deliverable will describe the results of these studies with the aim of improving the feeding protocol. [month 36]

D18.4) Determine the most effective culture system (RAS vs flow-through) for wreckfish larvae: Three culture systems will be tested; two are representative of intensive culture (RAS and FT) while the other is a semi-intensive (Mesocosm) system. This deliverable will present and define the culture protocols for the three systems : 1) Management of RAS and FT under intensive and semi-intensive conditions, 2) Different larval and prey densities, 3) feeding sequence, according to the system used, 4) control of the physical and chemical

# WT3: Work package description

parameters in the three systems, 5) feeding protocols for the three systems during the first 30 DAH. In addition, this deliverable will include information on larvae survival, growth, parameters on larval quality, deformations and size distribution. Samples will also be taken to determine biochemical profiles of the larvae (proteins, lipids and EFA contents). [month 48]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP19	Type of activity <sup>54</sup>	RTD
Work package title	Larval husbandry - grey mullet		
Start month	1		
End month	58		
Lead beneficiary number <sup>55</sup>	4		

## Objectives

1. Investigating environmental and nutritional factors that affect larval rearing.
2. Determine the effect of co-feeding ciliates and rotifers on digestive tract maturation and enzyme production.
3. Determine when to wean larvae and to feed weaning diet type according DT maturation and the shift from carnivorous to omnivorous feeding.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

Harel et al. (1998) found that the “greening of the larval grey mullet rearing tanks with *Isochrysis galbana*, an alga rich in docosahexaenoic acid (DHA, 22:6n-3), contributed more to larval survival than when adding *Nannochloropsis galbana* which has considerable levels of eicosapentaenoic acid (EPA, 20:5n-3). However, the most effective algal concentration during larval rearing and the long-term effects on later developmental stages such as metamorphosis still must be studied. In addition, the question remains if the benefits derived from the use of live microalgae would be conserved in frozen algal pastes, which would be more available and economical (Lubzens et al. 1995). This would be the case if the benefit of the use of microalgae is due solely to its contribution to water turbidity, which creates suitable background lighting for efficient prey capture, and is not imparting other biochemical and stimulatory advantages to the larvae (Rocha et al., 2008). In support of this, recent studies have claimed that ceramic clay may be a viable alternative to adding microalgae to the rearing tanks (Attramadal et al., 2012). As a result, the planned studies here will be to (1) compare the effect of microalgae type (*Nannochloropsis oculata* and *Isochrysis galbana*) and concentration (cells/ml) on larval rotifer ingestion rate, biochemical composition, digestive tract enzyme ontogeny and metamorphic synchrony; and (2) to determine if the benefit of live algal addition is due to its effect on tank turbidity that leads to efficient prey capture or other factors. (3) The cost-benefit analysis of using live algae versus frozen in order to establish an economical protocol.

It is widely accepted that marine fish larvae grown under extensive or semi-intensive mesocosm conditions result in high quality juveniles (Papandroulakis et al. 2004, 2005; Zouiten et al. 2004; Zaiss et al. 2006). This may be due, in part, to the larvae consuming a range of available zooplankton species that optimize development and growth. This, contrasts with conditions in intensive systems where fish are reared in high densities and fed only monocultured zooplankton and phytoplankton species that are not representative of their natural habitat and, apart from sub-optimal growth and survival, may adversely affect oxidative status and cause developmental and skeletal deformities. Importantly, the biochemical composition of the natural diet in the mesocosm pond can affect the larval enzymatic development and maturation process (Zambonino-Infante and Cahu, 2001; Buchet et al., 2000; Tovar et al., 2002; Savoie et al. 2006). On the other hand, mesocosm zooplankton and phytoplankton species can vary in type and concentration and the reduced egg stocking densities do not provide an answer to the production demands of the production industry. Recently, an Israeli start-up company (Zoopt) has developed technology to mass produce ciliates which represent one of the main organisms found in mesocosms. Recent studies conducted at the IOLR/NCM indicated a preference for ciliates when co-fed with rotifers (Koven et al. 2012) in the larvae of species such as the gilthead sea bream (*Sparus aurata*), white grouper (*Epinephelus aeneus*) and bluefin tuna (*Thunnus Thynnus*). Moreover, larval growth, survival and essential fatty acid profile were improved. The question remains if the co-feeding of ciliates and rotifers in intensive systems will similarly and positively affect DT maturation as when larvae are fed semi-intensive mesocosm organisms and rotifers.

# WT3:

## Work package description

The ontogeny of digestive tract development may be particularly important in grey mullet where the ontogeny of DT enzymes from larvae to juvenile would reflect the transition from a strict carnivorous diet to an omnivorous mode of feeding. In thick lipped grey mullet (*Chelon labrosus*) and similar to carnivorous species, a marked increase in trypsin activity was observed when a more dense protein weaning diet compared to *Artemia* was fed to 20 dph larvae. On the other hand, amylase activity in the larvae of this mullet species, unlike the larvae of carnivorous teleosts, continually increased from first feeding to 20 dph and was maintained at a high level (Zouiten et al. 2008). This suggested the inclusion of low cost starch or other amylolytic energetic compounds in the weaning diet should be considered (Lazo et al. 2007). During intestinal maturation, the cytosolic intestinal enzyme leucine alanine peptidase (leu-ala) decreases, while brush border alkaline phosphatase (AP) and aminopeptidase N (AN) increases. In the thick lipped grey mullet both the AP/leu-ala and AN/leu-ala ratios increased sharply at 8 dph indicative of rapid DT maturation (Zouiten et al. 2008) but decreased later signaling the switch from carnivorous to omnivorous diet. Kvåle et al. 2007 concluded that this digestive enzyme activity is genetically programmed to match ontogenetic shifts in diet. Knowing the ontogeny of key enzymes and when this shift in feeding mode occurs would contribute greatly to designing the composition of weaning diets and when to feed them.

EU Budget allocation: 35,000€ (IRTA), 55,000€ (IOLR) and 11,000€ (DOR)

Task 19.1 Effect of algal type and concentration on larval performance (led by IOLR).

Action 19.1.1 Determine the effect of algal type and concentration in rearing tanks on larval performance (led by IOLR). Two separate trials (IOLR) will be carried out in sixteen 1500 l conical tanks using the current IOLR protocol for grey mullet larviculture. In these studies, the effect of different *Nannochloropsis oculata* and *Isochrysis galbana* concentrations (0, 250, 500 and 1000 x 10<sup>3</sup> cells/ml and 0, 18, 36 and 72 x 10<sup>3</sup> cells/ml, respectively) will be tested on larval prey ingestion rate, growth, survival, body composition (lipid class, fatty acids, protein, free amino acids), metamorphic synchrony (percent "silvering") and digestive tract enzyme ontogeny. Previous studies (IOLR) showed that each pair of inter-specific *Nannochloropsis oculata* and *Isochrysis galbana* algal concentrations (0,0; 250,18; 500,36; 1000,72 x 10<sup>3</sup> cells/ml) gave the same turbidity in the larval rearing tanks. These trials will be carried out in sixteen 1500 l tanks where each algal concentration will be tested in 4 replicate tanks. This task will contribute to deliverable D19.1 Determine most effective type and concentration of algae used in grey mullet larval rearing.

Action 19.1.2 Determine if the benefit of algal addition to rearing tanks due to background lighting or other factors that contribute to larval performance (led by IOLR). The selected *Nannochloropsis oculata* and *Isochrysis Galbana* concentrations, from the first two studies, will be tested (IOLR) against a Kaolite (clay) treatment having the same turbidity as the algal treatments to determine if the benefit of the selected algal concentration is due only to its background lighting effect on rotifer ingestion rate or other factors that contribute to larval performance. These studies will be carried out in fifteen 1500 l conical tanks where each the Kaolite control and the two algal treatments will be tested in five replicate tanks each. This task will contribute to D19.1 Determine most effective type and concentration of algae used in grey mullet larval rearing.

Task 19.2 Comparing the selected microalgae type and protocol (Task 19.1) with lyophilized substitute (led by IRTA). The selected microalgae from Task 19.1 will be compared with a lyophilized substitute when used to "green tanks" during early grey mullet larviculture (IRTA). Larval performance will be evaluated in terms of larval growth, survival, rotifer ingestion rate, geometric morphology, whole body composition, maturation of the digestive tract and the incidence of skeletal deformities This task will contribute to deliverable D19.2 Evaluate the effectiveness of replacing live algae with lyophilized algae during grey mullet larval rearing.

Task 19.3 Determine the effect of co-feeding copepods and *Artemia* on digestive tract maturation and enzyme production (lead by IOLR). The maturation of the digestive tract (DT) of grey mullet, according to the results in previous tasks carried out in Diversify, occurs around 61 dph. In this task (lead by IOLR), the aim will be to determine the effect of co-feeding copepods and *Artemia* nauplii on DT maturation and enzyme production, particularly alkaline phosphatase (marker for brush border enzyme activity) and leucine alanine peptidase (marker for cytosol enzyme activity). This task will address a potential bottleneck in grey mullet larviculture, which is that the maturation of the DT during intensive rearing occurs relatively late as the fish transit from carnivory to omnivory. In omnivory, a substantial part of the diet is carbohydrate, which would reduce the cost of weaning and starter diets compared to diets for carnivores, which would have higher levels of more costly protein. Consequently, it would be more economical to bring gut maturation forward.

Copepods may contain nutrients (e.g. polyamines) that encourage cell proliferation and differentiation and reduce the time line to maturation. Three enriched *Artemia*: copepod treatments will be produced at the P4:IOLR facilities. These treatment ratios will be; 3 *Artemia* nauplii: 0 copepods/ml, 1.5 *Artemia* nauplii :1.5 copepods/ml,

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0 Artemia nauplii: 3 copepods/ml and will be fed to 20-50 dph mullet larvae stocked in fifteen 17 l aquaria (50 fish/aquaria) in a salinity and temperature computer controlled experimental system at P4:IOLR in Eilat. This will allow the testing of each treatment in replicates of 5 aquaria per treatment.

At 20, 35 and 50 dph, fish samples will be taken to determine growth as well as pancreatic, brush border and cytosol enzyme activity and gene expression of Pept1, the major peptide transporter of di- and tripeptides into the enterocyte. Enzyme analyses will be carried out by partner (P3.IRTA). In addition, Artemia and copepods will be sampled periodically and analyzed for polyamine content (e.g. spermidine, spermine) as well as fatty acid content. The survival will be determined at the end of the study. This task will contribute to deliverable D19.3 Determining the effect of co-feeding copepods and Artemia nauplii on digestive tract maturation and enzyme production.

Task 19.4 Determine when to wean larvae and to feed weaning diet type according to DT maturation and the shift from carnivorous to omnivorous feeding (Lead by IOLR). This study will test weaning diets (WD) varying in protein, carbohydrate and lipid levels at the suggested times for weaning and mode of feeding change (Action 19.1.3). Fifteen dph larvae grown on best performing ciliate-rotifer co-feeding protocol (Action 19.1.3) will be stocked in twenty 20 l aquaria and tested on an Artemia control and 3 MD treatments (MD1, MD2, MD3) in replicates of 5 aquaria/treatment from 15-40 dph. These treatments will consist of: (1) Control- Artemia fed to larvae from 15-40 dph. (2) Artemia fed until larvae weaned (highest AP/leu-ala ratio) onto WD1 (high protein: low carbohydrate : High lipid) until 40 dph. (3) Artemia fed until larvae weaned (highest AP/leu-ala ratio) onto WD1 and then (AP/leu-ala falls) onto WD2 (low protein: moderate carbohydrate: high lipid) until 40 dph. (4) Artemia fed until weaned (highest AP/leu-ala ratio) onto WD1 and then (AP/leu-ala falls) onto WD3 (low protein: high carbohydrate: low lipid) until 40 dph. IOLR will sample on 15 and 40 dph for gene expression of the enzyme alkaline phosphatase and peptide transporter PepT1 as well as growth, survival (40 dph) and fatty acid analysis. Samples (15 and 40 dph) will be sent for analyses (IRTA) for pancreatic (alkaline proteases, amylase, lipase, trypsin) stomach (pepsin), brush border (alkaline phosphatase), and cytosol (leucine- alanine peptidase) enzymes. This task will contribute to deliverable D19.4 Determine weaning time and type of feed according to the shift from carnivorous to omnivorous feeding.

Task 19.5 Testing the improved grey mullet larval rearing protocol in a commercial hatchery (led by DOR). The improved grey mullet larval rearing protocol resulting from Task 19.1 and Task 3.6.1 in the WP Nutrition will be tested at the Israeli commercial hatchery (DOR) in three 10 m3 round tanks in a flow through system. Larval performance will be evaluated in terms of growth and juvenile survival. This task will contribute to deliverable D19.5 Evaluate an improved grey mullet larval rearing protocol in a commercial hatchery.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
3	IRTA	2.00
4	IOLR	11.00
25	DOR	3.00
	Total	16.00

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D19.1	Determine most effective type and concentration of algae used in grey mullet larval rearing	4	3.00	R	PU	24

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D19.2	Determining the effect of co-feeding ciliates and rotifers on digestive tract maturation and enzyme production	4	3.00	R	PU	36
D19.3	Determining the effect of co-feeding copepods and Artemia nauplii on digestive tract maturation and enzyme production	4	3.00	R	PU	58
D19.4	Evaluate the effectiveness of replacing live algae with lyophilized algae during grey mullet larval rearing	3	4.00	R	PU	48
D19.5	Evaluate an improved grey mullet larval rearing protocol in a commercial hatchery	25	3.00	R	PU	55
Total			16.00			

## Description of deliverables

D19.1) Determine most effective type and concentration of algae used in grey mullet larval rearing: This deliverable will describe which selected algae (*Nannochloropsis oculata* or *Nannochloropsis galbana*) and its concentration (cells/ml) will be used to "green tanks" and improve the larval rearing protocol of mullet. It will deal specifically if the benefit of algal addition to tanks is due to the background lighting it provides, which improves prey detection, and/or imparts biochemical compounds that improves ingestion. The revised protocol will improve larval growth, prey ingestion and digestion, metamorphic synchrony and survival. [month 24]

D19.2) Determining the effect of co-feeding ciliates and rotifers on digestive tract maturation and enzyme production: Digestive tract (DT) maturation and enzyme production was found to be optimized when larvae were extensively grown in a mesocosm environment that was supplemented with rotifers and *Artemia*. It is then hypothesized that co-feeding copepods, that are representative of a dominant mesocosm species which can be intensively and dependably cultured, with *Artemia* might sufficiently mimic mesocosm conditions. In turn, this might provide optimum conditions for rapid digestive tract maturation. The results from these studies would contribute to an improved larval rearing protocol. [month 36]

D19.3) Determining the effect of co-feeding copepods and *Artemia* nauplii on digestive tract maturation and enzyme production: From examining the dynamics of DT developmental markers (e.g. alkaline phosphatase) in D19.3, we will be able to determine when the DT can produce pancreatic and brush border enzymes as well as the switch from a carnivorous mode of feeding to an omnivorous one. This would be very advantageous to determine when it is possible to start feeding a high protein and low carbohydrate weaning diet and when to switch to a feed with a low protein high carbohydrate diet. Clearly this knowledge would contribute greatly to a further improvement in the larval rearing protocol. [month 58]

D19.4) Evaluate the effectiveness of replacing live algae with lyophilized algae during grey mullet larval rearing: In order to make the improved protocol with the selected algae more widely available (not all farms have live algae culture) the live algae will be compared with a lyophilized preparation during larval rearing. If there is no significant difference between the two algal protocols in terms of larval growth, prey ingestion and digestion, metamorphic synchrony and survival, then the lyophilized algal supplement will be used in the revised larval rearing protocol. [month 48]

D19.5) Evaluate an improved grey mullet larval rearing protocol in a commercial hatchery: In order to evaluate the applicability of the improved larval rearing protocol under commercial conditions, it will be tested at the DOR facility. These trials will be in one of three commercial systems (depending on the number of eggs) that include 19 10-m<sup>3</sup> tanks, 8 7-m<sup>3</sup> tanks or 4 5-m<sup>3</sup> tanks. [month 55]



# WT3: Work package description

Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS38	Successful maturation and spawning of grey mullet broodstock to produce good quality eggs and larvae	4	9	
MS39	Successful maturation and spawning of grey mullet broodstock to produce good quality eggs and larvae	4	21	
MS40	Successful maturation and spawning of grey mullet broodstock to produce good quality eggs and larvae	4	33	
MS41	Successful maturation and spawning of grey mullet broodstock to produce good quality eggs and larva	4	45	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP20	Type of activity <sup>54</sup>	RTD
Work package title	Grow out husbandry - meagre		
Start month	6		
End month	42		
Lead beneficiary number <sup>55</sup>	3		

## Objectives

1. Adaptations in the existing methodology for grow out in cages related to the rearing environment (depth and light conditions) and improvements related to the size dispersion that is frequently observed,
2. Development of an appropriate feeding method that respects the species specificities.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

The tasks will address (a) the size variability presented during pre-growing (b) the effect of the rearing environment (depth, light) during cage rearing and (c) the development of an appropriate feeding system for the meagre.

EU Budget allocation: 231,179€ (HCMR), 130,000€ (IRTA), 120,000€ (ARGO) and 10,040€ (CULMAREX)(company exited the Consortium in March 2015)

Task 20.1 Size variability at juveniles (led by IRTA). Size variability in juvenile pre-grow out makes regular grading essential to avoid cannibalism and grades of smaller fish may be related to poor performance when transferred to sea cages. Experiments will be carried out with meagre juveniles of a mixture of 5-6 known families, to simulate the commercial hatchery situation and in order to study differences in growth rate. Juvenile fish will be stocked in triplicate tanks at the same initial density and fed the same commercial diet (IRTA). At the end of the experiment fish will be genetically characterised for parentage assignment (HCMR, Task 2.4 from WP2 Reproduction and genetics - meagre) to establish if differences in growth rate is a consequence of genetic origin. Fish with low growth rates will be used for compensatory growth studies to determine growth potential of small juveniles and estimate the economic cost of using these fish for production, compared to discarding and using only larger juveniles. This task will result in deliverable D20.1 Methodology to avoid size variability in meagre juveniles.

Task 20.2 Effect of rearing environment (led by HCMR)

Sub-task 20.2.1 Effect of cage depth. Trials in 180 (6x6x5) and 290 (6x6x8) m<sup>3</sup> cages will be performed in the HCMR pilot farm using two groups of sizes (200-600 g) and (800- 1.5 kg). In both cases the final stocking density will be 15 kg m<sup>3</sup>. The duration of each trial will be 8 months. Growth performance will be estimated with monthly samples while every second month haematological (hematocrite, hemoglobin), biochemical (osmotic pressure, glucose, lactic acid, free fatty acids), immunological (lysozyme, myeloperoxidase serum), hormonal (cortisol) evaluation will be performed. Also, the vertical distribution in cages will be monitored using an echo integrator. This Sub-task will contribute to deliverable D20.2 Definition of the optimum conditions for cage culture of meagre.

Sub-task 20.2.2 Effect of light intensity in the cage. Test cage rearing with and without shading at an SME farm (ARGO) applying standard commercial procedures for 2 rearing periods (each with 2 cages) with groups of different ages (200-600 g) and (800- 1.5 kg). In both cases the final stocking density will be 15 kg m<sup>3</sup>. The duration of each trial will be 8 months. Growth performance will be estimated on monthly basis, while the vertical distribution in cages will be monitored for a specific periods of time (2 weeks per trial) using an echo integrator (HCMR). This Sub-task will contribute to deliverable D20.2 Definition of the optimum conditions for cage culture of meagre.

# WT3: Work package description

Task 20.3 Development of feeding methodology (led by HCMR). The development of an appropriate feeding methodology will be implemented in 5 Steps.

Sub-task 20.3.1 (HCMR) Test of different feeding stimuli (mechanical, optical etc). Groups of two different individual sizes (50-100 and 700-900 g) at different tank sizes (500 and 5000 l respectively) will be used for testing mechanical and optical feeding stimuli for a period of 4 months (each group). Monitoring with video recordings will allow the definition of the optimal feeding stimuli.

Sub-task 20.3.2 (HCMR) Test of different feeding methods. Three methods will be tested with fish from two different ages (50-100 and 700-900 g) at different tank sizes (500 and 5000 l) for a period of 4 months (each group).

- Self feeder
- Automatic feeding three times per day
- Hand feeding

Monitoring with video recordings and records of the self-feeding activity will be performed.

Sub-task 20.3.3 (HCMR) Test in cages of 2 feed distribution methods. Feed distribution from the surface and from the bottom will be tested during two duplicated trials in the HCMR pilot farm in (6x6x8 m<sup>3</sup>) cages. Two size groups (200-400 g) and (1.0- 1.5 kg) will be used for a period of 4 months each. The final stocking density during the rearing will be 15 kg m<sup>-3</sup>. Growth performance will be estimated with monthly samples while every second month haematological (hematocrite, hemoglobin), biochemical (osmotic pressure, glucose, lactic acid, free fatty acids), immunological (lysozyme, myeloperoxidase serum), hormonal (cortisol) evaluation will be performed. Also, the vertical distribution in cages will be monitored using an echo integrator.

Sub-task 20.3.4 (IRTA) Comparison of automatic and demand type feeding in tanks. Comparison in each season of the year of (a) demand feeding and (b) feeding with automatic feeders programmed to follow the feeding routines that are used customarily in meagre cage farms (feeding in farms is now based mainly of automated feeding). Three replicate control tanks (automated feeding) will be compared to three experimental demand-feeding tanks. Video cameras and sensors will be installed to register the activity of the fish and behaviours related to feeding and aggression. Experimental conditions will be natural photoperiod and simulated natural temperature controlled to be similar to sea cage growing areas for the specific season. The parameters to be evaluated would be: feeding time, feed delivered, growth, size variation in the population, FCR, pattern of fish activity, level of aggressive behaviours and fin condition.

Advantages of proposed tank experiments:

- a) More robust results with replicated (3x) groups,
- b) Results can be expected to be more predictable or reliable compared to results from commercial trials,
- c) Better control of growth, variation in population and FCR,
- d) Daily and seasonal activity and feeding patterns,
- e) Wider range of parameters analysed to give clearer picture on behaviour and explain differences due to feeding methods.

As mentioned earlier, studies in other cultured species indicate that rhythms of appetite and activity in tanks are representative of cages. All these sub-tasks will contribute to deliverable D20.3 Methodology for meagre feeding.

Fin-clip samples for genetic studies of fast and slow growing fish (described under WP2.4) will be obtained from another commercial enterprise (ANDROMEDA, not a member of consortium) that has offered to work with DIVERSIFY in return for being involved in the genetic study on growth, and participation in the “open days” of our coordination meetings. The company has already provided samples for another genetic study (See Deliverable 2.2 Genetic characterization of different meagre captive broodstocks and evaluation of available variability, submitted) and we have developed an excellent relation with this company.

Sub-task 20.3.5 (HCMR) Development of feeding system for industrial application. The design and operation parameters of an industrial feeding system (HCMR, ARGO, IRTA) will be implemented by integrating the gathered information. This task will contribute to deliverable D20.3 Methodology for meagre feeding.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	48.50

# WT3: Work package description

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
3	IRTA	12.00
23	ARGO	30.00
30	CULMAREX	1.00
Total		91.50

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D20.1	Methodology to avoid size variability in meagre juveniles	3	12.00	R	PU	24
D20.2	Definition of the optimum conditions for cage culture of meagre (Report)	1	36.50	R	PU	39
D20.3	Methodology for meagre feeding	1	43.00	R	PU	42
Total			91.50			

## Description of deliverables

D20.1) Methodology to avoid size variability in meagre juveniles: A method will be developed to avoid size variability of meagre juveniles. The deliverable will define the influence of genetic origin on the size variability in juveniles and on the bases of this provide recommendations on how to avoid variability (e.g. genetic improvement and / or size grading including the possibility of recovering slow growing fish). The deliverable will include results from growth trails and genetic analysis of fast and slow growers to support the recommendations. [month 24]

D20.2) Definition of the optimum conditions for cage culture of meagre (Report): A methodological procedure will be developed for the optimum cage rearing of meagre including suggestions for the optimum (a) cage depth, and (b) lighting conditions in the cage. The deliverable will also present the results from the performed trials and the performance (growth and behavioral) of the reared groups. [month 39]

D20.3) Methodology for meagre feeding: A feeding method as a result of different tests that will be performed will be delivered. Also the results of the trials on (a) the appropriate feeding stimuli (b) the optimum feeding method (c) the distribution method and the performance of the reared groups will be presented. The gathered information will result in an integrated feeding system. [month 42]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS42	Results on feeding stimuli of meagre	3	18	
MS43	First cage trials (different volume and light conditions) with meagre implemented	3	24	
MS44	Results on feed distribution method in cages with meagre	3	24	

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP21	Type of activity <sup>54</sup>	RTD
Work package title	Grow out husbandry - greater amberjack		
Start month	8		
End month	57		
Lead beneficiary number <sup>55</sup>	1		

## Objectives

1. Development of appropriate rearing methods for cages including rearing volume and type of cage,
2. Development of feeding methods for fry and juveniles by identifying daily rhythms and feeding frequency.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

In this WP rearing methodologies for the cage rearing of the greater amberjack will be developed with emphasis on (a) the cage technology (depth and type), (b) the feeding method and (c) the husbandry practise (temperature, stocking density)

EU Budget allocation: 45,000€ (HCMR), 95,000€ (FCPCT), 60,000€ (IEO), 15,000 (ULL) 65,096€ (FORKYS) and 100,000€ (CANEXMAR)

Task 21.1 Development of rearing method in cages (led by FCPCT)

Action 21.1.1 (FORKYS) Effect of rearing volume (depth) on performance. Trials will be performed in commercial cages (FORKYS) of 10 m and 6 m depth (not duplicated) for a period of 12 months for two successive rearing periods. Final stocking density will be kept at 15kg m<sup>3</sup>. Growth performance will be estimated every second month together with a control of the muscle quality (HCMR). Additional evaluation (HCMR) of the performance will be done with haematological (hematocrite, hemoglobin), biochemical (osmotic pressure, glucose, lactic acid, free fatty acids), immunological (lysozyme, myeloperoxidase serum), and hormonal (cortisol) analysis, while the expression of heat shock proteins, glucocorticoid GR and mineralocorticoid MR receptors (in spleen and gills) will be also estimated. This Action will contribute to deliverable D21.2 Definition of optimum conditions for cage culture of greater amberjack

Action 21.1.2 (CANEXMAR) Effect of cage type on performance. A comparison of surface and submerged cages will be performed in trials with commercial cages (CANEXMAR) of 20 m diameter; 10 m depth, for 2 successive rearing periods of 12 months each. The final stocking density will be kept at 15 kg m<sup>3</sup>. Growth performance and health status will be estimated every second month (FCPCT). This Action will contribute to deliverable D21.2 Definition of optimum conditions for cage culture of greater amberjack.

Task 21.2 Development of feeding methods (led by IEO). Test of different feeding methods including estimation of daily rhythm and frequency (continuous vs fixed ratios) will be tested with individuals at different developmental stages of juveniles (5 g and 200 g individuals).

Action 21.2.1 (FCPCT) Definition of feeding pattern for 5 g fish reared in 500 l-tanks for 4 months. Monitoring will include growth performance, feed efficiency, k index, juvenile quality (morphological aspects) and haematological, histological, biochemical and immunological analysis. This Action will contribute to deliverable D21.1 Definition of optimum feeding methods for greater amberjack grow out.

Action 21.2.3 (IEO) Definition of feeding pattern for 200 g reared in 500 l-tanks for 4 months. Monitoring will include growth performance, feed efficiency, k index, juvenile quality (morphological aspects) and haematological, histological, biochemical and immunological analysis. This Action will contribute to deliverable D21.1 Definition of optimum feeding methods for greater amberjack grow out.

# WT3: Work package description

## Task 21.3 Development of appropriate husbandry practise (led by HCMR)

Action 21.3.1 (HCMR) Determination of minimum-maximum temperature ranges. The trial will be performed with different size individuals (starting at 5, 200 and 500 g), all trials will be conducted in triplicates. Rearing will be realized in 500-l, for the first two sizes, and 10 m3 for the third size, tanks at 2 different temperature ranges (a) 14-17°C representing the lower temperatures observed in Mediterranean open sea and (b) 26-29°C representing the upper temperatures observed in Mediterranean open sea. The trial with the 5 g and the 500g individuals will be conducted at the facility of FCPCT and the one with the 200 g individuals at HCMR. The duration of the trial will be 4 months. Monitoring will include growth performance, feeding activity, gut transit time, digesta sample analysis (protein, fat, dry matter, apparent digestibility, energy) and protease, trypsin, chymotrypsin, lipase enzyme activities. This Action will contribute to deliverable D21.2 Definition of optimum conditions for cage culture of greater amberjack.

Action 21.3.2 (IEO) Definition of optimal stocking density. Rearing trials at 3 different stocking densities will be performed with individual size of 5 g in 500 l-tanks (IEO) and 150 g in 4000 l tanks (IEO) for a period of 4 months. Monitoring will include growth performance, feed efficiency, k index, and quality including morphological aspects and haematological, histological, biochemical and immunological studies (IEO). Also analysis of oxidative stress enzymes will be evaluated (ULL). This Action will contribute to deliverable D21.2 Definition of optimum conditions for cage culture of greater amberjack.

### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	7.50
2	FCPCT	36.00
8	IEO	3.40
15	ULL	4.50
27	FORKYS	15.00
28	CANEXMAR	45.00
Total		111.40

### List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D21.1	Definition of optimum feeding methods for greater amberjack grow out	8	49.40	R	PU	42
D21.2	Definition of optimum conditions for cage culture of greater amberjack	2	62.00	R	PU	57
Total			111.40			

### Description of deliverables

D21.1) Definition of optimum feeding methods for greater amberjack grow out: A feeding methodology will be developed for the greater amberjack. This will include definition of (a) feeding method and (b) estimation of daily feeding rhythms at different size classes. The results of the trials, including the evaluation of the performance (growth and quality) of the reared groups, will be also presented. [month 42]

# WT3: Work package description

D21.2) Definition of optimum conditions for cage culture of greater amberjack: A methodological procedure will be developed for the optimum cage rearing of the species.. This will include (a) the definition of the optimal stocking density and (b) the determination of minimum-maximum temperature ranges, both at different size classes. The results of the relevant trials on the performance (growth, physiological) will be also delivered. [month 57]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS45	Feeding pattern of greater amberjack fry available	1	21	
MS46	First results on optimum husbandry practise (thermal ranges, stocking density) of greater amberjack	1	28	
MS47	First experimen on cage culture condition (net volume, cage type) of greater amberjack implemented	1	30	

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP22	Type of activity <sup>54</sup>	RTD
Work package title	Grow out husbandry – pikeperch		
Start month	8		
End month	48		
Lead beneficiary number <sup>55</sup>	16		

## Objectives

1. Effect of husbandry practices and environmental factors on pikeperch growth, immune and physiological status,
2. Characterization of pikeperch growth, immune and physiological status in farm conditions,
3. Effect of pikeperch domestication level and geographical origin on growth and stress sensitivity.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

In this WP the effects of husbandry practices on growth, physiological and immune status of pikeperch over a production cycle will be studied with additional consideration on the domestication level and the geographical origin of the pikeperch stocks.

EU Budget allocation: 131,000€ (UL), 161,008€ (FUNDP), 101,779€ (DTU), 25,396€ (ASIALOR, exited the consortium on 31/5/2016) and 43,090€ (F2B, joined the consortium in Aug 2016)

Task 22.1 Effect of husbandry practices and environmental factors on pikeperch growth, immune and physiological status (led by FUNDP). In order to identify the main stressful factors, pikeperch juveniles (80-100 g) will be exposed to various potentially stressful husbandry practices and environmental conditions (i.e. lightning, rearing density, handling, sorting, water temperature, salinity, pH, TAN, NO<sub>2</sub>-N, NO<sub>3</sub>-N, oxygen level, tank design/rearing system) (UL) and examined for physiological stress responses, immune competence and global resistance to infectious diseases (FUNDP). This first experiment will last around 8-12 months. At that level, the application of a multifactorial experimental design will be used as a first screening approach. We will test 8 factors (2 modalities per factor, using 16 experimental units). Based on physiological and immunological variables as outputs, that screening approach will allow identifying an optimal combination of environmental and husbandry factors that will apply on commercial farm conditions (ASIALOR/F2B) as a validation step.

The analytical approaches will include behavioral as well as biochemical, cellular and molecular techniques (2D-DIGE proteomic and RT-PCR) (FUNDP). Concerning behavior, a major objective will be the identification of specific behavioral traits or changes before the sudden mortality and the proposal of real-time behavioral indicators (video recording, UL). Pertaining to the possible immune consequences of stressors, bacterial challenges will be imposed to the fish and both internal organs/fluids involved in immune function (spleen, head-kidney, blood) and primary barriers (mucus, gills) will be investigated. In addition metabolic costs will be evaluated, using an approach where oxygen consumption is measured in fish following a stressful event, also known as post stress oxygen consumption, this provides valuable insight on the metabolic cost of recovering from different stressors, and the duration recovery (DTU). The approach allows for assessing different severities or durations or repetitions of a stress event that may occur during husbandry or transport, e.g. decreases in dissolved oxygen, increased ammonia concentrations, light exposure, crowding, etc. This task will result in deliverable D22.1 Effects of multiple variables on stress, immune response and growth performances and recommendations of optimal conditions for pikeperch grow out.

Task 22.2 Characterization of pikeperch growth, immune and physiological status in farm conditions (led by F2B). Based on the results from multifactorial experiments (see Task 22.1), growth and physio-immunological status of 2 or 3 batches of pikeperch at different developmental stages (from 10 g to 1.5 kg) will be compared, in farm conditions (ASIALOR/F2B), between standard husbandry conditions usually applied in routine by the



# WT3: Work package description

SME and the best rearing conditions identified in Task 22.1. From this task, the expected results will help (i) to recommend the best conditions applicable in pikeperch farming for reducing stress level and supporting maximal growth performances. This task will result in deliverable D22.2 Validation of optimal rearing variables under commercial farm conditions.

Task 22.3 Effect of pikeperch domestication level and geographical origin on growth and stress sensitivity (led by FUNDP). Due to recent intensive culture, pikeperch populations displaying various domestication levels are reared so far (Teletchea & Fontaine, 2012). Domestication highly affects the stress and immune status of fish in farm conditions (Verbeek et al., 2008; Zuberi et al., 2008; Douxfils et al., 2011). The effects of domestication process (wild vs domesticated strains) and geographical origin (freshwater vs brackish water strains) will be investigated. Different batches of juveniles (3 or 4 geographical origins, 1 or 2 populations of the same geographical origin with 2 levels of domestication) will be produced from larval stage in similar conditions (year 3, UL). The genetic variability and value of the different populations will be characterized (link with WP2.3). Then, using the optimal combination of factors identified on Task 22.1, fish will be reared and examined for physiological stress responses, immune competence and global resistance to infectious diseases (FUNDP). The analytical approaches are similar to the one presented for Task 22.1. This task will specify the effect of domestication level and geographical origin on pikeperch growth and stress sensitivity, allowing the further selection of pikeperch strain according to the rearing conditions of commercial fish farms. This task will result in deliverable D22.3 Effects of domestication level and geographical origin on stress, immune response and growth performances and strain recommendation.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
9	UL	18.00
16	FUNDP	26.00
21	DTU	7.00
29	ASIALOR	5.00
39	F2B	5.00
Total		61.00

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D22.1	Effects of multiple variables on stress, immune response and growth performances and recommendations of optimal conditions for pikeperch grow out	16	31.00	R	PU	24
D22.2	Validation of optimal rearing variables under commercial farm conditions	29	12.00	R	PU	42
D22.3	Effects of domestication level and geographical origin on stress, immune response and growth performances and strain recommendation	16	18.00	R	PU	48
Total			61.00			

# WT3: Work package description

## Description of deliverables

D22.1) Effects of multiple variables on stress, immune response and growth performances and recommendations of optimal conditions for pikeperch grow out: Multifactorial designs will be performed to characterize stress physiology, immune response to various husbandry conditions, and immune competence to infectious diseases. The results will enable to determine the major aquaculture stressors in pikeperch in order (a) to reduce stress sensitivity, (b) to increase disease resistance, (c) to improve growth performances. [month 24]

D22.2) Validation of optimal rearing variables under commercial farm conditions: The optimal rearing variables determined during the first phase research will be tested in a commercial RAS in order to validate and to recommend the best conditions applicable at a large-scale RAS for reducing stress level and supporting maximal growth performances. [month 42]

D22.3) Effects of domestication level and geographical origin on stress, immune response and growth performances and strain recommendation: Broodstocks of different domestication levels and geographic origins will be characterized by microsatellite approach in order: (a) To test the potential immunocompetence, (b) To determine the extent of fast/growers in pikeperch juveniles; (c) To establish basis for strain recommendation in pikeperch production. [month 48]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS48	Experiment on the definition of optimal conditions for pike perch on growing implemented	16	18	
MS49	First trials with different strains of pike perch implemented	16	40	

# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP23	Type of activity <sup>54</sup>	RTD
Work package title	Grow out husbandry - grey mullet		
Start month	9		
End month	40		
Lead beneficiary number <sup>55</sup>	4		

## Objectives

1. Evaluating the geographic range for grow-out of grey mullet in the Mediterranean basin,
2. Determine the cost-benefit of different weaning diets on the performance and health status of juvenile grey mullet.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

These tasks will examine the effect of (a) different stocking densities and (b) different juvenile origin on the performance of grey mullet during grow out at different locations in the Mediterranean.

EU Budget allocation: 20,000€ (HCMR), 25,000€ (IRTA), 20,000€ (IOLR), 32,425€ (CTAQUA), 30,000€ (DOR), 32,400€ (GEI) and 48,000€ (IRIDA)

Task 23.1. Determine the cost-benefit of different weaning diets on the performance and health status of wild juveniles (led by IRTA). Weaning strategy (e.g., type of diet and length of the co-feeding period with live prey and inert feeds during the weaning period) in grey mullet early juveniles (fry) will be tested using three available commercial diets for an experimental period of 75 days under standard rearing conditions (500 L tanks, 18-20°C, natural photoperiod, RAS). This period of time allows the proper adaptation of wild animals to experimental conditions and provides enough certainty that the observed results are due to the experimental design implemented, in addition, the timespan selected will allow fish to reach a significant size for tissue sampling. For achieving this goal and due to the unavailability of aquaculture produced grey mullet early juveniles, wild fry will be collected during their natural recruitment into coastal lagoons (Delta of the Ebro River), transported to IRTA's facilities and acclimated to experimental conditions (similar practices have been previously conducted by this partner with 90% of fish survival after acclimation conditions). Weaning strategies (i) long co-feeding period – 40 days; ii) short co-feeding period – 10 days, iii) intermediate length co-feeding period – 20 days, iv) direct transfer to inert feed – 0 days) and tested diets will be analysed in terms of fish growth, survival, quality, maturation of digestive system and fish health status (non-innate immune response). Non-innate immune response will be assessed by haematological parameters: serum lysozyme, complement, bacteriolytic activity and burst respiratory activity as described in the literature, the organization and functionality of the digestive system in different dietary groups will be conducted by standard histological procedures and biochemical spectrophotometric procedures for measuring the activity of pancreatic, gastric and intestinal enzymes. Each experimental group will be tested in triplicate. In addition, the economical cost/profitability of each weaning strategy will be evaluated with regards to the direct costs associated to fish rearing practices (feed and facilities) and their performance for the subsequent stages of fish rearing. This task will result in deliverable D23.1 Cost-effective weaning strategies for wild-caught grey mullet grow out and their effect on growth and health status.

Task 23.2 Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture of F1 juveniles stocked at two different densities in cement and earthen ponds (led by IOLR). Hatchery produced F1 juvenile grey mullet (IOLR) will be supplied to the SME (DOR). These fish will be stocked at two different densities (0.5 and 1 juvenile m<sup>2</sup>) in two earthen 6,000 m<sup>2</sup> ponds and fed an improved grey mullet extruded feed, (WP3 Nutrition). This feed will be custom-produced for all grey mullet tasks in this WP (IRIDA). In parallel, a 1 year density study (4 and 6 juveniles m<sup>2</sup>) using the project's grey mullet feed will be carried out on F1

# WT3: Work package description

juveniles (IOLR) in 4 circular cement ponds (30 m<sup>3</sup>), where each density will be tested in duplicate tanks. Fish performance will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival. At the DOR facility fish weighing will be done every 6 months while at the IOLR the fish will be weighed every 2 months. In addition, analyses of lipid class and fatty acid composition of selected tissues (liver, muscle and gonads) will be carried out at the IOLR. This task will contribute to deliverable D23.2 Stocking protocols for pond monoculture grow out of F1 and wild caught grey mullet.

Task 23.3 Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture of wild caught juveniles stocked at two different densities in cement ponds in Greece (led by HCMR). Wild caught grey mullet juveniles will be stocked at two different densities (4 and 6 juveniles/m<sup>2</sup>) in 6 cement ponds (20 m<sup>2</sup>) where each density will be tested in triplicate tanks over 1 year. The fish will be fed an improved grey mullet extruded feed as described in Task 23.1 (IRIDA). Fish performance will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival and the trial will be supervised by the HCMR. In addition, analyses of lipid class and fatty acid composition of selected tissues (liver, muscle and gonads) as well as proximate analyses of the fish and diet samples will be carried out at the HCMR. This task will contribute to deliverable D23.2 Stocking protocols for pond monoculture grow out of F1 and wild caught grey mullet.

Task 23.4 Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture of wild juveniles at two different densities in ponds in Spain (led by CTAQUA). Wild caught grey mullet juveniles will be stocked at two different densities (0.5 and 1 fish m<sup>-2</sup>) in 2 earthen ponds (1100 m<sup>2</sup>). The fish will be fed an improved grey mullet extruded feed as described in Task 23.1 (IRIDA). Fish performance will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival. In addition, analyses of lipid class and fatty acid composition of selected tissues (liver, muscle and gonads) as well as proximate analyses of feeds and fish samples will be carried out (CTAQUA). This task will contribute to deliverable D23.3 Comparison of the project's improved grey mullet grow-out feed under the different environmental and water conditions in Israel, Greece and Spain.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	7.00
3	IRTA	3.00
4	IOLR	2.00
18	CTAQUA	5.80
25	DOR	10.00
26	GEI	12.00
31	IRIDA	4.00
Total		43.80

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D23.1	Cost-effective weaning strategies for wild-caught grey mullet grow out and their effect on growth and health status	3	3.00	R	PU	18

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D23.2	Stocking protocols for pond monoculture grow out of F1 and wild caught grey mullet	4	28.00	R	PU	30
D23.3	Comparison of the project's improved grey mullet grow-out feed under the different environmental and water conditions in Israel, Greece and Spain	4	12.80	R	PU	40
Total			43.80			

## Description of deliverables

D23.1) Cost-effective weaning strategies for wild-caught grey mullet grow out and their effect on growth and health status: This report will include a) evaluation of wild fry to adapt to culture conditions; (b) development of weaning protocol for mullet fry (biological model to be applied in cultured fish); (c) comparison of weaning strategies (cultured vs wild fish). [month 18]

D23.2) Stocking protocols for pond monoculture grow out of F1 and wild caught grey mullet: Hatchery produced F1 juvenile grey mullet will be supplied to the SME DAGON at kibbutz Ma'agan Michael in northern Israel. These fish will be stocked at two different densities (0.5 and 1 juvenile m<sup>2</sup>) in two earthen 6,000 m<sup>2</sup> ponds and fed the improved grey mullet extruded feed as a result of studies in this project. In parallel, a 1 year density study (4 and 6 juveniles m<sup>2</sup>) using the project's grey mullet feed will be carried out on F1 juveniles (IOLR) in 4 circular cement ponds (30 m<sup>3</sup>), where each density will be tested in duplicate tanks. In contrast, wild caught grey mullet juveniles will be stocked at two different densities (4 and 6 juveniles/m<sup>2</sup>) in 6 cement ponds (20 m<sup>2</sup>) in Greece where each density will be tested in triplicate tanks over 1 year and fed the project's grey mullet grow out diet. Fish in all studies will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival. In addition, analyses of lipid class and fatty acid composition of selected tissues (liver, muscle and gonads) as well as proximate analyses of the fish and diet samples will be carried out. The project feed for these studies will be produced by the Greek company IRIDA. [month 30]

D23.3) Comparison of the project's improved grey mullet grow-out feed under the different environmental and water conditions in Israel, Greece and Spain: In order to evaluate the applicability of the improved grow out diet for grey mullet under the variable climatic and pond conditions throughout the Mediterranean basin, a comparison of the project's improved grey mullet grow out feed will be tested in Israel, Greece and Spain. All feed will be produced by IRIDA. Fish performance will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival. In addition, analyses of lipid class and fatty acid composition of selected tissues (liver, muscle and gonads) as well as proximate analyses of feeds and fish samples will be carried out. [month 40]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS50	Experimental trials of grey mullet in the three locations implemented	4	27	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP24	Type of activity <sup>54</sup>	RTD
Work package title	Fish health - meagre		
Start month	5		
End month	57		
Lead beneficiary number <sup>55</sup>	1		

## Objectives

1. Identify the causes of systemic granulomatosis (SG), and chronic ulcerative dermatopathy,
2. Investigate anti-parasite treatments in juvenile meagre,
3. Undertake preliminary characterisation of immune genes and study specific immune responses post-vaccination,
4. Evaluate the occurrence of Nocardia infections in meagre,
5. Optimization and evaluation of a vaccine for Vibrio,
6. Develop diagnostic-prevention-treatment protocols for diseases in meagre.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviation)

This WP will address a number of identified bottlenecks relating to meagre health. The tasks include (a) studies of key disease states, (b) development of appropriate treatments, and (c) a first characterisation of the meagre immune system/ immune responses required for future immune intervention.

EU Budget allocation: 207,080€ (HCMR), 75,000€ (FCPCT), 161,850€ (IRTA), 112,804€ (UNIABDN) and 36,000€ (SARC)

Task 24.1. Systemic Granulomatosis (led by HCMR). The most important disease of cultured meagre is a newly described condition under this provisional name (Katharios et al., 2011). It is characterized by multiple systemic visceral granulomas and is manifested progressively by calcified and necrotic liver and kidney. The aetiology of the disease is unknown however there is evidence that it may be a metabolic disorder. To investigate the hypothesis that the disease is related to nutrition we will run several feeding trials to assess the effect of vitamin D and Ca/P levels in feeds (HCMR), the effect of low/high protein levels in feeds and Mg inclusion (HCMR), the effect of plant ingredients in the feeds (HCMR), as well as the effect of minerals and vitamins levels (FCPCT).

Sub-task 24.1.1. Feeding trials (HCMR)

Trial 1. (HCMR) The effect of vitamin D (3 levels) inclusions in diets (SARC) will be examined in the development of Systemic Granulomatosis (SG). Meagre fry (1-5 g) will be used for this trial. Growth, feed efficiency, body composition, vitamin D determination in target tissue and specific biomarkers (CYP27, CYP24 enzymes which are involved in vitamin D metabolism and SOD, CAT and GSH) will be evaluated (HCMR). Samples will be provided for pathological assessment at HCMR (Sub-task 24.1.2). Together with the health and pathological assessment in Sub-task 24.1.2, this Trial will result in deliverable D24.1 The effect of vitamin D inclusions in diets in the development of Systemic Granulomatosis in meagre.

Trial 2. (HCMR) The effect of Ca/P ratio in the diet (SARC) will be examined, as well as the Low/High protein content of the diet will be investigated (SARC). For the experimentation (HCMR), 50 fish/per tank (each treatment in triplicate) weighing 1 g will be used until they reach about 20 g (3-4 months feeding) in weight. During the experimental period, samplings will be performed every 20 days for the detection of SG and samples will be taken for pathological assessment at HCMR (Sub-task 24.1.2). Body weight, liver weight and feed consumption will be recorded for calculation of growth rates, hepatosomatic index and feed conversion ratio. Body composition, Ca/P determinations and specific biomarkers (CYP27, CYP24 enzymes) will be studied

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(HCMR). Together with the health and pathological assessment in Sub-task 24.1.2, this Trial will result in deliverable D24.2 The effect of Ca/P ratio in the diet in the development of Systemic Granulomatosis in meagre.

Trial 3. (HCMR) The effect of high plant protein diets will be examined against a control fishmeal based diet on gut morphology and the potential appearance of SG. Three to five fish per tank in a non-fasting state will be sampled from each treatment for histology of intestine (HCMR). Sampling will be done at the beginning of the experiment and after 30 and 60 days. Samples will be taken for pathological assessment at HCMR (Sub-task 24.1.2) as described in Sub-task 24.1.2 below. Together with the health and pathological assessment in Sub-task 24.1.2, this Trial will result in deliverable D24.5 The effect of high plant protein diets in the development of Systemic Granulomatosis in meagre.

Trial 4. (FCPCT) The combined effect of vitamins E, C and carotenoids will be examined in SG prevention. Diets produced by SARC will contain several combinations of these antioxidants to determine optimum levels required to reduce or completely prevent the occurrence of SG. The effect of these nutrients on survival and growth parameters (FCPCT) and on several biomarkers of fish metabolism in relation to these nutrients, such as oxidative stress enzymes and health, will be also studied (FCPCT). Meagre welfare biomarkers will also be examined, including Heat Shock Proteins (FCPCT). Fish and samples will be provided for health studies in Sub-task 24.1.2, where FCPCT will conduct health analysis and pathological assessment. The deliverable corresponding to this trial (D24.6) will describe the recommended levels of pro and antioxidant nutrients to prevent SG. Together with the health and pathological assessment in Sub-task 24.1.2, this Trial contribute to deliverable D24.11 Recommended levels of pro- and anti-oxidant nutrients to prevent Systemic Granulomatosis in meagre.

Trial 5. (FCPCT) The effect of Se, Mn and Fe will be examined in SG prevention. Diets produced by SARC will contain several combinations of these minerals in relation to the polyunsaturated fatty acid contents of the diets to determine optimum levels required to reduce or completely prevent the occurrence of SG. The effect of these nutrients on survival and growth parameters (FCPCT) and on several biomarkers of fish metabolism in relation to these nutrients, such as oxidative stress enzymes and health, will be also studied (FCPCT). Meagre welfare biomarkers will also be examined (FCPCT). Fish and samples will be provided for health studies (Sub-task 24.1.2) where FCPCT will conduct health analysis and pathological assessment. The deliverable corresponding to this trial will describe the recommended levels of antioxidant nutrients to prevent SG (D24.6). Together with the health and pathological assessment in Sub-task 24.1.2, this Trial will contribute to deliverable D24.11 Recommended levels of pro- and anti-oxidant nutrients to prevent Systemic Granulomatosis in meagre.

Sub-task 24.1.2. Health and pathological assessment (HCMR). Depending on size, samples will include whole fish (fish <2 g) or dissected organs and tissues, which will be preserved in various fixatives. Analyses will include histology (HCMR, FCPCT) for the description of the development of SG using standard and special staining techniques (Bennett et al., 1976; McDowell and Trump, 1976), Transmission Electron Microscopy (HCMR) with X-ray microanalysis (Suzuki et al., 1997), Scanning Electron Microscopy (HCMR) and tissue biochemical analysis (HCMR). Hepatic and renal subacute toxicity will be evaluated by measuring enzyme activity or concentrations of: alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total cholesterol, gamma-glutamyltransferase, glucose, potassium, sodium, blood urea nitrogen and uric acid (HCMR). At least 10 fish per treatment will be included in this analysis. Trials at HCMR will provide samples for the analyses at HCMR, and trials at FCPCT will provide samples for analyses in FCPCT. A limited number of samples will be exchanged among Partners, for selected analyses not available to both Partners. The task will result in a full description of the pathology of SG and will assess the impact of the various feed/nutritional conditions to the development of the disease (HCMR, FCPCT). The deliverable associated with this task (D24.1) will include the description of the disease, the time of first appearance of the granulomas and the relationship to the various diets. This Sub-task contributes directly to deliverables D24.1, D24.2, D24.5, D24.11 (see above) and D24.14 Diagnostics protocol for Systemic Granulomatosis in meagre and aetiological factors and D24.15 Report for the prevention/treatment of Systemic Granulomatosis in meagre.

Task 24.2. Chronic Ulcerative Dermatopathy (led by HCMR). This is a newly described condition affecting the lateral line canals of many cultured fishes. From previous studies, it is established that Chronic Ulcerative Dermatopathy (CUD) is directly associated with the use of borehole water (Katharios et al 2011). Two parallel rearing trials of meagre in borehole and natural seawater will be conducted in order to study the development of CUD. Rearing using water from the two sources will be performed in duplicate 500L tanks (HCMR). The development of the disease in the different environments will be studied using histological and molecular techniques focusing on the enzymatic activity (TRAP, ALP, cathepsin K etc) in the affected area (Nemoto et al., 2007). Fish will be sampled every 5-10 days until lesions become visible. The study will also involve

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physicochemical analysis and comparison of the two water sources, in order to identify the aetiological agent of the condition. The analysis includes monitoring of pH, carbon dioxide, salinity and selected heavy metals such as Zn, Cu, Pb and Cd. Recovery studies will be performed using fish with visible lesions originally grown in borehole water that will be transferred in sea cages. This group will be compared against fish that will continue to grow in borehole water in inland facilities (HCMR). The resolution of lesions will be studied during 5 months following transfer to seawater using visual inspection and histology. The deliverables associated with this Task will include D24.7 Diagnostics protocol for Chronic Ulcerative Dermatopathy in meagre and aetiological factors, and D24.8 Report on the prevention/treatment of Chronic Ulcerative Dermatopathy in meagre.

Task 24.3. Anti-parasitic treatments (led by IRTA). Parasites such as *Sciaenacotyle panceri*, a monogenean found on the gills of meagre (Merella et al., 2009), are also known to cause mortality in farms in the Mediterranean and require development of appropriate treatments. A range finding test will be performed to evaluate the tolerance of the fish to each chemical product that will be considered as a potential treatment (IRTA). At the end of each test, fish will be sacrificed humanely and samples of the gills and blood taken. Gills will be fixed and analysed by histology in order to evaluate any damage caused at the tissue level either by the potential treatment or by the parasite should they be encountered in the test population during the course of the treatment trials. Plasma will be collected from the blood and general stress indicators will be measured, such as cortisol, glucose and lactate. All potential treatments will be repeated and a minimum number of 15 fish per treatment will be analysed. It is anticipated that treatments will be given either through medicated feed or medicated bath, depending on the compound being tested. Likely candidates are praziquantel, benzalkonium chloride, copper sulphate, gracilin, and magnesium sulphate among others. This task will result in deliverable D24.9 Determination of effective treatments for common monogenean parasites in meagre, and compliance with relevant EU and national regulatory frameworks will be considered.

Task 24.4. Nocardia infection in meagre (led by HCMR).

Sub-task 24.4.1 Isolation and characterization of the pathogen (HCMR). Nocardia infection has been reported recently in large cultured meagre and may pose a significant threat and bottleneck for its production, since Nocardiosis is a chronic disease which is hard to eradicate and apart from the direct mortalities may also adversely affect the final product quality. Since there is only one report in the literature for Nocardiosis in meagre and in the Mediterranean aquaculture in general, in this WP we will monitor fish from various geographic localities (Greece and Spain) for the presence of the bacterial pathogen. The screening (HCMR, IRTA) involves isolation using standard microbiological techniques (isolation in various nutrient media such as TSA, BHIA and Nutrient Agar) and physiological characterization based on colony morphology, bacterial morphology, Gram and Ziehl-Neelsen staining, antibiotic sensitivity, biochemical profiling using API ZYM and BIOLOG Microsystems. Isolated strains will be also genetically characterized based on DGREA analysis and sequencing of 16s rRNA following PCR with universal or Nocardia-specific primers, along with the putative virulence of the chosen strains, based on clinical data (mortalities, morbidity) (HCMR, IRTA). With this task we will assess the existence of Nocardia in meagre and its genetic and phenotypic variability that will be important for future vaccine design, along with the putative virulence of the chosen strains, based on clinical data (mortalities, morbidity). This Sub-task will result in deliverables D24.4 Isolation and characterization of Nocardia from infected meagre and D24.6 Experimental vaccine for meagre.

Sub-task 24.4.2. Preparation of an autogenous vaccine (HCMR). Based on the results of Sub-task 24.4.1, a monovalent or polyvalent vaccine was originally proposed to be prepared using formalin inactivated bacterin (HCMR). The vaccine would have been tested for sterility and toxicity in HCMR and then sent to IRTA for vaccination/challenge experiments. However, since Nocardia has not been isolated so far in subtask 24.4.1 (after 30 mo in the project), subtask 24.4.2 will be performed with a commercial *Vibrio* vaccine since it is anticipated that vibriosis will become a health issue for meagre culture. Challenge will be initially optimised with the isolated strain or with a virulent *Vibrio anguillarum* strain, and subsequently this method will be used to determine the effectiveness of the pilot vaccine after prior immunisation of the fish for 8 weeks, using fish of 10-20 g. The challenge will be carried out for up to a month, when samples will be collected from peripheral blood, spleen and head kidney (IRTA) for gene expression analysis (IRTA, UNIABDN) to assist in describing the immune system (eg analysis of IFN-g, MyD88, TNF- $\alpha$ ), together with other immune genes identified as necessary for documentation of the ontogeny of the immune system in Task 24.5. We will also (1) isolate the organism from diseased fish if available, and grow it in pure culture, and (2) confirm it is the same species as that used in the challenge. This Sub-task will result in deliverables D24.12 Determination of efficacy of vaccination of meagre against Vibriosis and D24.13 Description of immune gene expression pre- and post-immunization of meagre with a *Vibrio* vaccine.



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Task 24.5. First characterisation of the immune system (led by UNIABDN). Anticipating that future management of disease issues in meagre will require vaccines as part of the arsenal of approaches used, we will undertake a characterisation of the immune system to identify key immune molecules, as potential markers of immune system development and induction of antiviral and antibacterial responses (UNIABDN, IRTA). For markers of the adaptive immune system, we will clone RAG1/2, Ig and TcR genes from meagre, and determine their expression during development, to allow an analysis of when to vaccinate as the immune system matures. In addition, we will clone marker genes of inflammation (IL-1B, TNFa), antibacterial responses (antimicrobial peptides, such as piscidins and defensins) and antiviral response (interferon genes, Mx). The latter will require prior stimulation of fish/cells, since these genes show low constitutive expression but can be markedly induced upon infection. Pathogen Associated Molecular Patterns (PAMPs) will be used for this purpose and will include bacterial LPS, polyI:C (a synthetic double stranded RNA) and  $\beta$ -glucan. In each case qPCR assays will be established for future gene expression profiling following vaccination or immunostimulant treatment. This Task will result in deliverable D24.3 Cloning of key marker genes of innate and adaptive immune responses in meagre (UNIABDN).

Task 24.6. Monitor specific immune responses (led by UNIABDN). In this task we will monitor meagre specific immune responses following vaccination with a bacterin (UNIABDN, IRTA). Classically antibody responses are measured post-vaccination, and we will develop anti-meagre IgM and anti-meagre IgT antibodies for this purpose. In addition, a range of cytokines associated with helper-T cell responses will be cloned (such as IL-2, IFN- $\gamma$ , IL-4, IL-17 or IL-22) to allow analysis of cell-mediated responses. Meagre will be vaccinated with a vaccine to *Vibrio anguillarum* (IRTA), a common Gram-negative bacterium found in the marine environment, and blood/mucus samples (for antibody analysis) and tissue samples (gill/kidney) for cytokine analysis (UNIABDN) will be collected at a range of times post-vaccination and post-exposure to the pathogen. This Task will result in deliverable D24.10 Kinetics of antibody and cytokine production established post-pathogen exposure or stimulation with PAMPs.

Task 24.7. Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in meagre (led by FCPCT). Increase in meagre production, as it occurs in other warm water species, may lead to the emergence of bacterial diseases such as vibriosis or photobacteriosis and some viral diseases such as nodavirus and lymphocystis disease. In this task we will monitor meagre throughout the project to establish the seasonality of the potential diseases. External morphology, necropsies and samples of liver, spleen, kidney, gut and nervous system will be studied to determine the presence of pathogenic organisms and histopathological damage to the different organs (FCPCT). These samples will be processed for determination of bacterial diseases by classical microbiological techniques, and by PCR. For viral diseases, cell cultures suited to each disease (eg SS-1, SAF-1) will be used for virus propagation, following by RT-PCR or PCR verification depending on the specific virus. Susceptibility of meagre will be set against field and reference strains of the following pathogens: *Vibrio anguillarum*, *Photobacterium damsela* subsp. *piscicida*, and betanodavirus. Susceptibility values will be set by challenge test using intraperitoneal or intramuscular injections for bacteria or nodavirus respectively, in studies conducted in a biosecurity facility (FCPCT). When outbreaks of disease occur, methods will be established to determine the causal agent of the disease and attempt to find appropriate preventive and therapeutic measures for control. Specific diagnosis protocols will range from molecular (PCR, RT-PCR) to antibody based (ELISA, Immunohistochemistry, Immunofluorescence) methods. Treatment protocols will be established with selected antibiotics (depending on the limitation of their use) for each pathogen isolated from natural outbreaks of disease, by studying the MICs of the different pathogens, as well as the best routes of administration, depending on the type of fish (age, type, etc.). The results obtained from this task will produce deliverable D24.16 Diagnostics manual for the major bacterial and viral diseases found in meagre, and where useful treatments have been developed, complete protocols for their implementation will be provided.

Task 24.8 Diagnostic-recommendation manual for meager health (led by HCMR). This will be a practical diagnostic manual and recommendation guide for meager health issues targeted to fish health specialists and aquaculture scientists and producers. The manual will be the synopsis of major findings of WP24. It will be published in electronic format (pdf file) and uploaded in the project website, and will be freely available for the public. The manual will be organized in chapters describing the major diseases of the species with original photographic material, epidemiological and pathological data. Responsible for the compilation and organization of the manual will be HCMR, and all partners involved (FCPCT, IRTA, UNIABDN) will contribute according to their participation with chapters, photographic material, diagnostic keys etc. This task will result in deliverable D24.17. Diagnostic-recommendation manual for meager fish health.

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## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	73.10
2	FCPCT	29.00
3	IRTA	23.00
5	UNIABDN	20.00
20	SARC	3.30
Total		148.40

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D24.1	The effect of vitamin D inclusions in diets in the development of Systemic Granulomatosis in meagre	1	11.00	R	PU	20
D24.2	The effect of Ca/P ratio in the diet in the development of Systemic Granulomatosis in meagre	1	11.00	R	PU	24
D24.3	Cloning of key marker genes of innate and adaptive immune responses in meagre	5	12.00	R	PU	26
D24.4	Efforts towards the isolation and characterization of Nocardia from infected meagre	1	6.00	R	PU	36
D24.5	The effect of high plant protein diets in the development of Systemic Granulomatosis in meagre	1	13.00	R	PU	36
D24.6	Testing of commercial Vibrio vaccine	1	5.00	R	PU	42
D24.7	Diagnostics protocol for Chronic Ulcerative Dermatopathy in meagre, aetiological factors and solutions	1	11.00	R	PU	44
D24.8	Report on the prevention/treatment of Chronic Ulcerative Dermatopathy in meagre	1	4.00	R	PU	44
D24.9	Determination of effective treatments for common monogenean parasites in meagre	3	10.00	R	PU	48
D24.10	Kinetics of antibody and cytokine production established post-pathogen exposure or stimulation with PAMPs	5	12.00	R	PU	48
D24.11	Recommended levels of pro- and anti-oxidant nutrients to prevent Systemic Granulomatosis in meagre	2	6.00	R	PU	54

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D24.12	Determination of the efficacy of vaccination of meagre against Vibriosis	3	5.00	R	PU	54
D24.13	Description of immune gene expression post-immunisation and challenge of meagre with a Vibrio vaccine	3	6.00	R	PU	54
D24.14	Diagnostics protocol for Systemic Granulomatosis, causes and solutions in meagre	1	14.40	R	PU	54
D24.15	Report on the prevention/treatment of Systemic Granulomatosis in meagre	1	10.00	R	PU	54
D24.16	Report of the major bacterial and viral diseases found in meagre, and where useful treatments have been developed, complete protocols for their implementation by the industry will be provided	2	6.00	R	PU	57
D24.17	Diagnostic-recommendation manual for meagre fish health	1	6.00	R	PU	57
		Total	148.40			

## Description of deliverables

D24.1) The effect of vitamin D inclusions in diets in the development of Systemic Granulomatosis in meagre: The deliverable will be a report where the effects of vitamin D inclusion will be evaluated together with the responses of the specific biomarkers CYP27 and CYP24 which are involved in vitamin D metabolism as well as the antioxidant enzymatic activities of SOD, CAT and GSH content, in respect to the development of granulomatosis. [month 20]

D24.2) The effect of Ca/P ratio in the diet in the development of Systemic Granulomatosis in meagre: The deliverable will present evidence whether or not Ca/P ratio in the diet affects the appearance of Systemic Granulomatosis in meagre. Under the different dietary regimes the development or not of Systemic Granulomatosis will be presented over time. In addition the deliverable will provide data on specific growth rate of fish and feed:gain ratio, feed consumption as well as body indexes (hepatosomatic and viscerosomatic) and blood biochemistry data in combination with the responses of the specific biomarkers CYP27 and CYP24 (which are regulated by dietary P intake levels). [month 24]

D24.3) Cloning of key marker genes of innate and adaptive immune responses in meagre: The innate and adaptive immune response can only be exploited for the health benefit of animals in culture when it is understood in its proper context. Immune memory in particular, as part of the adaptive immune response, is an aspect of immunity that is important for the implementation of vaccines. For a vaccine to be useful it needs to be administered after a period at which the immune system is sufficiently mature to have memory cells which can be activated. However to achieve this understanding markers of the ontogenetic development of the immune system will be identified for studying when and how the immune system develops for the proper administration of vaccines. The cloned genes will be sequenced for identifying useful sites within the sequence for development of gene expression assays for the study of the functioning of the immune system. [month 26]

D24.4) Efforts towards the isolation and characterization of Nocardia from infected meagre: The deliverable is a report on the findings of the diversity of Nocardia infection in cage-cultured meagre in Greece and Spain and

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will include the microbiological, biochemical and genetic characterization of the Nocardia isolates together with the epidemiological data obtained from the affected fish farms towards the development of a Nocardia vaccine based on the most appropriate and virulent strain. [month 36]

D24.5) The effect of high plant protein diets in the development of Systemic Granulomatosis in meagre: The deliverable will present evidence whether or not a high plant protein diet affects the appearance of Systemic Granulomatosis in meagre. Under the different dietary regimes the development or not of Systemic Granulomatosis will be presented over time. In addition the deliverable will provide data on specific growth rate of fish and feed:gain ratio, feed consumption as well as body indexes (hepatosomatic and viscerosomatic) and blood biochemistry data. The effect of plant diet on liver and gut histology will be presented. [month 36]

D24.6) Testing of commercial Vibrio vaccine: A commercially available Vibrio vaccine will be tested in meagre. [month 42]

D24.7) Diagnostics protocol for Chronic Ulcerative Dermatopathy in meagre, aetiological factors and solutions: Chronic Ulcerative Dermatopathy is a newly described disease affecting cultured meagre. The disease results in severe ulceration of the lateral line canals of the head and the trunk of the fish, when they are cultured in borehole water. Following rearing trials in natural seawater and borehole water we will describe the disease and we will investigate the aetiological factors and possible solutions. The deliverable is a report that will contain the information about the disease development and manifestation and its correlation with specific water quality parameters such as pH, CO<sub>2</sub> and selected heavy metals. The description of the pathology will be based on histological and histochemical techniques. [month 44]

D24.8) Report on the prevention/treatment of Chronic Ulcerative Dermatopathy in meagre: Based on the results from the study of the Chronic Ulcerative dermatotopathy, this deliverable will comprise a set of recommendations for the prevention and treatment of the disease. [month 44]

D24.9) Determination of effective treatments for common monogenean parasites in meagre: A range finding test will have been performed to evaluate the tolerance of the fish to each chemical product considered a potential treatment for parasites such as *Sciaenacotyle panceri*. Following administration by medicated feed or medicated bath, histological analysis will have confirmed any damage caused by the treatments and plasma cortisol, glucose and lactate will have been measured as general stress indicators. [month 48]

D24.10) Kinetics of antibody and cytokine production established post-pathogen exposure or stimulation with PAMPs: This deliverable will determine the ability of meagre to respond to common pathogen associated molecular patterns (PAMPs) as a means to evaluate the magnitude of the responses seen and the kinetics of the responses. Data will be collected on responses to killed bacteria, and the PAMPs LPS, polyI:C and beta-glucan, with assays optimised to detect antibody level and key immune gene expression, the latter based on the results of D24.3. [month 48]

D24.11) Recommended levels of pro- and anti-oxidant nutrients to prevent Systemic Granulomatosis in meagre: The deliverable will present evidence whether or not the balance pro/anti-oxidant nutrients in diet affects the appearance of Systemic Granulomatosis in meagre. A description on the occurrence of systemic Granulomatosis will be done under the different dietary regimes will be presented. [month 54]

D24.12) Determination of the efficacy of vaccination of meagre against Vibriosis: For evaluation of the efficacy of a vaccine it is necessary to run a trial experiment to determine if the vaccine has provided sufficient and specific stimulus of the immune memory to provide protection against subsequent infection. We will use growth curve data acquired in deliverable D24.4 to perform an experiment to determine the lethal dose needed to kill half of infected individuals (LD<sub>50</sub>) and this dosage will be used for challenging the meagre post-vaccination. Survivors will be counted to determine the efficacy of the vaccine. Portions of the tissues displaying lesions or granulomas will also have been used to re-isolate the bacterial challenge organism to fulfil Koch's postulates. [month 54]

D24.13) Description of immune gene expression post-immunisation and challenge of meagre with a Vibrio vaccine: An important aspect of vaccine development is evaluation of safety but also efficacy. As a part of the vaccine evaluation we will be using the genetic markers developed in deliverable D24.3 to monitor the immune response of fish challenged with a commercial Vibrio vaccine. We will examine markers of immune response, both innate and adaptive, from samples collected of spleen head kidney and peripheral blood (key tissues of the adaptive immune system) pre-challenge and post-challenge to evaluate how effectively the vaccine stimulates an immune response. The pre challenge will also include a time series of samples collected during juvenile development to examine how the maturation of the immune system alters specific expression of certain genes. [month 54]

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D24.14) Diagnostics protocol for Systemic Granulomatosis, causes and solutions in meagre: Systemic granulomatosis is the most significant disease affecting almost every cultured meager. The disease is characterized by multiple visceral granulomas that progressively develop extensive tissue calcification. This deliverable contains the pathological assessment performed by two partners, HCMR and UPLGC on various fish samples derived from the nutritional experiments performed in the previous task of the WP. The assessment will be based on visual inspection, histopathology, Electron microscopy but also on the assessment of selected blood biochemical parameters. [month 54]

D24.15) Report on the prevention/treatment of Systemic Granulomatosis in meagre: Based on the results from the study of the systemic granulomas, this deliverable will comprise a set of recommendations for the prevention and treatment of the disease. [month 54]

D24.16) Report of the major bacterial and viral diseases found in meagre, and where useful treatments have been developed, complete protocols for their implementation by the industry will be provided: A compilation will be done of the potential bacterial/viral diseases in meagre related with annual seasonality. The deliverable will present the description of diagnosis tools of the different bacterial/viral diseases of natural occurrence in the meagre. In addition, the deliverable will include a detailed description of the symptoms found after challenge test against the most common pathogens, as a tool for diagnosis of those diseases. All those results will be compiled as a diagnosis manual for the major bacterial and viral diseases found in meagre. Finally, this manual will include developed protocols for the implementation of useful treatments, which could be implemented by the aquaculture industry [month 57]

D24.17) Diagnostic-recommendation manual for meagre fish health: This will be a practical diagnostic manual and recommendation guide for meager health issues targeted to fish health specialists and aquaculture scientists and producers. The manual will be the synopsis of major findings of WP24. It will be published in electronic format (pdf file) and uploaded in the project website, and will be freely available for the public. The manual will be organized in chapters describing the major diseases of the species with original photographic material, epidemiological and pathological data. Responsible for the compilation and organization of the manual will be HCMR, and all partners involved will contribute according to their participation with chapters, photographic material, diagnostic keys etc. [month 57]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS51	Design of primers for amplification of meagre target gene DNA sequences	5	12	
MS52	Grow-out of larvae and collection of samples from immune ontogeny time-line	5	24	
MS53	Amplification and sequencing of target gene sequences from stimulated tissues	5	30	
MS54	Completion of challenge and collection of samples for study of immune gene modulation	5	36	
MS55	Complete preparation of cDNA synthesis from all meagre samples	5	40	
MS56	Complete gene expression analysis for immune ontogeny	5	42	
MS57	Complete gene expression analysis for immune stimulus /response	5	45	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP25	Type of activity <sup>54</sup>	RTD
Work package title	Fish health - greater amberjack		
Start month	6		
End month	57		
Lead beneficiary number <sup>55</sup>	5		

## Objectives

1. Provide early diagnosis tools for Epitheliocystis,
2. Develop “antiparasite diets” to be used prior to sea cage culture,
3. Begin characterisation of the immune system, with a focus on mucosal (skin/gill) defences,
4. Develop anti-monogenean parasites infection rearing protocols.
5. Develop diagnostic-prevention-treatment methods for diseases in greater amberjack.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviation)

This WP will address a number of bottlenecks relating to amberjack disease control. The tasks include development of (a) dietary regimes that improve larval and adult disease resistance, (b) diagnostic tests for several major pathogens, and (c) immune markers to aid selection of resistance, with a focus on mucosal defences.

EU Budget allocation: 40,000€ (HCMR), 160,000 € (FCPCT), 94,508€ (UNIABDN), 20,000€ (IEO) and 32,000€ (ULL).

Task 25.1. Study of Epitheliocystis during larval rearing (led by HCMR). This is an infectious disease recorded in greater amberjack larvae. It is caused by Chlamydia-related organisms and is considered mostly as benign, since it is associated with low if any mortality when it is detected in juvenile or adult fish. Epitheliocystis, however is a fatal disease when it occurs at early stages with mortalities reaching 100% overnight (Katharios, 2008); in these incidences epitheliocystis may be misdiagnosed due to the rapid deterioration of the delicate fish larvae. Previous experience with greater amberjack larval has shown that epitheliocystis is the major pathological problem at the early stages, especially when mesocosm technology is used. Therefore development of early diagnosis methodology is essential. This methodology will include, firstly, sampling of rearing water, live feeds and fish during mesocosm rearing of greater amberjack, in order to screen for Chlamydia (HCMR). Water samples from rearing water (HCMR) will be fractionated using serial size pore meshes. Water fractions, live feeds and fish larvae will be screened with specific primers for Chlamydia. Validation of epitheliocystis disease will be performed using PCR, histology and/or in situ immunofluorescence with specific antibodies. Early diagnosis tools will be developed (HCMR) using new molecular probes that allow early detection of the pathogenic strains, based on the sequences that will be obtained from the Chlamydia screening. These probes will be applied during the mesocosm larval rearing (HCMR) under WP4.2 Larval husbandry – greater amberjack as an early diagnostic tool. The efficacy of the new tool will be assessed by screening water samples and monitoring fish for Chlamydia infection in mesocosm rearing greater amberjack. The deliverables associated with this task are D25.2 Mucus defences of greater amberjack analysed and immune potential characterized (FCPCT) and D25.4 Protocol for early diagnosis of epitheliocystis during early stages of greater amberjack culture (HCMR).

Task 25.2. Promoting resistance to parasitic incidence on greater amberjack (led by FCPCT). A number of parasitic diseases occur during on-growing of amberjack juveniles, inducing growth retardation, low feed efficiency, immune-suppression and increased mortality. Parasitic infection is difficult to control in sea cages in an effective way. One potential effective way to improve resistance to parasitic infection is through the increase of mucus efficacy and immune potential using dietary mucus stimulation products. In addition, an

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increase of knowledge on the physiological and physical barriers for parasites will help increase the efficacy of the treatments. Different levels of mucus stimulation products, such as some prebiotics (MOS, beta- glucans) or certain phytobiotics, will be studied during the early on-growing of juvenile amberjack under controlled conditions. Fish health will be monitored and samples will be provided for Task 25.3. Growth, survival and immune markers (those determined in Task 25.3) will be monitored. Resistance to opportunistic pathogens and to monogenean infection will be also conducted with infection experiments by cohabitation with monogenean parasites infected animals (*Benedenia seriolae* or the most frequent parasite found in task 25.5). Morphometric and ultrastructure of target tissues (i.e. gills, intestine, etc), including several morphometric variables, will be studied and evaluated by image analysis. These will include height of folds, goblet cell density, lamina propria width (light microscopy studies), branchial arches, etc. Electron microscopy studies prior and post infection with parasites will be undertaken, to determine the immune potential of mucus after treatments with mucus stimulatory products. This Task will result in deliverable D25.5 Impact of oral administration of greater amberjack with mucus stimulation products on immune resistance to parasitic infections and development of molecular markers for its evaluation.

Task 25.3. Identification of immune markers (led by UNIABDN). A number of important immune molecules that are essential for initiating and effecting mucosal defences, including IL-17 and IL-22, antimicrobial peptides, iNOS and IgT will be cloned (FCPCT, UNIABDN). The impact of the different dietary treatments on expression of these molecules will be examined at mucosal sites and during development. Ways to induce these molecules will also be studied using gill leucocyte primary cultures and/or gill explants, as a key mucosal tissue affected by these diseases. Lastly, the cutaneous mucus layer itself will be analysed and its immune potential characterised in terms of antimicrobial activity, protease activity, presence of lectins and immunoglobulins. This Task will result in deliverables D25.1 Marker genes of mucosal immunity in greater amberjack cloned and ways to increase their expression level determined and D25.5 Impact of different dietary regimes in greater amberjack on immune marker gene expression determined. The Task will also contribute to deliverable D25.2 Mucus defences of greater amberjack analysed and immune potential characterised.

Task 25.4. Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites (led by IEO). The monogenean parasites (*Benedenia seriolae* and *Zeuxaptaseriolae*) cause high mortalities in cultures of *Seriola* sp. The implementation of measures, including biosecurity practices, inhibition of oncomiracidia attaching capacity, as well as reinforcement of the fish immune system against stressful rearing conditions must be developed to obtain uninfected fish. PIT-tagged juveniles of greater amberjack reared in two different stocking densities will be subjected (or not) to baths of lectin type anti-oncomiracidia attaching substances. Fish growth and hematological and biochemical indicators of health and welfare (triglycerides, cholesterol, protein, enzymes (GPT, GOT, alkaline phosphatase, cholinesterase and amylase) as well as cortisol, glucose, lactate, osmolality and electrolytes) will be studied (IEO). Tank collector devices will be placed, and regularly checked, for evaluation of presence and density of parasites (IEO). At the beginning and end of the trials, a representative number of fish will be killed to establish the intensity and sites preference of infection. Isolation of cells from skin, gills and gut for viability and integrity evaluation as well as ATPase activity studies of osmoregulatory epithelia will be performed (ULL). This task will result in deliverable D25.6 Rearing protocol against monogenean parasites.

Task 25.5. Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in amberjack (led by FCPCT). Increase in fish production, particularly in fast growing species such as greater amberjack, may lead to the emergence of bacterial diseases such as vibriosis or photobacteriosis, lactococcosis, streptococcosis and some viral diseases such as, nodavirus, limphocystis, iridovirus and aquabinnavirus disease. In this task, greater amberjack will be monitored throughout the year to establish the seasonality of the potential diseases. External morphology, necropsies and samples of liver, spleen, kidney, gut and nervous system will be studied to determine the presence of pathogenic organisms and histopathological damage to the different organs (FCPCT). These samples will be processed for determination of bacterial diseases by classical microbiological techniques, and by PCR. For viral diseases cell cultures suited to each disease (eg SS-1, SAF-1) will be used for virus propagation, following by RT-PCR or PCR verification depending on the specific virus. Susceptibility of greater amberjack will be set against field and reference strains of the following pathogens: *Vibrio anguillarum*, *Photobacterium damsela* subsp. *piscicida*, and betanodavirus. Susceptibility values will be set by challenge test (intraperitoneal or intramuscular injections for bacteria or nodavirus respectively). These studies will be conducted in the biosecurity station of the FCPCT. When outbreaks of disease occur, methods will be established to determine the causal agent of the disease and attempt to find appropriate preventive and therapeutic measures for control. Specific diagnosis protocols will range from molecular (PCR, RT-PCR) to antibody based (ELISA, Immunohistochemistry, Immunofluorescence) methods. Treatment protocols will be

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established with selected antibiotics (depending on the limitation of their use) for each pathogen isolated from natural outbreaks of disease, by studying the MICs of the different pathogens, as well as the best routes of administration, depending on the type of fish (age, type, etc.). The results obtained from this task will produce the deliverable D25.7 Report on the major bacterial and viral diseases found in greater amberjack, and where useful treatments have been developed, complete protocols for their implementation by the industry will be provided

Task 25.6 Diagnostic-recommendation manual for greater amberjack health (led by HCMR). This will be a practical diagnostic manual and recommendation guide for greater amberjack health issues targeted to fish health specialists and aquaculture scientists and producers. The manual will be the synopsis of major findings of WP24. It will be published in electronic format (pdf file) and uploaded in the project website, and will be freely available for the public. The manual will be organized in chapters describing the major diseases of the species with original photographic material, epidemiological and pathological data. Responsible for the compilation and organization of the manual will be HCMR, and all partners involved (FCPCT, UNIABDN, IEO, ULL) will contribute according to their participation with chapters, photographic material, diagnostic keys etc. This task will result in deliverable D24.17. Diagnostic-recommendation manual for greater amberjack fish health.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	17.00
2	FCPCT	55.00
5	UNIABDN	17.00
8	IEO	1.10
15	ULL	5.00
	Total	95.10

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D25.1	Marker genes of mucosal immunity in greater amberjack cloned and ways to increase their expression level determined	5	16.00	R	PU	39
D25.2	Mucus defences of greater amberjack analysed and immune potential characterised	2	7.00	R	PU	39
D25.3	Impact of dietary regime on parasite resistance and mucosal defences of greater amberjack juveniles	5	20.00	R	PU	42
D25.4	Protocol for early diagnosis of epitheliocystis during early stages of greater amberjack culture	1	8.00	R	PU	44
D25.5	Impact of oral administration of greater amberjack with mucus stimulation products on immune resistance to parasitic infections and	2	28.00	R	PU	57



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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	development of molecular markers for its evaluation					
D25.6	Rearing protocol against monogenean parasites	8	6.10	R	PU	57
D25.7	Report on the major bacterial and viral diseases found in greater amberjack, and where useful treatments have been developed, complete protocols for their implementation by the industry will be provided	2	5.00	R	PU	57
D25.8	Diagnostic-recommendation manual for greater amberjack fish health	1	5.00	R	PU	57
<b>Total</b>			<b>95.10</b>			

## Description of deliverables

D25.1) Marker genes of mucosal immunity in greater amberjack cloned and ways to increase their expression level determined: Key immune molecules effecting mucosal defences will be cloned and sequenced, with targets including IL-17 and IL-22, antimicrobial peptides, iNOS and IgT. The impact of the different dietary treatments on expression of these molecules will have been determined. The potential to induce these molecules will have been established using gill leucocyte primary cultures and/or gill explants. [month 39]

D25.2) Mucus defences of greater amberjack analysed and immune potential characterised: The immune potential of the cutaneous mucus layer will have been determined, in terms of its antimicrobial activity, protease activity, and presence of lectins and immunoglobulins. [month 39]

D25.3) Impact of dietary regime on parasite resistance and mucosal defences of greater amberjack juveniles: The deliverable will present evidence whether the inclusion of Mucus Stimulation Ingredients increases the resistance to parasitic infection in amberjack juveniles. The incidence of parasitic infection by cohabiting fish and preference of site of infection will be determined. The immune potential of the mucosal defenses will be also determined. [month 42]

D25.4) Protocol for early diagnosis of epitheliocystis during early stages of greater amberjack culture: A method will be developed for the early diagnosis of epitheliocystis in greater amberjack larval mesocosm tanks. The method includes the development of specific molecular probes that cover the diversity of chlamydia-like organisms that are responsible for epitheliocystis disease. The method will be applied during the larval rearing trials and the deliverable will be a report with the description of the tools and the results obtained following their application. [month 44]

D25.5) Impact of oral administration of greater amberjack with mucus stimulation products on immune resistance to parasitic infections and development of molecular markers for its evaluation: The deliverable will present evidence whether the inclusion of Mucus Stimulation Ingredients modulates the gene expression of selected immune markers. The idoneous immune markers will be selected. [month 57]

D25.6) Rearing protocol against monogenean parasites: A treatment protocol against monogenean parasites which infect and cause mortalities in greater amberjack, based in the use of anti-oncomiracidia attaching substances will be developed. The results of the trials with two different stock densities subjected or not to antiparasitic treatment will be presented including (a) intensity and sites preference of infection, (b) survival, (c) fish growth, (d) haematological, immunological and biochemical indicators of health and welfare. In addition, results of the evaluation of the viability and integrity as well as ATPase activity of osmorregulatory epithelia (skin, gills and gut) will be presented. [month 57]

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D25.7) Report on the major bacterial and viral diseases found in greater amberjack, and where useful treatments have been developed, complete protocols for their implementation by the industry will be provided. A compilation will be done of the potential bacterial/viral diseases in greater amberjack related with annual seasonality. The deliverable will present the description and diagnosis of the different bacterial/viral diseases of natural occurrence in greater amberjack. In addition, a detailed study on the symptoms after challenge test against the most common pathogens will provide important tools for diagnosis of the incidence of those diseases. All those results will be compiled as a diagnosis manual for the major bacterial and viral diseases found in greater amberjack. Finally, this manual will include the developed protocols for the implementation of useful treatments, which will be available to the aquaculture industry. [month 57]

D25.8) Diagnostic-recommendation manual for greater amberjack fish health: This will be a practical diagnostic manual and recommendation guide for greater amberjack health issues targeted to fish health specialists and aquaculture scientists and producers. The manual will be the synopsis of major findings of WP24. It will be published in electronic format (pdf file) and uploaded in the project website, and will be freely available for the public. The manual will be organized in chapters describing the major diseases of the species with original photographic material, epidemiological and pathological data. Responsible for the compilation and organization of the manual will be HCMR, and all partners involved will contribute according to their participation with chapters, photographic material, diagnostic keys etc [month 57]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS58	Design of primers for amplification of amberjack target gene DNA sequences	5	18	
MS59	Successful Chlamydia screening and sequencing	5	30	
MS60	Samples collected from stimulated primary cultures/explants, ready for immune gene expression analysis	5	30	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP26	Type of activity <sup>54</sup>	RTD
Work package title	Fish health - Atlantic halibut		
Start month	4		
End month	40		
Lead beneficiary number <sup>55</sup>	7		

## Objectives

1. Determine the effect of delivering recombinant capsid protein during late larval stages on protection to nodavirus (VNN).

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviation)

This WP will address a key bottleneck relating to Atlantic halibut larval health, namely nodavirus infections (Viral Neural Necrosis, VNN) outbreaks in larval and juvenile stages. The task includes (a) production of the VNN capsid protein in *E. coli*, tobacco plants and possibly microalgae, and (b) oral delivery of the recombinant capsid protein in *Artemia* to late larval stages, and (c) assessment of the degree of protection obtained with the different formulations, assessed by histology and immunohistochemistry with antibodies to NVV. This WP will liaise closely with the TargetFish programme (EU 7th FP), where VNN vaccines are being developed for use in other farmed fish species (see letter from TargetFish coordinator).

EU Budget allocation: 87,481€ (IMR).

Task 26.1 Production of VNN capsid protein (led by IMR). VNN outbreaks are mainly observed during early larval and juvenile stages of Atlantic halibut, when the immune system is not fully developed, and thus developing vaccines against this virus is especially challenging. We have several vaccine candidates: A vaccine with recombinant nodavirus capsid protein expressed in *E. coli*, which has previously been shown to elicit protection in turbot (*Scophthalmus maximus*), and to induce an immune response in Atlantic halibut with a protective character. Recombinant capsid protein has also been expressed in tobacco plants, from which it can be isolated as virus like particles (VLP) for integration in a vaccine. This is interesting as the plant (as a eukaryote), will presumably give post-translational modifications more in line with virus propagated in fish cells. We will work with two other eukaryotic expression systems; microalgae and a protozoa (*Leishmania tarentolae*), both suited for integration in an oral vaccine system. At least the two former will be tested in this project. A formulation of the capsid protein of betanodavirus expressed recombinantly in *E. coli* has been shown to induce certain extent of protection in juvenile halibut (Øvergård et al., 2013). We will test the system for delivery of vaccine candidates through *Artemia* to Atlantic halibut larvae during late larval stages and the larvae will be challenged with VNN during early juvenile stages to assess for protection. This Task will result in deliverables D26.1 Assess the use of two eukaryotic expression systems; microalgae and a protozoa (*Leishmania tarentolae*) for production of nodavirus capsid protein and D26.2 Testing of the delivery of vaccine candidates through *Artemia* to halibut larvae.

Task 26.2 Monitor and assess immune response and protection (led by IMR). We will monitor specific immune responses, viral load and possibly mortality at various time points following vaccination and further challenge with nodavirus. Samples will be taken after VNN exposure and examined by histology and immunohistochemistry for the presence of virus using specific antibodies against VNN. Since it is difficult to collect enough blood from juveniles in their early phase just after end metamorphosis, we will only concentrate on characterisation of specific cell mediated response by analysing a range cytokines associated with T cell responses (such as IL-12 $\beta$ , IFN- $\gamma$ , IL-1 $\beta$ ) using qPCR assays for detection of virus and immune related genes that have already been established (Patel et al., 2008, 2009; Øvergård et al., 2009, 2012). Brain samples will be

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analysed for viral load. This Task will result in deliverable D26.3 Determine immune response and effectiveness of orally delivered VNN capsid protein on protection of halibut larvae.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
7	IMR	4.16
Total		4.16

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D26.1	Assess the use of two eukaryotic expression systems; microalgae and a protozoa ( <i>Leishmania tarentolae</i> ) for production of nodavirus capsid protein	7	1.02	R	PU	24
D26.2	Testing of the delivery of vaccine candidates through <i>Artemia</i> to Atlantic halibut larvae	7	1.40	R	PU	36
D26.3	Determine immune response and effectiveness of orally delivered VNN capsid protein on protection of Atlantic halibut larvae	7	1.74	R	PU	40
Total			4.16			

## Description of deliverables

D26.1) Assess the use of two eukaryotic expression systems; microalgae and a protozoa (*Leishmania tarentolae*) for production of nodavirus capsid protein: We will work on several alternatives for the expression of nodavirus capsid protein in this task. We have two vaccine candidates: A vaccine with recombinant nodavirus capsid protein expressed in *E.coli*, which previously has been shown to elicit protection in turbot, and to induce an immune response in Atlantic halibut with a protective character. Recombinant capsid protein has also been expressed in tobacco plants, from which it can be isolated as virus like particles (VLP) for integration in a vaccine. Production of capsid protein using two other eukaryotic expression systems; microalgae and a protozoa (*Leishmania tarentolae*), both which will be suited for integration in an oral vaccine system will be assessed. [month 24]

D26.2) Testing of the delivery of vaccine candidates through *Artemia* to Atlantic halibut larvae: Uptake of recombinant capsid protein expressed either in *E.coli*, tobacco plants or by the two eukaryotic expression systems into *Artemia* and further to late larval stages of Atlantic halibut will be assessed. If the protein is effectively delivered and uptake of protein can be successfully determined, then this delivery system will be optimised. [month 36]

D26.3) Determine immune response and effectiveness of orally delivered VNN capsid protein on protection of Atlantic halibut larvae: Uptake of recombinant capsid protein expressed either in *E.coli*, tobacco plants or by the two eukaryotic expression systems into *Artemia* and further to late larval stages of Atlantic halibut will be assessed. If the protein is effectively delivered and uptake of protein can be successfully determined, then this delivery system will be optimised. [month 40]

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## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP27	Type of activity <sup>54</sup>	RTD
Work package title	Socioeconomics – Institutional and organizational context		
Start month	1		
End month	12		
Lead beneficiary number <sup>55</sup>	6		

## Objectives

1. To give insight in the competitive field of and market developments in the European aquaculture market with a focus on the species selected in DIVERSIFY (meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet),
2. To assess the obstacles for growth in the current aquaculture production chains and for these selected species,
3. To identify market opportunities for future growth of the European aquaculture sector for the selected species.
4. Propose a certification framework for the species addressed in DIVERSIFY

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

In this work package the institutional and organizational context will be analysed. This will be done by looking at the macro-environmental and micro-environmental (competitive) factors that influence supply and demand in the aquaculture production chains in general, and the chains of the considered species that are currently in production (meagre, halibut, pikeperch and mullet) or are supplied by the capture fishery (wreckfish and greater amberjack). This analysis will serve as the basis of WPs 28, 29 and 30, since they define the opportunities and threats for product development, market development and the development of successful new product marketing strategies and the feasibility study.

EU Budget allocation: 68,000€ (SWR/DLO), 40,776€ (TU/e), 20,000€ (AU) and 40,037€ (APROMAR)

Task 27.1 External environmental analysis (led by SWR/DLO)

Sub-task 27.1.1 (SWR/DLO) With the PESTEL-model (Gillespie, 2011) the social, technological, economic, ecological and political context of European aquaculture is analysed with a focus on the selected species. This validated methodology provides a framework of macro-environmental factors that affect or will affect the production chains of amberjack, halibut, meagre, mullet, pikeperch and wreckfish. This Sub-task will result in Deliverable D27.1 Report on external environmental factors that affect or will affect the production chains of meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet.

Sub-task 27.1.2 (SWR/DLO) Analysis of current certification schemes and standards and their business dynamics in the different domestic and international supply chains (SWR/DLO, APROMAR). In order to get insight in the level playing field of the selected species in relation to wild fish and similar species from other world regions, insight in certification schemes and standards are necessary. This Sub-task will lead to Deliverable D27.2 Report on current certification schemes and standards and their business dynamics in the fish supply chain and on potential interesting certification schemes for the species in Diversify.

Task 27.2 Competitive analysis (led by SWR/DLO)

Sub-task 27.2.1 (SWR/DLO) With the Porter five forces model (Porter, 1985; Porter, 1998), a competitive analysis will be carried out for the selected species. For each production chain the current suppliers, customers and markets, substitutes and potential entrants in the market will be described. These five forces provide insight in the market structure and competitive situation of the five selected species in the market (incl. different products and market segments). This Sub-task will result in deliverable D27.3 Report on competitive analysis for the supply chains of meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet.

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Sub-task 27.2.2 (SWR/DLO) Short- and long-term trend mapping (less/more than 5 years) will be carried out to identify the main trends for European aquaculture, the seafood sector and the protein market in the near future (SWR/DLO, APROMAR). These trends will focus on the selected species, cultured and captured fish, seafood in general and meat and other novel proteins. This study includes consumption trends (incl. quality standards and certification schemes), trends in product development and value-addition within the European seafood sector and the global seafood market. The results of the trend mapping will be presented in deliverable D27.4 Report on trend mapping for the European aquaculture, seafood sector and protein market in the (near) future.

Sub-task 27.2.3 (SWR/DLO) International survey in selected countries (UK, France, Spain, Germany and Italy) to investigate the industrial buyers preferences (i.e., purchase managers of up to 10 retailers and food service companies will be interviewed in each country), in order to gain insights into their buying criteria, cultured fish perception (SWR/DLO, TU/e). More specific, industrial buyers' preferences in terms of country of origin, suppliers, production circumstances, types of products, pricing and quality will be covered in the survey. These insights have to identify dimensions for expansion of the category. Deliverable D27.5 will present the results of the international survey on industrial buyers' attitudes and perceptions regarding cultured fish.

Task 27.3 Opportunities and barriers for growth (led by SWR/DLO )

Sub-task 27.3.1 (SWR/DLO) A success-failure study of comparative cases will be carried out in order to identify critical success factors for market acceptance, given the legal, organizational, competitive and trend context as analysed in Tasks 27.1 and 27.2. (SWR/DLO, APROMAR, AU). This Sub-task will result in D27.6 List of critical success factors for market acceptance.

Sub-task 27.3.2 (SWR/DLO) Using the Business Model Canvas approach (Osterwalder & Pigneur, 2010), a business model and supply chain analysis of the participating SME's will be made, by organizing a workshop, in order to identify the presence or absence of the identified critical success factors and opportunities for improvement (SWR/DLO, TU/e). At the end of the project (in WP30), this analysis will allow an assessment of the contribution of project innovations, to the competitiveness of the aquaculture sector (SWR/DLO, APROMAR, AU). The final outcome of WP 27 will be made available for decision-making purposes in the activities provision further in WP 28 and 29 and will be presented in a report on the analysis of the business models and supply chains of the participating SME's (Deliverable D27.7)

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
6	DLO	7.00
10	TU/e	5.50
11	AU	2.15
12	APROMAR	9.00
	Total	23.65

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D27.1	Report on external environmental factors that affect or will affect the production chains of meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet	6	1.00	R	PP	3
D27.2	Report on current certification schemes and standards and their	6	5.00	R	PP	3

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	business dynamics in the fish supply chain					
D27.3	Report on competitive analysis for the supply chains of meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet	6	2.00	R	PU	12
D27.4	Report on trend mapping for the European aquaculture, seafood sector and protein market in the (near) future	6	5.00	R	PU	12
D27.5	Report with results of international survey on industrial buyers' attitudes and perceptions regarding cultured fish	6	4.00	R	PU	12
D27.6	List of critical success factors for market acceptance	6	3.00	R	PU	12
D27.7	Report on the analysis of the business models and supply chains of the participating SME's	6	3.65	R	PU	12
Total			23.65			

## Description of deliverables

D27.1) Report on external environmental factors that affect or will affect the production chains of meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet: This report will present the external factors that influence the market for aquaculture on basis of desk research en expert knowledge. As external factors will be taken into account: technical factors (state of the art of processing procedures in the EU in relation to the rest of the world, development of investments and the development of regulation regarding processing), legal factors (EU legislation on production and imports and exports of aquaculture), economic factors (like civilian income distribution in the EU and income distribution in the sector), demographic developments in the EU, development in the natural environment (availability and needs of natural resources for production), political developments regarding aquaculture and cultural developments in consumption of aquaculture. [month 3]

D27.2) Report on current certification schemes and standards and their business dynamics in the fish supply chain: In this report an overview of certification schemes and standards relevant for the aquaculture sector will be presented based on desk research and expert knowledge. Per certification scheme will the following information will be presented: background of the scheme, claims, implementation and the results. [month 3]

D27.3) Report on competitive analysis for the supply chains of meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet: With the five forces model of Porter model the competitive situation in the aquaculture sector will be described. In this model the competitive rivalry within the industry is described and determined on basis of four other forces: bargaining power of supplier, bargaining power of consumers, threat of new entrants and threat of substitute products. All these elements are described detailed on basis of expert knowledge and desk research. [month 12]

D27.4) Report on trend mapping for the European aquaculture, seafood sector and protein market in the (near) future: The trend analysis in this deliverable will present the major trends in consumption of fish in general and more specific on aquaculture. The results will be based on consumption data and trend reports of trend watchers and expert opinions. [month 12]



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D27.5) Report with results of international survey on industrial buyers' attitudes and perceptions regarding cultured fish: This report presents the results of an international survey of retailers and traders in aquaculture. In the report the methodology of the survey will be presented as well as the expected trends in this market, the buying factors, the buying behavior, patterns in the market, identifiable retail and consumer segments, perception of the product portfolio and strengths and weaknesses of the product portfolio. [month 12]

D27.6) List of critical success factors for market acceptance: In this report an analysis will the outcomes of the previous deliverables in WP 27 (D27.1 to D27.5) will be brought together in a summary of the most important success factors for new product entries in the market of fish and aquaculture. [month 12]

D27.7) Report on the analysis of the business models and supply chains of the participating SME's: In this document the current business models of the envolved SME's will be presented on basis of the Canvas business model theory. These models will be used as input for WP 30, where new strategies are developed for the new products on basis of the new species. [month 12]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
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# WT3: Work package description

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP28	Type of activity <sup>54</sup>	RTD
Work package title	Socioeconomics – New product development		
Start month	13		
End month	58		
Lead beneficiary number <sup>55</sup>	3		

## Objectives

1. To develop new product concepts from selected species, by incorporating consumer and expert input,
2. To select product ideas and develop physical new products from selected species,
3. To monitor the quality of new products in terms of organoleptic characteristics and nutrition-rearing history,
4. To make a technical assessment of the products.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

This work package covers the development of new products from the selected species meagre, greater amberjack, pikeperch and grey mullet. Atlantic halibut will not be taken into account in this work package since the assortment is already broad.

Wreckfish will be taken into account, but since there is no EU cultured product available when this work package is planned, wild wreckfish will be used. Use of wild wreckfish is considered “sub-optimal” in providing clear and reliable data in relation to the future cultured wreckfish. Taking into account the intrinsic quality differences between the wild individuals and the farmed counterparts (when the latter are achieved in the future), there are two issues to address and in the following the methodological approach is presented:

1. To assess the product quality in a global way, i.e. to get to know the characteristic quality features, for the technical quality (Sub-task 28.2.1 somatometric features & technological losses) and the chemical quality (Sub-task 28.3.1). For this reason a range of commercial fish sizes, and fishing regions and seasons will be covered, whenever possible, when assessing the technical and chemical quality of the fish. This way the effects of extrinsic (environmental) parameters on fish quality will be included in the overview.

2. To obtain products of uniform quality (for the subtask 28.2.2 physical prototypes of the product). Fish that will be obtained for product development (28.2.2) and sensory analysis ( ) will be, at a certain degree, uniform in size and biological – fishing characteristics (fishing season, fishing area, freshness). All technical solutions will be used to achieve uniform products (equal sizes, appearance, and nutritional characteristics – mainly fat content).

Based on the previous, the quality of wreckfish will be assessed adequately and new products will be developed, by using wild individuals.

The market insights of WP 27, and the consumer insights of Task 29.1 will be the basis for new product development. The other tasks of WP 29, will get the input from this work package. The results of the technical assessment will be input for WP 30.

EU Budget allocation: 95,302€ (HCMR), 95,000€ (IRTA), 8,000€ (SWR/DLO), 10,000€ (TU/e), 20,000€ (AU), 35,000€ (ULL), 28,320€ (CTAQUA) and 45,020 (HRH)

Task 28.1 Product concept development: technical and consumer-driven (led by AU)

Sub-task 28.1.1 (AU) Qualitative research (i.e., focus groups) with consumers and experts in selected countries (UK, D, ES, F, I) to generate input for new product development (IRTA, SWR/DLO, HRH). The choice for the countries is determined by the following characteristics: largest EU markets for cultured fish (ES, F, I), important growing EU markets for cultured fish (UK, D). This will result in Deliverable D28.1 Report with results of focus groups with consumers and experts regarding ideas for new products.

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Sub-task 28.1.2 (HCMR) Screening of ideas, based on the market data of WP 27 and Sub-task 28.1.1 and using technical, economic and market assessment criteria, among others (HCMR, IRTA, TU/e, AU, SWR/DLO, HRH). The ability of ideas to attract customer demand and generate cash flow will be emphasised, given the objective is to boost the competitiveness of the sector in terms of products and market. This Sub-task will result in Deliverable D28.2 List of ideas for new product development.

## Task 28.2 New Product Development (led by IRTA)

Sub-task 28.2.1 (HCMR) An estimation of optimum fish sizes for developing new products identified in Sub-task 28.1.2 based on basic somatometric measurements and evaluation of losses (HCMR, IRTA, CTAQUA). Further examination of chemical-mechanical properties of fish species during cutting and minimal processing will be performed, which will result in a definition of process solutions for each species based on technological, physical and sensory characteristics (HCMR, IRTA, CTAQUA). This will result in Deliverable D28.3 Report on product and process solutions for each species based on technological, physical and sensory characteristics.

Sub-task 28.2.2 (IRTA) Physical prototypes of new products will be developed from meagre, greater amberjack, pikeperch, wreckfish and grey mullet (IRTA, CTAQUA). On the basis of their organoleptic characteristics, potential products from the specific species (up to three products of varying degree of processing incorporated per species) will be made available for further testing in WP 29. Species and product selection criteria will be decided according to technical processing limitations, fish availability and similar products availability in markets (see WP29 below, and the market potential of the new species as established in WP 27). The final outcome of WP28 will be made available for further consumer acceptance testing in WP 29. These physical prototypes of new products from the selected species meagre, greater amberjack and pikeperch and grey mullet are defined as Deliverable D28.4.

## Task 28.3 Monitoring technical quality of the products (led by HCMR)

Sub-task 28.3.1 (HCMR) A quality evaluation study will be performed on basic quality characteristics of the products developed, which will include somatometric measurement, fat deposition evaluation, proximate fillet composition and fatty acid analysis (HCMR, IRTA, ULL, CTAQUA). Next to that, a sensory descriptive analysis will be done in order to analyse and describe the sensory characteristics of the developed products (HCMR, IRTA). This will result in two deliverables: D28.5 Report on results of quality evaluation study on basic quality characteristics of the developed products; and D28.6 Report on results of sensory descriptive analysis of the developed products.

Sub-task 28.3.2 (ULL) Correlation of technical quality characteristics with previous nutritional - rearing history will take place by studying the quality of different fish groups from varying farming histories (ULL, HCMR). It will provide input for value positioning statement and communication claims. This results in Deliverable D28.7 Report on correlation of technical quality with nutritional - rearing history

Sub-task 28.3.3 (IRTA) On the basis of the results of the former Sub-tasks in WP 28 a technical assessment will be done for all selected species (meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet). In this technical assessment the technical performance of the new products will be concluded from all Sub-tasks before and be related to the technical performance of products already in the market (IRTA, HCMR, SWR/DLO). This technical assessment covers: a) a general description of the products; b) the list of essential characteristics relevant for high quality consumption and intended use of the products; c) the methods and criteria for assessing the performance of the product in relation to those essential characteristics; and d) principles and conditions for the production, control and packaging of the new products to guarantee quality of consumption. This Sub-task will result in Deliverable D28.8 Technical assessment of selected species, which will be part of the feasibility study of Sub-task 30.3.1.

### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	35.00
3	IRTA	18.25
6	DLO	2.85
10	TU/e	0.50

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## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
11	AU	2.15
15	ULL	6.50
18	CTAQUA	7.30
38	HRH	5.30
Total		77.85

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D28.1	Report with results of focus groups with consumers and experts regarding ideas for new products	11	4.00	R	PU	14
D28.2	List of ideas for new product development	1	8.75	R	PU	16
D28.3	Report on product and process solutions for each species based on technological, physical and sensory characteristics	1	20.00	R	PU	18
D28.4	Physical prototypes of new products from the selected species meagre, greater amberjack, wreckfish, pikeperch and grey mullet	3	8.00	R	PU	26
D28.5	Report on results of quality evaluation study on basic quality characteristics of the developed products	1	12.00	R	PU	54
D28.6	Report on results of sensory descriptive analysis of the developed products	1	12.00	R	PU	54
D28.7	Report on correlation of technical quality with nutritional - rearing history	15	6.00	R	PU	54
D28.8	Technical assessment of selected species	1	7.10	R	PP	58
Total			77.85			

## Description of deliverables

D28.1) Report with results of focus groups with consumers and experts regarding ideas for new products: In this report the results of the focus groups with consumers with respect to expectations with regard to the aquaculture market are presented. The report describes the methodology used, the buying factors, the selection criteria, the selection process and the quality expectations of fish and aquaculture products. [month 14]

D28.2) List of ideas for new product development: A combination of the market perceptions(D.28.1), the technical limitations and the economical prospects efficiencies (i.e. within a socio-techno-economic study), will be used to generate a pool of ideas about potential products. Within this report the perspectives of scientists

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## Work package description

from different scientific areas will be included in order to justify feasibility for potential products. The results of this report will feed together with D28.3 to generate the actual physical products (D28.4), i.e. to process the fish respectively. [month 16]

D28.3) Report on product and process solutions for each species based on technological, physical and sensory characteristics: A report will be delivered, where the following will be mentioned: (a) commercial sizes of fish (from the studied species) suitable for processing. (b) which sizes achieve best edible proportions and filleting yields. (c) potential product range for the various commercial sizes. (d) based on the fact that size and composition (especially fat contents) affect the technological properties of the fish, there will be included correlations between these quality attributes and evaluation of crucial parameters influencing the processing yields and the quality of the processed product (e.g. colour, potential gapping, general appearance, texture, water binding capacity & water losses etc.). [month 18]

D28.4) Physical prototypes of new products from the selected species meagre, greater amberjack, wreckfish, pikeperch and grey mullet: Four selected species (meagre, greater amberjack, pikeperch and grey mullet) will be the raw material for new product development. A maximum of 3 physical prototypes per species of new products of varying degree of processing in commercial format (size, packaging, presentation) will be delivered. The physical prototypes will be developed based on: information provided by WP 27 (market potential of the new species), Task 28.1 (product concept development: technical and consumer driven), Task 29.1 (consumer value perceptions and segmentation), physico-chemical characteristics of each raw material, technical properties of the product and the process, and similar product availability in the market. Guidelines to obtain the new products will be provided including inputs, processing conditions, technical specifications, and troubleshooting. In addition, packaging, conservation conditions, product shelf life and consumer handling/cooking specifications will be provided. These new product prototypes will be the input for consumer sensory acceptability evaluation in WP 29. [month 26]

D28.5) Report on results of quality evaluation study on basic quality characteristics of the developed products: The report will refer to the total proximate composition of the products (protein, lipid moisture, inorganic content and carbohydrates), the energy contents of the selected products and the quantitative nutritional value in aspects of fatty acids. [month 54]

D28.6) Report on results of sensory descriptive analysis of the developed products: The report will refer: (a) to the selection and training of panelists that will consist the group of experts evaluating the sensory properties of the products. Selection and training based on specific sensory tests. (b) to the development of vocabulary consisting of specific terms describing the fish products. Terms will be refer to all sensory qualities describing the products (external appearance, odour, taste, flavor, texture, aftertaste). (c) to the main evaluation procedures of the products, including the complete experimental setup. (d) to the rating of the products for each of the attributes (vocabulary terms). A full map of the organoleptic qualities of each product will be presented. Also comparisons between different products will be made were applicable. [month 54]

D28.7) Report on correlation of technical quality with nutritional - rearing history: This report will include both proximate composition and analytic fatty acid profiles of fish received different dietary treatments. The effect of dietary treatment on end product quality will be analyzed within its frames, by correlations between individual quality attributes and the dietary history (e.g. dietary fat and protein levels, or fat sources etc.) or other rearing parameters (e.g. different rearing temperature). The dietary or the environmental parameters that will be correlated to the end product quality for each species, will largely depend on the GWP 5 (Grow out husbandry) and the choices of parameters that will be made within this package. A technical quality evaluation of developed products from selected species will be assessed in relation to farming history. A specific dossier per product, including (a) nutritional value in terms of protein, fat and w-3 contents and (b) sensory characteristics, regarding fish origin, rearing conditions and feeding regime will be presented. [month 54]

D28.8) Technical assessment of selected species: This report will present: a general description of the products; the list of essential characteristics relevant for high quality consumption and intended use of the products; the methods and criteria for assessing the performance of the product in relation to those essential characteristics; principles and conditions for the production, control and packaging of the new products to guarantee quality of consumption. [month 58]

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## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS61	Ideas for new products	1	18	
MS62	Optional physical new products	1	26	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP29	Type of activity <sup>54</sup>	RTD
Work package title	Socioeconomics – Consumer value perceptions and behavioural change		
Start month	1		
End month	44		
Lead beneficiary number <sup>55</sup>	38		

## Objectives

1. To analyse and understand overall value perceptions of consumers with regard to cultured fish in general and the DIVERSIFY fish species in particular, and undertake a value-based segmentation study,
2. To evaluate consumer sensory perceptions towards the newly developed DIVERSIFY species' products,
3. To optimize the DIVERSIFY species' newly developed products in terms of ideal extrinsic product attribute combinations that have the potential to generate ideal consumer value perceptions,
4. To determine the effectiveness of market communication in consumer behaviour change in relation to the DIVERSIFY species considered and the new raw and other value added products developed.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

The analysis of the institutional context in WP 27 and the new product development activity in WP28 are the starting points for the analysis of the consumer value perceptions. This WP will test the new products for consumer acceptability and will incorporate extrinsic quality attributes (i.e., product labelling elements such as quality certification, sustainability and health claims) to define optimal intrinsic-extrinsic product attribute combinations. In addition, WP 29 will provide elements for Sub-taskable product-market combinations for the selected species and identify pricing ceilings per product based on customer value perceptions.

EU Budget allocation: 29,900€ (HCMR), 38,000€ (IRTA), 101,456€ (SWR/DLO), 173,353€ (AU), 50,010€ (CTAQUA) and 175,049 (HRH)

Task 29.1 Consumer value perceptions and segmentation (led by AU).

Sub-task 29.1.1 (SWR/DLO) An international online consumer survey in the 5 countries selected (UK, Germany, Spain, France, Italy) with an n=500 at minimum per country (nationally representative samples) will be conducted to investigate consumers' associations with and perceptions of the new products developed, attitudes towards established and new aquaculture as opposed to wild fish, buying intentions current/future fish consumption, willingness to buy and pay, and overall value perceptions (SWR/DLO, IRTA, HRH). This information will determine consumer perceived value as a trade-off between gains (i.e. benefits) and losses (i.e., sacrifices) from the purchasing/consumption of the examined new products (SWR/DLO, IRTA, AU, HRH). This will result in Deliverable D29.1 Dataset of consumers' perceptions, attitudes, buying intentions, consumption, willingness to buy and pay, and value perceptions towards the selected species

Sub-task 29.1.2 (AU) On the basis of the dataset generated through Sub-task 29.1.1, a segmentation study will be conducted that will give insights into consumer sub-markets (i.e., segments) across and within the 5 countries examined with the highest potential for maximised consumer value perceptions (AU, SWR/DLO, IRTA, HRH). This will be reported in Deliverable D29.2 Segmentation analysis based on consumer value perceptions about the selected species in the five countries investigated.

Task 29.2 Consumer sensory perceptions (led by IRTA). In a hedonic sensory test, consumer sensory perceptions of the new products (from WP 28) will be evaluated in 5 countries (n=80 per country/segment at min). The sensory tests will take place at segments selected in Task 29.1, based on the different potential the new products are expected to have in different national and international segments (HCMR, IRTA, HRH). The products used are provided by Sub-task 28.2.2 and transferred to actual product samples from the selected

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species for the sensory testing with consumers (Deliverable D29.3) in the five countries investigated. By acceptability ratings on visual attractiveness, taste, odour, mouth feeling and some hedonic characteristics, each product will be evaluated in a sensory lab (CTAQUA). This will result in a rating of the most attractive products per segment on sensory grounds and the most desired sensory characteristics for the evaluated products (HCMR). This will be reported in Deliverable D29.4 Report on the actual products' sensory profiling in the five countries investigated.

Task 29.3 Optimization of intrinsic-extrinsic attribute combinations (led by HRH).

Sub-task 29.3.1 (HRH) A number of extrinsic quality attributes (i.e., product labelling elements) will be incorporated into the physical product prototypes developed in WP 28 to develop experimental product mock-ups with optimal intrinsic-extrinsic attribute combinations (IRTA, AU, CTAQUA, HRH). Deliverable D29.5 includes the product mock-ups for use in the experimentation with consumers.

Sub-task 29.3.2 (HRH) A number of experimental set-ups will be established on-line to test the mock-ups developed above. The experiments will run with 3 new products resulting from the new species as tested and screened in previous tasks (see Tasks 28.1, 28.2 and 29.2). All 3 products will be tested with consumer samples (n=300 per country), with 150 participants in each country belonging to the two segments with the highest/best value perceptions per product (the "traditionals" and the "innovators", see Task 29.1) in order to achieve a best match between ideal extrinsic/intrinsic attribute combinations and high-potential segments (HRH, AU). This work will result in Deliverable D29.6 Report on the experimentation with product mock-ups in the five countries investigated and identification of the optimal intrinsic-extrinsic product quality profiles for targeted segments.

Task 29.4 Communication effectiveness in behavioural change (led by AU). In addition to product acceptance and optimal [product attributes X segments] combinations (Sub-tasks 29.1.1 to 29.3.2), the effects of different forms of label communication will be tested for their effectiveness on consumer buying intentions and willingness to pay (AU, IRTA, HRH). More specifically, task 29.4 examines the communication parameters (i.e., message type, process process, and source) have the ability to influence consumer value perceptions and cause attitudinal change, as well as purchasing intentions, willingness to pay and actual behaviour, thus causing behavioural change. Deliverable D29.7 includes those communication parameters' combinations as experimental stimulus that will be used in the communication experiments in the five countries investigated. A second round of experimental set-ups with samples similar to Sub-task 29.3.2 will be designed and implemented on-line (IRTA, AU, HRH), in order to test for communication effects. This results in Deliverable D29.8 Report on the results of the experimentation with the communication stimulus and evaluation of their effectiveness in changing consumers attitudes and behaviour.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	7.00
3	IRTA	8.00
6	DLO	12.50
11	AU	21.88
18	CTAQUA	8.20
38	HRH	17.90
	Total	75.48



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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D29.1	Dataset of consumers' perceptions, attitudes, buying intentions, consumption, willingness to buy and pay, and value perceptions towards the selected species in the five	6	7.38	R	PU	9
D29.2	Report on the segmentation analysis based on consumer value perceptions about the selected species in the five countries investigated (value-based segmentation task)	11	7.00	R	PU	24
D29.3	Development of the actual product samples from the selected species for the sensory testing with consumers in the five countries investigated	3	7.50	R	PU	28
D29.4	Report on the actual products' sensory profiling in the five countries investigated	3	14.20	R	PU	29
D29.5	Development of the product mock-ups for use in the experimentation with consumers in the five countries investigated	11	9.20	R	PP	30
D29.6	Report on the experimentation with product mock-ups in the five countries investigated and identification of the optimal intrinsic-extrinsic product quality profiles for targeted segments	11	11.20	R	PU	36
D29.7	Development of the stimulus (i.e. written and broadcasted information material) that will be used in the communication experiments in the five countries investigated	11	9.50	R	PP	42
D29.8	Report on the experimentation with the communication stimulus and evaluation of their effectiveness in changing consumers attitudes and behaviour towards the products coming from the selected	11	9.50	R	PU	44
		Total	75.48			

## Description of deliverables

D29.1) Dataset of consumers' perceptions, attitudes, buying intentions, consumption, willingness to buy and pay, and value perceptions towards the selected species in the five: The dataset will contain the entries generated from the international online consumer surveys in the 5 countries selected (UK, D, ES, F, IT) with an n=500 at minimum per country (nationally representative samples). The surveys will be conducted to investigate

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consumers' associations with and perceptions of the new products developed, attitudes towards established and new aquaculture as opposed to wild fish, buying intentions current/future fish consumption, willingness to buy and pay, and overall value perceptions. This information will determine consumer perceived value as a trade-off between gains (i.e. benefits) and losses (i.e., sacrifices) from the purchasing/consumption of the examined new products. The fieldwork will be subcontracted to a company that has country-specific panels. [month 9]

D29.2) Report on the segmentation analysis based on consumer value perceptions about the selected species in the five countries investigated (value-based segmentation task): The specific report will provide the results of the segmentation study that will be conducted on the data generated in D29.1. The report will give insights into consumer sub-markets (i.e., segments) across and within the 5 countries examined (i.e., national and international segments) with the highest potential for maximised consumer value perceptions, thus relevant for exploitation in subsequent activities of WP 29. [month 24]

D29.3) Development of the actual product samples from the selected species for the sensory testing with consumers in the five countries investigated: The different physical prototypes developed and tested in Tasks 28.2 and 28.3 will be manufactured according to the amount needed and following strict hygienic conditions. These samples will be the basis for the acceptability test to be done in task 29.2. This deliverable will contain all the information needed to handle, store and prepare the different samples, the statistical design to follow in each location (order of presentation, sample distribution among participants, etc.) as well as some practical recommendations to carry out the test and recruit the participants properly. [month 28]

D29.4) Report on the actual products' sensory profiling in the five countries investigated: Consumer sensory evaluation of the new products developed in WP 28 will be conducted in the five countries selected by means of a central location test under controlled conditions. This deliverable will contain the overall acceptability for each product, a quantitative assessment of relevant and simple sensory attributes made by participants and supplementary qualitative descriptive information obtained by means of a CATA (Check-All-That-Apply) questions. In addition, sensory motives driving consumers' acceptance will be identified by integrating sensory descriptive data provided by trained panelists with consumers' acceptability. Results will be described and presented taking into account socio-demographic data (country, gender, etc.) and the previous segmentation information obtained in Task 29.1. [month 29]

D29.5) Development of the product mock-ups for use in the experimentation with consumers in the five countries investigated: This deliverable incorporates the work done in WPs 28 and in Tasks 29.1 and 29.2. above. In this respect, a number of extrinsic quality attributes (i.e., product labelling elements that function as quality cues, for instance quality certifications, health claims etc.) will be incorporated into the physical product prototypes developed in WP7.2 to develop experimental product mock-ups with optimal intrinsic-extrinsic attribute combinations. The mock-ups will be tested in experimentation in Action 29.3.2. [month 30]

D29.6) Report on the experimentation with product mock-ups in the five countries investigated and identification of the optimal intrinsic-extrinsic product quality profiles for targeted segments: This report will provide the results from a number of experimental set-ups (i.e., Conjoint or Discrete Choice models) that will be established on-line to test the mock-ups developed above. The experiments will run with consumer samples (n=80 per country/segment at minimum) with participants belonging to the segments (national and international) with the highest/best value perceptions per product defined above (i.e., Action 29.1.1), in order to achieve a best match possible between ideal extrinsic/intrinsic attribute combinations and high-potential markets/segments. [month 36]

D29.7) Development of the stimulus (i.e. written and broadcasted information material) that will be used in the communication experiments in the five countries investigated: In addition to product acceptance and optimal [product attributes X segments] combinations (Actions 29.1.1 to 29.3.2), the effects of different forms of textual and broadcasted communication will be tested for their effectiveness on consumer buying intentions and willingness to pay. The specific deliverable will select the single communication parameters (i.e., message, process, source, medium) and their combinations (i.e., message content such as emotional/relational/gains-losses, clarity/uniformity of the process, trustworthiness of the source, etc.) that will be tested in Task 29.1 [month 42]

D29.8) Report on the experimentation with the communication stimulus and evaluation of their effectiveness in changing consumers attitudes and behaviour towards the products coming from the selected: This report will provide the results from a number of experimental set-ups, where the above-selected single communication parameters and their combinations will be tested in relation to their ability to influence consumer value perceptions (and cause attitudinal change), purchasing intentions, willingness to pay and actual behaviour, thus causing behavioural change. A second round of experimental set-ups after those in D29.6 with samples

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similar to above in terms of size and structure will be designed and implemented on-line, in order to test for communication effects. [month 44]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS63	Insights in the consumer and B2B market for cultured fish	11	12	
MS64	Selection of new products, with good sensory perception	11	30	
MS65	Intrinsic and extrinsic attributes related to the new products	11	36	
MS66	Communication concept for behavioral change to cultured fish	11	44	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP30	Type of activity <sup>54</sup>	RTD
Work package title	Socioeconomics – Business model and marketing strategy development		
Start month	43		
End month	60		
Lead beneficiary number <sup>55</sup>	10		

## Objectives

1. To identify business models for sustainable profitability and improved competitiveness of the sector for all the DIVERSIFY species,
2. To devise marketing strategies for the newly developed products from the DIVERSIFY species, aiming to develop a market that is as large and profitable as possible,
3. To come up with policy/strategy recommendations for further development and market expansion.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

Following the analysis of the supply and demand structure, and sensory and other tests from WP28 and 29, business models will be developed and successful new product marketing launch strategies proposed. This will be done in collaboration with the participating SMEs and using the business model concept. This Business Model Canvas approach distinguishes several components, including (i) resources/infrastructure, (ii) value proposition and delivery and (iii) costs and finances.

EU Budget allocation: 30,000€ (IRTA), 104,502€ (SWR/SWR/DLO), 213,000€ (TU/e), 72,500€ (AU), 35,000€ (APROMAR), 15,000€ (CTAQUA), 3,000€ (ARGO), 3,000€ (DOR), 3,000€ (CANEXMAR) and 2,260€ (HRH) and 3,000€ (F2B, joined the consortium in Oct 2016). ITTICAL and ASIALOR exited the consortium in May 2016)

Task 30.1 Business models (led by TU/e).

Sub-task 30.1.1 (TU/e) Firstly, the value proposition for the producers and partners (involved SMEs) will be described and specified for specific customer segments targeted in close cooperation with the SMEs. The information gathered in Task 27.3, will be used as a basis. This will include quantitative elements of price and efficiency, and qualitative matters of overall customer experience and outcome (TU/e, SWR/DLO APROMAR). This Sub-task will result in Deliverable D30.1 Report on value propositions for the producers and Partners.

Sub-task 30.1.2 (TU/e) Secondly, the resources necessary to create value for the customer will be described. It identifies the partner network and determines the actual resources that are required in order to optimize operations and reduce risks of a business model. Guidelines for the organizations to cultivate buyer-supplier relationships will be developed. Complementary business alliances will be explored, including options for joint ventures to expand globally (TU/e, APROMAR). This will be finished in Deliverable D30.2 Report on indications of resources for creating customer value for the specific products.

Sub-task 30.1.3 (TU/e) Finally, cost structures and possibilities to further drive down costs will be analysed together with the SME Partners. The way different companies along the value stream are involved and will get an income from cooperation or customer segments will be described and analysed (TU/e, APROMAR). It will be linked to price decisions to allow for estimating revenue streams. Several ways to generate revenue streams will be explored. The effort will draw on market data and trends from Task 27.1. Deliverable D30.4 Revenue (pricing & costs structures) model per species will present the results of this Sub-task.

Task 30.2 New product marketing strategy development (led by TU/e).

Sub-task 30.2.1 (TU/e) Development of a new product marketing strategy including actionable product-market combinations, new product launch, new market entry and timing, stimulating consumer adoption and encouraging diffusion across EU markets (TU/e, IRTA, AU, SWR/DLO, APROMAR, CTAQUA, HRH), drawing

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on the results of the segmentation of the market (Sub-task 29.1.2), the experimental studies (Sub-tasks 29.3.2 and 29.4.1) and the sensory evaluation (Sub-task 29.2.1). The focus will be on the five countries selected for the Tasks in WP7. Results from market tests (Sub-task 30.2.2) conducted will be used to further improve these strategies. These strategies will be reviewed by the involved SME's. In Deliverable D30.3 Guidelines to cultivate buyer-supplier relationships per species, the results of the product market phase are presented.

Sub-task 30.2.2 (TU/e) Testing of the proposed market strategy. In cooperation with the SMEs involved, a market test will be performed in the 5 countries selected (i.e. UK, D, ES, F, I). The network of the SMEs will be used to perform this test. These small-scale consumer tests will run in mid-size to large cities of these countries (TU/e, SWR/DLO, IRTA, AU, HRH). Locations will be chosen in accordance with the target segment, channel selection and using the marketing (e.g., communications, packaging) guidelines from WP29, and options available. Based on test market guidelines for actual rollout will be optimized. Furthermore, market simulations will be performed to estimate and facilitate the market launch and diffusion. The modelling will happen using data from WP27 and using system dynamics modelling. It will help SMEs to make decisions regarding their sales & operations planning (so called S&OP). As the choice of value products developed will impact the nature of the markets that will be targeted and parties involved, the efforts of market testing and simulation should be expected to vary by species, product and country. In Deliverable D30.5 New product marketing strategies per species and product, the definite marketing strategies are presented, while in Deliverable D30.6 Report on results of test markets per species are summarised.

Task 30.3 Recommendations for industry development and international market expansion ( by SWR/DLO). Sub-task 30.3.1 (SWR/DLO) In the feasibility study, an analysis on basis of the technical assessment (WP 28), market information (WP 29), resource and cost analysis (Task 30.1) and the results of the tested strategies (Task 30.2) will be delivered (SWR/DLO, IRTA, TU/e, HCMR). This study covers a financial analysis, an assessment of return on investment and a definition of efforts needed, a risk assessment, technological assessment (WP 28), political analysis of potential risks of implementation, environmental impact assessment (with information from GWP5 Grow out husbandry), a sociological and market impact assessment and a stakeholder identification to introduce the products in the market. This feasibility study will be reviewed by the participating SMEs (ARGO, DOR, CANEXMAR and F2B). The results will be presented in Deliverable D30.7 Feasibility study.

Sub-task 30.3.2 (SWR/DLO) Finally, a global market approach will be developed based on input from Task 30.2. Based on market similarities and existing contacts of the EU fish industry in foreign countries, opportunities for the new products developed in WP 28 will be identified and suggestions developed on how to further promote growth and market expansion (SWR/DLO, AU, HRH). The development of these plans will involve experts from the industry and the respective countries, as well as the experience (and networks) of the SMEs involved in DIVERSIFY. On the basis of the analysis, policy (macro-level) and strategy (micro-level) recommendations will be provided (SWR/DLO, AU, APROMAR, CTAQUA, HRH) with the potential to make the European aquaculture sector more competitive, and to provide a level playing field with respect to production in developing countries. Based on the above input we will again develop system dynamics simulation models that help predict the diffusion, of the EU produced fish species of this study, internationally. The models will factor in SMEs' international relations and other (e.g., cultural) linkages between geographical markets. The results will be published in Deliverable D30.8 Report on EU and international market development plans and recommendations.

Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
3	IRTA	3.00
6	DLO	12.83
10	TU/e	21.00
11	AU	7.82
12	APROMAR	8.14
18	CTAQUA	1.00

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## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
23	ARGO	0.10
25	DOR	0.10
28	CANEXMAR	0.10
38	HRH	1.00
39	F2B	0.10
Total		55.19

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D30.1	Report on value propositions for the producers and Partners	10	5.00	R	PU	46
D30.2	Report on indications of resources for creating customer value for the specific products	10	8.00	R	PU	46
D30.3	Guidelines to cultivate buyer-supplier relationships per species	10	5.32	R	PU	48
D30.4	Revenue (pricing & costs structures) model per species	10	6.00	R	PU	48
D30.5	New product marketing strategies per species and product	10	6.40	R	PU	52
D30.6	Report on results of test markets per species	10	9.16	R	PU	54
D30.7	Feasibility study	6	9.25	R	PU	60
D30.8	Report on EU and international market development plans and recommendations	10	6.06	R	PU	58
Total			55.19			

## Description of deliverables

D30.1) Report on value propositions for the producers and Partners: Deliverables D30.1 to D30.4. results in business models for the selected species, and more in detail for the ones for which new products are developed. It includes a description of the rationale of how organizations in the supply chain create, deliver, and capture value in the market place. In accord with the Canvas model the target customer segments will be outlined and value propositions developed. [month 46]

D30.2) Report on indications of resources for creating customer value for the specific products: Deliverables D30.1 to D30.4. results in business models for the selected species, and more in detail for the ones for which new products are developed. This report presents a part of the business model development effort, resources and processes necessary to sustain and support the business model. Information on e.g., the level of financial, physical and intellectual/human resources needed will be provided. [month 46]

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D30.3) Guidelines to cultivate buyer-supplier relationships per species: Deliverables D30.1 to D30.4. results in business models for the selected species, and more in detail for the ones for which new products are developed. Similarly, current relationships in the supply chain and opportunities to cultivate buyer-supplier relationships to develop the business will be identified and reported. Also bottlenecks will be studied and identified and potential solutions suggested. [month 48]

D30.4) Revenue (pricing & costs structures) model per species: Deliverables D30.1 to D30.4. results in business models for the selected species, and more in detail for the ones for which new products are developed. As part of this business model development effort, the revenue model will be specified, also identifying --based on e.g. also 30.3. opportunities to further drive down cost levels. Next all elements will be integrated in an overall business model reporting, with conclusions for the SME partners on how to proceed their business development process for the species involved. [month 48]

D30.5) New product marketing strategies per species and product: Identification of a set of alternative marketing strategies and selection of the best options for market testing in the selected countries accounting for competitive conditions (drawing on results of the consumer studies and business modelling efforts). These will be used for the test reported in deliverable D30.6. [month 52]

D30.6) Report on results of test markets per species: Empirical evidence of the effectiveness of the proposed marketing strategies. Suggestions for changes or optimization of these marketing strategies are included. Suggestions on prioritization of markets are made. [month 54]

D30.7) Feasibility study: Feasibility study. In the feasibility study assessments will be presented on several themes: financial, return on investments, efforts needed, risks, technological, political (of potential risks of implementation) environmental impact, sociological and market impact and a stakeholder identification. These assessments will be based on the results of WPs 27, 28 and 20, and the previous tasks in WP 30 [month 60]

D30.8) Report on EU and international market development plans and recommendations: Report on EU and international market development plans and recommendations: The diffusion studies show the effectiveness of alternative options of international market expansion and growth. Conclusions regarding the best options for internationalization help the industry/SMEs to share their international expansion strategies. Suggestions for policies that many stimulate growth/internationalization, based on potential bottlenecks identified [month 58]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS67	Business models to market the new products	10	48	
MS68	Product marketing strategy	10	54	
MS69	Marketable new products	10	60	

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Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## One form per Work Package

Work package number <sup>53</sup>	WP31	Type of activity <sup>54</sup>	OTHER
Work package title	Dissemination		
Start month	1		
End month	60		
Lead beneficiary number <sup>55</sup>	18		

## Objectives

1. Disseminate the knowledge acquired to the scientific community, to promote further research,
2. Disseminate the knowledge acquired to the aquaculture sector, to enhance feed back acquisition,
3. Promote implementation of new husbandry methods, protocols and products developed by DIVERSIFY by the aquaculture industry and the seafood processors,
4. Enhance awareness of the diversification efforts of the project to the general public, with special attention to the food industry and consumer's organizations,
5. Promote investment opportunities making available the species feasibility studies to the industry,
6. Provide documented information to fish producers, fish processors and consumers on the new farmed aqua products from DIVERSIFY.

## Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants (Partner abbreviations)

Strong emphasis will be put in disseminating the results of the project and in engaging with relevant stakeholders and the society. The consortium will keep the EC's scientific officer of the project informed in a timely manner about any dissemination/communication activity and WS related to the project's implementation and results. In addition the consortium will inform (and when relevant will send a copy to) the project's EC scientific officer about any publication, leaflet, and other dissemination/communication outcome as soon as it is produced, during and after the implementation of the project. All the activities, deliverables, publications and project outcomes will clearly indicate the EU financial support and when applicable will display the appropriate EU logos.

EU Budget allocation: 92,613€ (HCMR), 26,211€ (IRTA), 21,000€ (IMR), 19,200€ (IEO), 21,120€ (UL), 29,024€ (APROMAR), 19,312€ (UNIBA), 88,368€ (CTAQUA), 13,200€ (FGM), 20,381€ (BVFfi), 13,800€ (MASZ), 15,104€ (ANFACO) and 37,680€ (EUFIC).

Task 31.1 Project website and brochure (led by CTAQUA). Prepared from the start of the project (CTAQUA, APROMAR, HCMR), the web site will include:

- (a) information on the objectives and main tasks,
- (b) a newsletter with regular updates on the project (CTAQUA),
- (c) downloadable documentation for the general public, video podcasts and/or radio podcasts with interviews of project scientists and/or experts in the project scope (APROMAR, CTAQUA). At least two podcasts per year, including interviews with the PC (first and last interview) and with the SLs will be available in the web.
- (d) links to abstracts, presentations and of scientific articles (CTAQUA, HCMR),
- (e) a blog page for interactions with scientists and aquaculturists, for the acquisition of feedback on DIVERSIFY (CTAQUA, APROMAR),
- (f) links to related national and EU projects,
- (g) highlights and breakthroughs (from DIVERSIFY and beyond) in the fields covered by the project

Content development will be done by CTAQUA, APROMAR and HCMR in collaboration with the GWP leaders (GWPL) and the Species leaders (SL). A project brochure (CTAQUA, APROMAR) will be prepared and distributed to the sector through the partners that represent producer and processor associations, such as APROMAR, EUFIC, BVFi, ANFACO, MASZ and FGM (M6). The actions mentioned in this task will result in Deliverable D31.1. Establishment of the programme web site (fishDIVERSIFY.eu) where information about



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the programme and release of knowledge will be done; Deliverable D31.2. Project logo and release of project brochure and Deliverables D31.4, D31.7, D31.8, D31.12, D31.13, D31.15, D31.17, D31.22 and D31.35 Production and release of downloadable audiovisual material

Task 31.2 Annual Coordination Meetings (led by HCMR). The annual coordination meetings (See also WP 1) will serve also a dissemination purpose. The first two days of the meetings will be open to the public and will include invited speakers involved in the production of any of the species studied (both from Europe and world-wide). The presentations made will provide both Partners and invited guests with an overview of the work carried out in each species. The annual coordination meeting report including the advances of the project will be included in the project web, contributing to the release of knowledge of Deliverable D31.1.

Task 31.3 Presentation of DIVERSIFY at the AQUA EUROPE meetings (led by HCMR). The Program Coordinator (HCMR) will make a summary presentation at the EAS's annual meeting (EU Forum), to present the major achievements of DIVERSIFY. Likewise, the SLs (IRTA-meagre, HCMR-greater amberjack, UL-pikeperch, IMR-Atlantic halibut, IEO-wreckfish and IOLR-grey mullet) will provide more detailed summaries of the work specific for each species achieved at Y2 and Y4 of the project. These presentations will be available in the project's web site and will also be sent to international fish producers magazines (e.g., Fishing News, Greece; MisPeces, Spain; Fish Farming Xpert, Norway; Aquaculture Europe, Europe; Fish Farmer International, Global Aquaculture Advocate). This task will result in Deliverables D31.6, D31.9, D31.14, D31.19, and D31.28 Annual presentations of DIVERSIFY at the Aqua Europe meetings (EU Forum) by the Project Coordinator (Y1-5) and D31.10 and D31.20. Presentation of DIVERSIFY at the Aqua Europe meetings (Diversification Sessions) by the Species leaders at Y2 and Y4.

Task 31.4 Scientific presentations and submission of manuscripts (led by HCMR). The work carried out for the different species in the various work packages will be presented at relevant European or international conferences, and when appropriate the work will be prepared for submission to international ISI-indexed scientific journals, and when possible to "open access journals". It is expected to have an output of a minimum of 2 scientific articles per species per year (a total of 60 articles for the whole program). This task will result in Deliverable D31.11. Scientific publications in relevant journals.

Task 31.5 Full-day seminars on "Know-how Trasfer" of the aquaculture of each of the DIVERSIFY species (led by CTAQUA and the Species Leader Partner). These seminars will be organized at Y5 and will include 30 min presentations on selected aspects (e.g., reproduction and spawning induction, final product diversification and quality, socioeconomic issues and marketing, etc.), given by DIVERSIFY Partners, but also from any authorities in the species (European or world-wide depending on the species), whose work was not part of the project. Aquaculturists (mainly), but also European aquaculture support companies (feed, pharmaceutical, equipment, engineering, etc.), researchers and educators, government organizations and other important institutions (FAO, Globefish) will be invited to attend these meetings. The cost of the invited speakers and the registration of the participants will be covered by the programme (max 50 participants). The workshops will be organized by the SLs (HCMR, IRTA, UNIBA, IMR, IEO, UL) in countries where the particular species are cultured --or has the potential to be cultured -- and/or is located centrally in a region with interested aquaculture operations. One seminar will be organized for each of the selected species. The considered countries of choice are: Spain, France, Italy and Greece for meagre, greater amberjack, wreckfish and grey mullet; France, Belgium or Denmark for pikeperch; and Norway for Atlantic halibut. The result of this task will be Deliverables D31.29 to 34. Species-specific "Know-how transfer" seminars for the aquaculture industry, presenting the progress achieved through DIVERSIFY in the production technology. Just prior to these seminars, a summary audio-visual document will be also produced for communicating the projects activities and main achievements (all species and scientific disciplines) to the relevant industries, and will be provided to the seminar participants and sent to professional associations. This will be Deliverable D31.25 Audio-visual document with the project's activities and main achievements.

Task 31.6 Promotional workshops (led by CTAQUA). Specialized 1-day workshops will be organized in 4 strategic countries (Spain, Greece, UK and Italy) for the promotion of the project activities and results (CTAQUA, APROMAR). These workshops will be held during the last two years of the project, Y4 and Y5. Workshops will focus on specific audience, such as fish producers, processors and retailers, consumer organizations, and fisheries and aquaculture authorities. For each workshop, relevant European speakers from specialized consumer's organizations and/or professional associations will be invited to address among others the following topics: consumer perception of aquaculture fish, general trends in farmed fish markets, value added products from farmed fish, labelling and certification, imbalance in the market value chain of aquaculture products due to the dominance of large retailers/buyers, processing and freshness and food safety This task will benefit from the

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expertise of Partners from GWP 7 Socio-economic. Special attention will be given to the products developed in WP 28 and WP 29. This task will result in Deliverables D31.16, D31.18, D31.23, D31.27. Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK and Italy).

Task 31.7 Dissemination to the food industry and consumers (led by APROMAR and EUFIC). The projects results will be promoted to processors, retailers, caterers, food scientists, etc. Establishment of direct contacts will be made with main European Associations in these fields, the European Association of Supermarkets (COOPERNIC), European consumer organizations such as EUROCONSUMERS and BEUC, and the platform Global Initiative for Life and Leadership through Seafood (GILLS). The actions will include linkage of the DIVERSIFY web site to COOPERNIC, BEUC, EUROCONSUMER and GILLS web sites and at least, one meeting with each association to implement global communication from research through industry to consumers. EUFIC will prepare and publish in their website two dedicated articles for the project, one at the beginning (M6) to create awareness of the project and another one towards the end (M54) with the conclusions and main findings of DIVERSIFY. This task will result in Deliverables D31.3 Publication of the first of two articles in Food Today, electronic journal of EUFIC and Deliverable D31.26 Audio-visual popularization document and publication of the second article in Food Today, electronic journal of EUFIC. During the five years of the project, a summary of project activities will be presented in the annual meetings of the Partners representing associations of fish producers, fish processors and consumers, contributing to the popularization of the project findings and results. Finally, from the above material, an audio-visual popularization publication will be prepared as well, address to a non-specialist audience, contributing also to Deliverable D31.26 Audio-visual popularization document and publication of the second article in Food Today, electronic journal of EUFIC (M54)

At Y3, DIVERSIFY will be participating in the annual seafood conference European Seafood Exposition (Seafood Brussels) (APROMAR, CTAQUA) promoting and offering all the information on the DIVERISIFY fish species and their products developed within the project. As soon as available, technical information on the culture of DIVERSIFY fish species will be compiled and presented to the industry in the form of technical leaflets (one per species) and distributed to the sector. The leaflets will be translated to the language of the different associations included in the WP, Greek (FGM), German (BVFi), Hungarian (MASZ) and Spanish (ANFACO). The participating SMEs will also assist in the development and translation of these technical leaflets, in their own language (French, Norwegian, Italian, Hebrew). If technical information is available, the technical leaflets will be also distributed during the exposition. The results of this task will be Deliverable D31.5 Collaboration agreement with food industry and consumer organization; linkage of websites, Deliverable D31.24 Technical leaflets with main technical information on the culture of DIVERSIFY fish species and Deliverable D31.21. Presentation of DIVERSIFY at the European SEAFOOD Exposition.

## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	HCMR	8.00
3	IRTA	1.00
7	IMR	1.00
8	IEO	0.70
9	UL	1.00
12	APROMAR	2.74
13	UNIBA	1.50
18	CTAQUA	13.10
33	FGM	2.50
34	BVFi	0.42
35	MAHAL	3.00
36	ANF	2.25

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## Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
37	EUFIC	3.00
	Total	40.21

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
D31.1	Establishment of web site (fishDIVERSIFY.eu)	18	1.20	R	PU	4
D31.2	Project logo and brochure	18	1.00	R	PU	6
D31.3	Publication of the first of two articles in Food Today	37	0.50	R	PU	6
D31.4	Production and release of audiovisual material	18	0.25	R	PU	6
D31.5	Collaboration agreement with food industry and consumer organization; linkage of websites	18	0.27	R	PU	9
D31.6	Annual presentation of DIVERSIFY (Y1) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator	1	0.30	R	PU	9
D31.7	Production and release of audiovisual material	18	0.50	R	PU	12
D31.8	Production and release of audiovisual material	18	0.50	R	PU	18
D31.9	Annual presentation of DIVERSIFY (Y2) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator	1	0.50	R	PU	21
D31.10	Presentations of DIVERSIFY at the Aqua Europe meetings (Diversification Sessions) by the Species leaders (Y2)	1	2.50	R	PU	21
D31.11	Scientific publications in relevant journals	1	1.00	R	PU	60
D31.12	Production and release of audiovisual material	18	0.50	R	PU	24
D31.13	Production and release of audiovisual material	18	0.50	R	PU	30
D31.14	Annual presentation of DIVERSIFY (Y3) at a relevant conference (mainly	1	0.50	R	PU	33

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## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	Aqua Europe meetings, EU Forum) by the Project Coordinator					
D31.15	Production and release of audiovisual material	18	0.50	R	PU	36
D31.16	Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK or Italy) (1st workshop)	18	1.50	R	PU	37
D31.17	Production and release of audiovisual material	18	0.50	R	PU	42
D31.18	Promotional workshops (2nd) for specialized audience in fish market sector (Spain, UK, Italy or Greece)	18	1.50	R	PU	43
D31.19	Annual presentation of DIVERSIFY (Y4) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator	1	0.50	R	PU	44
D31.20	Presentations of DIVERSIFY at the Aqua Europe meetings (Diversification Sessions) by the Species leaders (Y4)	1	2.85	R	PU	44
D31.21	Presentation of DIVERSIFY at the European SEAFOOD Expo	18	0.50	R	PU	44
D31.22	Production and release of audiovisual material	18	0.50	R	PU	48
D31.23	Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK or Italy) (3rd workshop)	18	1.00	R	PU	49
D31.24	Technical leaflets	18	5.24	R	PU	54
D31.25	Audio-visual document with the project's activities and main achievements	1	0.50	R	PU	54
D31.26	Audio-visual popularization document and publication of the second article in Food Today, electronic journal of EUFIC	37	0.50	R	PU	54
D31.27	Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK or Italy) (4th workshop)	18	1.50	R	PU	55
D31.28	Annual presentations of DIVERSIFY at the Aqua Europe meetings (EU	1	0.50	R	PU	57

# WT3: Work package description

## List of deliverables

Deliverable Number <sup>61</sup>	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature <sup>62</sup>	Dissemination level <sup>63</sup>	Delivery date <sup>64</sup>
	Forum) by the Project Coordinator (Y5)					
D31.29	“Know-how Transfer” seminar for the aquaculture industry (Spain), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet	3	1.80	R	PU	57
D31.30	“Know-how Transfer” seminar for the aquaculture industry (Greece), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet	1	2.00	R	PU	57
D31.31	Pikeperch “Know-how Transfer” seminar for the aquaculture industry (potential location: France, Belgium, Denmark), presenting the progress achieved through DIVERSIFY in the production technology	9	2.00	R	PU	58
D31.32	Atlantic halibut “Know-how Transfer” seminar for the aquaculture industry (potential location: Norway), presenting the progress achieved through DIVERSIFY in the production technology	7	2.00	R	PU	58
D31.33	“Know-how Transfer” seminar for the aquaculture industry ( Spain), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet	8	2.00	R	PU	59
D31.34	“Know-how Transfer” seminar for the aquaculture industry (Italy), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet	13	2.00	R	PU	59
D31.35	Production and release of audiovisual material	18	0.80	R	PU	60
	<b>Total</b>		40.21			

## Description of deliverables

D31.1) Establishment of web site (fishDIVERSIFY.eu): Establishment and launch of a dynamic web site (fishDIVERSIFY.com) where information about the programme and release of knowledge will be available; video content, Facebook/Linkedin profiles and discussion groups will be developed. The web will include cross-links to specialist aquaculture websites and it will be maintained for one year after the project end. [month 4]

# WT3: Work package description

D31.2) Project logo and brochure: A unique project logo will be designed and available for all the partners to be used in all the communications as identification of the project. A brochure with the description of the main objectives of the project will be designed and sent to the partners for further distribution to other institutions and aquaculture representatives in their respective country. [month 6]

D31.3) Publication of the first of two articles in Food Today: EUFIC will elaborate and published (with the input from the WPL), one of two dedicated articles for the project. This one will be the one at the beginning to make awareness for the project (another one will be elaborated and published at the end with the conclusions and main findings of DIVERSIFY). [month 6]

D31.4) Production and release of audiovisual material: Downloadable documentation for the general public, video podcasts and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 6]

D31.5) Collaboration agreement with food industry and consumer organization; linkage of websites: CTAQUA and APROMAR will meet the European Association of Supermarkets (COOPERNIC), the European consumer organization (EUROCONSUMERS) and the platform Global Initiative for Life and Leadership through Seafood (GILLS) to create awareness of the project among their associates and to establish collaboration agreements in terms of including DIVERSIFY results and updates in their websites. [month 9]

D31.6) Annual presentation of DIVERSIFY (Y1) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator: The Project Coordinator will present a project summary with the major objectives and activities of and achievements of DIVERSIFY during the first year at the EAS's annual meeting (EU Forum). [month 9]

D31.7) Production and release of audiovisual material: Downloadable documentation for the general public, video podcasts and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 12]

D31.8) Production and release of audiovisual material: Downloadable documentation for the general public, video podcasts and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 18]

D31.9) Annual presentation of DIVERSIFY (Y2) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator: The project Coordinator will present a project summary with the major achievements of DIVERSIFY up-to-date at the EAS's annual meeting (EU Forum). The summary will be cumulative to allow the new audience to know the project from the start, as well as to offer a global view of DIVERSIFY research evolution. [month 21]

D31.10) Presentations of DIVERSIFY at the Aqua Europe meetings (Diversification Sessions) by the Species leaders (Y2): The species leaders (IRTA-meagre, HCMR-greater amberjack, UL-pikeperch, IMR-Atlantic halibut, IEO-wreckfish and IOLR-grey mullet) will provide a detailed summary of the work specific for each species achieved during the 4 years of the project at the Aqua Europe meeting of that year. These presentations will be available in the project website and will be sent to international fish producers magazines [month 21]

D31.11) Scientific publications in relevant journals: From the work carried out within the different work packages it is expected to produce at least, two scientific publication per species per year (meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet) with a total output of 60 scientific articles for the whole program. [month 60]

D31.12) Production and release of audiovisual material: Downloadable documentation for the general public, video podcasts and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 24]

D31.13) Production and release of audiovisual material: Downloadable documentation for the general public, video podcasts and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 30]

D31.14) Annual presentation of DIVERSIFY (Y3) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator: The project Coordinator will present a project summary with the major achievements of DIVERSIFY up-to-date at the EAS's annual meeting (EU Forum). The summary will be cumulative to allow the new audience to know the project from the start, as well as to offer a global view of DIVERSIFY research evolution. [month 33]

# WT3:

## Work package description

D31.15) Production and release of audiovisual material: Downloadable documentation for the general public, video podcastas and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 36]

D31.16) Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK or Italy) (1st workshop): Promotional workshops of 1 day duration, will be organize after the first 3 years of the project life. Workshops will be organized with an interval of 6 months. Target audience of the workshop is fish producers, fish processors, fish retailers, consumer organization and fisheries and aquaculture authorities. Relevant speakers from the professional associations and/or consumer organization will be invited to address to address among others the following topics: consumer perception of aquaculture fish, general trends in farmed fish markets, value added products from farmed fish, labelling and certification, imbalance in the market value chain of aquaculture products due to the dominance of large retailers/buyers, processing and freshness and food safety. [month 37]

D31.17) Production and release of audiovisual material: Downloadable documentation for the general public, video podcastas and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 42]

D31.18) Promotional workshops (2nd) for specialized audience in fish market sector (Spain, UK, Italy or Greece): Promotional workshops of 1 day duration. Target audience of the workshop is fish producers, fish processors, fish retailers, consumer organization and fisheries and aquaculture authorities. Relevant speakers from the professional associations and/or consumer organization will be invited to address to address among others the following topics: consumer perception of aquaculture fish, general trends in farmed fish markets, value added products from farmed fish, labelling and certification, imbalance in the market value chain of aquaculture products due to the dominance of large retailers/buyers, processing and freshness and food safety. [month 43]

D31.19) Annual presentation of DIVERSIFY (Y4) at a relevant conference (mainly Aqua Europe meetings, EU Forum) by the Project Coordinator: The project Coordinator will present a project summary with the major achievements of DIVERSIFY up-to-date at the EAS's annual meeting (EU Forum). The summary will be cumulative to allow the new audience to know the project from the start, as well as to offer a global view of DIVERSIFY research evolution. [month 44]

D31.20) Presentations of DIVERSIFY at the Aqua Europe meetings (Diversification Sessions) by the Species leaders (Y4): The species leaders (IRTA-meagre, HCMR-greater amberjack, UL-pikeperch, IMR-Atlantic halibut, IEO-wreckfish and IOLR-grey mullet) will provide a detailed summary of the work specific for each species achieved during the 4 years of the project at the Aqua Europe meeting of that year. These presentations will be available in the project website and will be sent to international fish producers magazines. [month 44]

D31.21) Presentation of DIVERSIFY at the European SEAFOOD Expo: As part of the dissemination of the project to the seafood industry, at Y3, DIVERSIFY will be participating at the annual seafood conference European Seafood Exposition (Seafood Brussels). APROMAR and CTAQUA will organize the promotion of the project in this forum. All the available information on the DIVERISIFY fish species and their products developed within the project will be publicized. [month 44]

D31.22) Production and release of audiovisual material: Downloadable documentation for the general public, video podcastas and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 48]

D31.23) Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK or Italy) (3rd workshop): Promotional workshops of 1 day duration. Target audience of the workshop is fish producers, fish processors, fish retailers, consumer organization and fisheries and aquaculture authorities. Relevant speakers from the professional associations and/or consumer organization will be invited to address to address among others the following topics: consumer perception of aquaculture fish, general trends in farmed fish markets, value added products from farmed fish, labelling and certification, imbalance in the market value chain of aquaculture products due to the dominance of large retailers/buyers, processing and freshness and food safety. [month 49]

D31.24) Technical leaflets: As soon as the technical information on the culture of DIVERSIFY fish species is available, it will be compiled and presented to the industry in the form of technical leaflets (one per species) and distributed to the aquaculture production sector. The leaflets will be translated to the language of the different associations included in the WP, Greek (FGM), German (BVF), Hungarian (MASZ) and Spanish (ANFACO). The participating SMEs will also assist in the development and translation of these technical leaflets, in their own language (French, Norwegian, Italian, Hebrew). [month 54]

# WT3:

## Work package description

D31.25) Audio-visual document with the project's activities and main achievements: An audio-visual documentation (brochure/folder) for the general public, video podcasts and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 54]

D31.26) Audio-visual popularization document and publication of the second article in Food Today, electronic journal of EUFIC: A popularized audio-visual document (brochure/folder) will be prepared by the Dissemination leader and EUFIC (with the input from the GWPL), for dissemination to non-specialized audiences. The material will also be used for the second dedicated articles for the project at the end with the conclusions and main findings of DIVERSIFY. [month 54]

D31.27) Promotional workshops for specialized audience in fish market sector (Spain, Greece, UK or Italy) (4th workshop): Promotional workshops of 1 day duration. Target audience of the workshop is fish producers, fish processors, fish retailers, consumer organization and fisheries and aquaculture authorities. Relevant speakers from the professional associations and/or consumer organization will be invited to address among others the following topics: consumer perception of aquaculture fish, general trends in farmed fish markets, value added products from farmed fish, labelling and certification, imbalance in the market value chain of aquaculture products due to the dominance of large retailers/buyers, processing and freshness and food safety. [month 55]

D31.28) Annual presentations of DIVERSIFY at the Aqua Europe meetings (EU Forum) by the Project Coordinator (Y5): The project Coordinator will present a project summary with the major achievements of DIVERSIFY during the five years and present them at the EAS's annual meeting (EU Forum). The summary will be cumulative to allow the new audience to know the project from the start as well as to offer a global view of DIVERSIFY research evolution. [month 57]

D31.29) "Know-how Transfer" seminar for the aquaculture industry (Spain), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet: A full-day seminar directed to the Mediterranean aquaculture industry in Spain, covering the knowledge obtained in DIVERSIFY for meagre, greater amberjack, wreckfish and grey mullet. The emphasis on each species will depend on the country and their potential for aquaculture. The seminar will include 30 min presentations on selected aspects (e.g., reproduction and spawning induction, final product diversification and quality, socioeconomic issues and marketing, etc.), given by DIVERSIFY Partners, but also from any authorities in the species, whose work was not part of the project. Aquaculturists (mainly), but also European aquaculture support companies (feed, pharmaceutical, equipment, engineering, etc.), researchers and educators, government organizations and other important institutions (FAO, Globefish) will be invited to attend these meetings. [month 57]

D31.30) "Know-how Transfer" seminar for the aquaculture industry (Greece), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet: A full-day seminar directed to the Mediterranean aquaculture industry in Greece covering the knowledge obtained in DIVERSIFY for meagre, greater amberjack, wreckfish and grey mullet. The emphasis on each species will depend on the country and their potential for aquaculture. The seminar will include 30 min presentations on selected aspects (e.g., reproduction and spawning induction, final product diversification and quality, socioeconomic issues and marketing, etc.), given by DIVERSIFY Partners, but also from any authorities in the species, whose work was not part of the project. Aquaculturists (mainly), but also European aquaculture support companies (feed, pharmaceutical, equipment, engineering, etc.), researchers and educators, government organizations and other important institutions (FAO, Globefish) will be invited to attend these meetings. [month 57]

D31.31) Pikeperch "Know-how Transfer" seminar for the aquaculture industry (potential location: France, Belgium, Denmark), presenting the progress achieved through DIVERSIFY in the production technology: A full-day seminar directed to the freshwater aquaculture industry, covering the knowledge obtained in DIVERSIFY for pikeperch (potential location: France, Belgium, Denmark). The seminar will include 30 min presentations on selected aspects (e.g., reproduction and spawning induction, final product diversification and quality, socioeconomic issues and marketing, etc.), given by DIVERSIFY Partners, but also from any authorities in the species, whose work was not part of the project. Aquaculturists (mainly), but also European aquaculture support companies (feed, pharmaceutical, equipment, engineering, etc.), researchers and educators, government organizations and other important institutions (FAO, Globefish) will be invited to attend these meetings. [month 58]

D31.32) Atlantic halibut "Know-how Transfer" seminar for the aquaculture industry (potential location: Norway), presenting the progress achieved through DIVERSIFY in the production technology: A full-day seminar directed to the coldwater aquaculture industry, covering the knowledge obtained in DIVERSIFY for Atlantic halibut (potential location: Norway). The seminar will include 30 min presentations on selected aspects (e.g., reproduction and spawning induction, final product diversification and quality, socioeconomic issues and



# WT3: Work package description

marketing, etc.), given by DIVERSIFY Partners, but also from any authorities in the species, whose work was not part of the project. Aquaculturists (mainly), but also European aquaculture support companies (feed, pharmaceutical, equipment, engineering, etc.), researchers and educators, government organizations and other important institutions (FAO, Globefish) will be invited to attend these meetings. [month 58]

D31.33) “Know-how Transfer” seminar for the aquaculture industry ( Spain), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet: A full-day seminar directed to the Mediterranean aquaculture industry in Northern Spain or France, covering the knowledge obtained in DIVERSIFY for meagre, greater amberjack, wreckfish and grey mullet. The emphasis on each species will depend on the country and their potential for aquaculture. The seminar will include 30 min presentations on selected aspects (e.g., reproduction and spawning induction, final product diversification and quality, socioeconomic issues and marketing, etc.), given by DIVERSIFY Partners, but also from any authorities in the species, whose work was not part of the project. Aquaculturists (mainly), but also European aquaculture support companies (feed, pharmaceutical, equipment, engineering, etc.), researchers and educators, government organizations and other important institutions (FAO, Globefish) will be invited to attend these meetings. [month 59]

D31.34) “Know-how Transfer” seminar for the aquaculture industry (Italy), presenting the progress achieved in DIVERSIFY in the technology for meagre, greater amberjack, wreckfish and/or grey mullet: A full-day seminar directed to the Mediterranean aquaculture industry in Italy covering the knowledge obtained in DIVERSIFY for meagre, greater amberjack, wreckfish and grey mullet. The emphasis on each species will depend on the country and their potential for aquaculture. The seminar will include 30 min presentations on selected aspects (e.g., reproduction and spawning induction, final product diversification and quality, socioeconomic issues and marketing, etc.), given by DIVERSIFY Partners, but also from any authorities in the species, whose work was not part of the project. Aquaculturists (mainly), but also European aquaculture support companies (feed, pharmaceutical, equipment, engineering, etc.), researchers and educators, government organizations and other important institutions (FAO, Globefish) will be invited to attend these meetings. [month 59]

D31.35) Production and release of audiovisual material: Downloadable documentation for the general public, video podcasts and/or radio podcasts including interviews with project scientists and/ experts in the project scope [month 60]

## Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS70	Agreement on project logo for website and publications, this will provide a recognizable image of DI	1	6	
MS71	Design and printing of project brochure (hard-copy) including the project logo, inserts with project	18	6	
MS72	Agreements with food industry and consumers association for web linkage	18	9	
MS73	Agreement on the Promotional workshop (1st) program	18	32	
MS74	Agreement on the Promotional workshop (2nd) program	18	32	
MS75	Agreement on the Promotional workshop (3rd) program	18	42	
MS76	Agreement on the Promotional workshop (4th) program	18	48	

# WT3: Work package description

Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS77	Agreement on the one-day State-of-the-art seminar program for meagre	3	49	
MS78	Agreement on the one-day State-of-the-art seminar program for greater amberjack	1	49	
MS79	Agreement on the one-day State-of-the-art seminar program for pike perch	9	50	
MS80	Agreement on the one-day State-of-the-art seminar program for Atlantic halibut	7	50	
MS81	Agreement on the one-day State-of-the-art seminar program for wreckfish	8	51	
MS82	Agreement on the one-day State-of-the-art seminar program for grey mullet	4	52	

# WT4: List of Milestones

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## List and Schedule of Milestones

Milestone number <sup>59</sup>	Milestone name	WP number <sup>53</sup>	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS1	Kick-off and annual coordination meeting (for Y1)	WP1	1	1	
MS2	Consortium Agreement	WP1	1	3	
MS3	Annual coordination meeting (for Y2)	WP1	1	14	
MS4	Periodic Report (Mo1-12) to DG RTD, including financial and administrative reports	WP1	1	18	
MS5	Annual coordination meeting (for Y3)	WP1	1	26	
MS6	Periodic Report (Mo13-30) to DG RTD, including financial and administrative reports	WP1	1	36	
MS7	Annual coordination meeting (for Y4)	WP1	1	38	
MS8	Annual coordination meeting (for Y5)	WP1	1	50	
MS9	Final coordination meeting	WP1	1	58	
MS10	Periodic Report (Mo30-48) to DG RTD, including financial and administrative reports	WP1	1	60	
MS11	Periodic Report (Mo4-608) to DG RTD, including financial and administrative reports	WP1	1	60	
MS12	Final Report to DG RTD	WP1	1	60	
MS16	SNP library with candidate SNPs potentially	WP2	3	18	

# WT4: List of Milestones

Milestone number <sup>59</sup>	Milestone name	WP number <sup>53</sup>	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
	associated to growth of meagre				
MS17	Database of genetic variability of pikeperch	WP4	1	12	
MS18	Documentation of ovulatory cycles in wild and F1 halibut broodstock	WP5	7	30	
MS19	Basic diet formulation for meagre grow-out studies	WP8	2	12	
MS20	Digestive utilization of experimental weaning diets for meagre	WP8	2	24	
MS21	Basic formulation for amberjack grow-out studies	WP9	2	12	
MS22	Definition of reproductive quality parameters to be studied in amberjack	WP9	2	12	
MS23	Definition of parameters for skeleton studies in pike perch	WP10	21	12	
MS24	Influence of salinity or temperature on LC-PUFAs synthesis in pike perch	WP10	21	36	
MS25	Ranges of digestive enzymes activities in Atlantic halibut	WP11	7	32	
MS26	Obtain viable gametes (oocytes and sperm) for larvae production in wreckfish	WP12	19	36	
MS27	Definition of methodology to study cost-benefit of grey mullet weaning diets	WP13	4	12	
MS28	Protocol for weaning meagre larvae	WP14	3	18	

# WT4: List of Milestones

Milestone number <sup>59</sup>	Milestone name	WP number <sup>53</sup>	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS29	Successful maturation and spawning of eastern Atlantic or Mediterranean Sea wild, F1 generation gre	WP15	2	6	
MS30	Successful maturation and spawning of eastern Atlantic or Mediterranean Sea wild, F1 generation gre	WP15	2	18	
MS31	Protocol for tank design, lighting and probiotics of larval rearing of amberjack	WP15	2	24	
MS32	Successful maturation and spawning of eastern Atlantic or Mediterranean Sea wild, F1 generation gre	WP15	2	30	
MS33	Successful maturation and spawning of eastern Atlantic or Mediterranean Sea wild, F1 generation gre	WP15	2	42	
MS34	Successful maturation and spawning of wreckfish to produce good quality eggs	WP6	8	5	This will allow implementation of larval rearing experiments (WP 18)
MS35	Successful maturation and spawning of wreckfish to produce good quality eggs	WP6	8	17	This will allow implementation of larval rearing experiments (WP 18)
MS36	Successful maturation and spawning of wreckfish to produce good quality eggs	WP6, WP6	8	29	This will allow implementation of larval rearing experiments (WP 18)
MS37	Successful maturation and spawning of	WP6	8	41	This will allow implementation of larval

# WT4: List of Milestones

Milestone number <sup>59</sup>	Milestone name	WP number <sup>53</sup>	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
	wreckfish to produce good quality eggs				rearing experiments (WP 18)
MS38	Successful maturation and spawning of grey mullet broodstock to produce good quality eggs and larvae	WP19	4	9	
MS39	Successful maturation and spawning of grey mullet broodstock to produce good quality eggs and larvae	WP19	4	21	
MS40	Successful maturation and spawning of grey mullet broodstock to produce good quality eggs and larvae	WP19	4	33	
MS41	Successful maturation and spawning of grey mullet broodstock to produce good quality eggs and larva	WP19	4	45	
MS42	Results on feeding stimuli of meagre	WP20	3	18	
MS43	First cage trials (different volume and light conditions) with meagre implemented	WP20	3	24	
MS44	Results on feed distribution method in cages with meagre	WP20	3	24	
MS45	Feeding pattern of greater amberjack fry available	WP21	1	21	
MS46	First results on optimum husbandry practise (thermal ranges, stocking density) of greater amberjack	WP21	1	28	
MS47	First experiment on cage culture condition (net volume, cage type)	WP21	1	30	

# WT4: List of Milestones

Milestone number <sup>59</sup>	Milestone name	WP number <sup>53</sup>	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
	of greater amberjack implemented				
MS48	Experiment on the definition of optimal conditions for pike perch on growing implemented	WP22	16	18	
MS49	First trials with different strains of pike perch implemented	WP22	16	40	
MS50	Experimental trials of grey mullet in the three locations implemented	WP23	4	27	
MS51	Design of primers for amplification of meagre target gene DNA sequences	WP24	5	12	
MS52	Grow-out of larvae and collection of samples from immune ontogeny time-line	WP24	5	24	
MS53	Amplification and sequencing of target gene sequences from stimulated tissues	WP24	5	30	
MS54	Completion of challenge and collection of samples for study of immune gene modulation	WP24	5	36	
MS55	Complete preparation of cDNA synthesis from all meagre samples	WP24	5	40	
MS56	Complete gene expression analysis for immune ontogeny	WP24	5	42	
MS57	Complete gene expression analysis for immune stimulus /response	WP24	5	45	
MS58	Design of primers for amplification of amberjack	WP25	5	18	

# WT4: List of Milestones

Milestone number <sup>59</sup>	Milestone name	WP number <sup>53</sup>	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
	target gene DNA sequences				
MS59	Successful Chlamydia screening and sequencing	WP25	5	30	
MS60	Samples collected from stimulated primary cultures/explants, ready for immune gene expression analysis	WP25	5	30	
MS61	Ideas for new products	WP28	1	18	
MS62	Optional physical new products	WP28	1	26	
MS63	Insights in the consumer and B2B market for cultured fish	WP29	11	12	
MS64	Selection of new products, with good sensory perception	WP29	11	30	
MS65	Intrinsic and extrinsic attributes related to the new products	WP29	11	36	
MS66	Communication concept for behavioral change to cultured fish	WP29	11	44	
MS67	Business models to market the new products	WP30	10	48	
MS68	Product marketing strategy	WP30	10	54	
MS69	Marketable new products	WP30	10	60	
MS70	Agreement on project logo for website and publications, this will provide a recognizable image of DI	WP31	1	6	
MS71	Design and printing of project brochure (hard-copy) including	WP31	18	6	



# WT4: List of Milestones

Milestone number <sup>59</sup>	Milestone name	WP number <sup>53</sup>	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
	the project logo, inserts with project				
MS72	Agreements with food industry and consumers association for web linkage	WP31	18	9	
MS73	Agreement on the Promotional workshop (1st) program	WP31	18	32	
MS74	Agreement on the Promotional workshop (2nd) program	WP31	18	32	
MS75	Agreement on the Promotional workshop (3rd) program	WP31	18	42	
MS76	Agreement on the Promotional workshop (4th) program	WP31	18	48	
MS77	Agreement on the one-day State-of-the-art seminar program for meagre	WP31	3	49	
MS78	Agreement on the one-day State-of-the-art seminar program for greater amberjack	WP31	1	49	
MS79	Agreement on the one-day State-of-the-art seminar program for pike perch	WP31	9	50	
MS80	Agreement on the one-day State-of-the-art seminar program for Atlantic halibut	WP31	7	50	
MS81	Agreement on the one-day State-of-the-art seminar program for wreckfish	WP31	8	51	

# WT4: List of Milestones

Milestone number <sup>59</sup>	Milestone name	WP number <sup>53</sup>	Lead beneficiary number	Delivery date from Annex I <sup>60</sup>	Comments
MS82	Agreement on the one-day State-of-the-art seminar program for grey mullet	WP31	4	52	

# WT5:

## Tentative schedule of Project Reviews

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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### Tentative schedule of Project Reviews

Review number <sup>65</sup>	Tentative timing	Planned venue of review	Comments, if any
RV 1	30	Brussels	<p>The project Review should take place after the scheduled Mid-term evaluation by the Steering Committee, Group Work Package leaders (GWPL) and Species Leaders (SL): A mid-term progress evaluation will be undertaken at Y3 in a joined meeting of the SC and SLs. The achieved work will be evaluated vis-à-vis the Technical Annex and any deviations will be addressed. We will examine if there is a need to modify the planned work and take any corrective measures. A report will be produced and be available to the EU (D1.5).</p>

## Project Effort by Beneficiary and Work Package

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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### Indicative efforts (man-months) per Beneficiary per Work Package

Beneficiary number and short-name	WP 1	WP 2	WP 3	WP 4	WP 5	WP 6	WP 7	WP 8	WP 9	WP 10	WP 11	WP 12	WP 13	WP 14	WP 15	WP 16	WP 17	WP 18	WP 19
-35 - MASZ	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
-17 - NIFES	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1 - HCMR	58.00	15.00	24.00	4.00	0.50	7.90	0.50	0.00	6.00	0.00	0.00	0.00	0.00	0.00	10.50	0.00	0.00	6.50	0.00
2 - FCPCT	5.00	7.00	19.00	0.00	0.00	0.00	0.00	20.00	18.00	5.00	0.00	2.00	20.00	0.00	40.00	0.00	0.00	0.00	0.00
3 - IRTA	1.35	15.90	0.00	0.00	0.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	1.10	2.40	0.00	2.00	0.00	0.00	2.00
4 - IOLR	2.00	0.00	14.00	0.00	0.00	0.00	7.00	0.00	0.00	0.00	0.00	0.00	38.00	0.00	0.00	0.00	0.00	0.00	11.00
5 - UNIABDN	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6 - DLO	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7 - IMR	0.50	0.00	0.00	0.00	8.00	0.00	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	13.00	0.00	0.00
8 - IEO	0.50	0.00	5.60	0.00	0.00	6.60	0.00	0.00	2.80	0.00	0.00	1.10	0.00	0.00	5.50	0.00	0.00	4.20	0.00
9 - UL	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.00	0.00	0.00	0.00
10 - TU/e	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11 - AU	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12 - APROMAR	0.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13 - UNIBA	0.50	0.00	30.00	0.00	0.00	0.00	3.70	0.00	0.00	0.00	0.00	0.00	5.30	0.00	0.00	0.00	0.00	0.00	0.00
14 - IFREMER	0.25	2.75	2.50	0.00	0.00	3.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15 - ULL	0.50	0.00	3.80	0.00	0.00	0.90	3.40	4.50	5.50	4.00	4.50	0.00	0.00	1.30	8.50	0.00	0.00	0.00	0.00
16 - FUNDP	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17 - IMR/NIFES	0.50	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	6.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00

# WT6:

## Project Effort by Beneficiary and Work Package

Beneficiary number and short-name	WP 1	WP 2	WP 3	WP 4	WP 5	WP 6	WP 7	WP 8	WP 9	WP 10	WP 11	WP 12	WP 13	WP 14	WP 15	WP 16	WP 17	WP 18	WP 19
18 - CTAQUA	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.75	0.00	0.00	0.00	0.00	0.00	0.00
19 - CMRM	0.50	0.00	0.00	0.00	0.00	11.70	0.00	0.00	0.00	0.00	0.00	3.50	0.00	0.00	0.00	0.00	0.00	3.10	0.00
20 - SARC	0.50	0.00	0.00	0.00	0.00	0.00	0.00	1.50	2.50	0.00	1.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21 - DTU	0.50	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	6.50	0.00	0.00	0.00	0.00	0.00	4.50	0.00	0.00	0.00
22 - SWH	0.50	0.00	0.00	0.00	11.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.00	0.00	0.00
23 - ARGO	0.50	0.00	80.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24 - ITTICAL	0.29	0.00	9.00	0.00	0.00	0.00	3.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25 - DOR	0.10	0.00	0.00	0.00	0.00	0.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00
26 - GEI	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27 - FORKYS	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.00	0.00	0.00	0.00	0.00
28 - CANEXMAR	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
29 - ASIALOR	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.50	0.00	0.00	0.00	0.00	0.00	7.50	0.00	0.00	0.00
30 - CULMAREX	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31 - IRIDA	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32 - MC2	0.25	0.00	0.00	0.00	0.00	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.01	0.00
33 - FGM	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
34 - BVFi	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35 - MAHAL	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
36 - ANF	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37 - EUFIC	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
38 - HRH	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39 - F2B	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.00	0.00	0.00	0.00	0.00	0.00	7.70	0.00	0.00	0.00
40 - GMF	0.50	0.00	9.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

# WT6:

## Project Effort by Beneficiary and Work Package

Beneficiary number and short-name	WP 1	WP 2	WP 3	WP 4	WP 5	WP 6	WP 7	WP 8	WP 9	WP 10	WP 11	WP 12	WP 13	WP 14	WP 15	WP 16	WP 17	WP 18	WP 19
Total	84.01	40.65	197.40	5.00	20.50	34.16	23.30	27.00	46.80	38.00	14.20	6.60	68.15	3.70	79.50	34.70	20.00	14.81	16.00

# WT6:

## Project Effort by Beneficiary and Work Package

Beneficiary number and short-name	WP 20	WP 21	WP 22	WP 23	WP 24	WP 25	WP 26	WP 27	WP 28	WP 29	WP 30	WP 31	Total per Beneficiary
-35 - MASZ	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
-17 - NIFES	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1 - HCMR	48.50	7.50	0.00	7.00	73.10	17.00	0.00	0.00	35.00	7.00	0.00	8.00	336.00
2 - FCPCT	0.00	36.00	0.00	0.00	29.00	55.00	0.00	0.00	0.00	0.00	0.00	0.00	256.00
3 - IRTA	12.00	0.00	0.00	3.00	23.00	0.00	0.00	0.00	18.25	8.00	3.00	1.00	97.00
4 - IOLR	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00
5 - UNIABDN	0.00	0.00	0.00	0.00	20.00	17.00	0.00	0.00	0.00	0.00	0.00	0.00	38.00
6 - DLO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.00	2.85	12.50	12.83	0.00	37.18
7 - IMR	0.00	0.00	0.00	0.00	0.00	0.00	4.16	0.00	0.00	0.00	0.00	1.00	28.66
8 - IEO	0.00	3.40	0.00	0.00	0.00	1.10	0.00	0.00	0.00	0.00	0.00	0.70	31.50
9 - UL	0.00	0.00	18.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	34.00
10 - TU/e	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.50	0.50	0.00	21.00	0.00	27.50
11 - AU	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.15	2.15	21.88	7.82	0.00	34.50
12 - APROMAR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.00	0.00	0.00	8.14	2.74	20.49
13 - UNIBA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50	41.00
14 - IFREMER	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.00
15 - ULL	0.00	4.50	0.00	0.00	0.00	5.00	0.00	0.00	6.50	0.00	0.00	0.00	52.90
16 - FUNDP	0.00	0.00	26.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	37.50
17 - IMR/NIFES	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.50
18 - CTAQUA	0.00	0.00	0.00	5.80	0.00	0.00	0.00	0.00	7.30	8.20	1.00	13.10	39.65
19 - CMRM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.80
20 - SARC	0.00	0.00	0.00	0.00	3.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.50
21 - DTU	0.00	0.00	7.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.50
22 - SWH	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.50
23 - ARGO	30.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	110.60

# WT6:

## Project Effort by Beneficiary and Work Package

Beneficiary number and short-name	WP 20	WP 21	WP 22	WP 23	WP 24	WP 25	WP 26	WP 27	WP 28	WP 29	WP 30	WP 31	Total per Beneficiary
24 - ITTICAL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.49
25 - DOR	0.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	16.20
26 - GEI	0.00	0.00	0.00	12.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.50
27 - FORKYS	0.00	15.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.50
28 - CANEXMAR	0.00	45.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	57.60
29 - ASIALOR	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.25
30 - CULMAREX	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.10
31 - IRIDA	0.00	0.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.25
32 - MC2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.32
33 - FGM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.50	2.75
34 - BVFi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.58
35 - MAHAL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	3.25
36 - ANF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.25	3.00
37 - EUFIC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	3.25
38 - HRH	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.30	17.90	1.00	0.00	24.80
39 - F2B	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	20.10
40 - GMF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00
<b>Total</b>	<b>91.50</b>	<b>111.40</b>	<b>61.00</b>	<b>43.80</b>	<b>148.40</b>	<b>95.10</b>	<b>4.16</b>	<b>23.65</b>	<b>77.85</b>	<b>75.48</b>	<b>55.19</b>	<b>40.21</b>	<b>1,602.22</b>



## Project Effort by Activity type per Beneficiary

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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### Indicative efforts per Activity Type per Beneficiary

Activity type	Part. -35 MASZ	Part. -17 NIFES	Part. 1 HCMR	Part. 2 FCPCT	Part. 3 IRTA	Part. 4 IOLR	Part. 5 UNIABDN	Part. 6 DLO	Part. 7 IMR	Part. 8 IEO	Part. 9 UL	Part. 10 TU/e	Part. 11 AU	Part. 12 APROMAR
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1. RTD/Innovation activities														
WP 2	0.00	0.00	15.00	7.00	15.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 3	0.00	0.00	24.00	19.00	0.00	14.00	0.00	0.00	0.00	5.60	0.00	0.00	0.00	0.00
WP 4	0.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
WP 5	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	8.00	0.00	0.00	0.00	0.00	0.00
WP 6	0.00	0.00	7.90	0.00	2.00	0.00	0.00	0.00	0.00	6.60	0.00	0.00	0.00	0.00
WP 7	0.00	0.00	0.50	0.00	2.00	7.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 8	0.00	0.00	0.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 9	0.00	0.00	6.00	18.00	0.00	0.00	0.00	0.00	0.00	2.80	0.00	0.00	0.00	0.00
WP 10	0.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00
WP 12	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	1.10	0.00	0.00	0.00	0.00
WP 13	0.00	0.00	0.00	20.00	1.10	38.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 14	0.00	0.00	0.00	0.00	2.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 15	0.00	0.00	10.50	40.00	0.00	0.00	0.00	0.00	0.00	5.50	0.00	0.00	0.00	0.00
WP 16	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	13.00	0.00	0.00	0.00
WP 17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.00	0.00	0.00	0.00	0.00	0.00
WP 18	0.00	0.00	6.50	0.00	0.00	0.00	0.00	0.00	0.00	4.20	0.00	0.00	0.00	0.00
WP 19	0.00	0.00	0.00	0.00	2.00	11.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 20	0.00	0.00	48.50	0.00	12.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 21	0.00	0.00	7.50	36.00	0.00	0.00	0.00	0.00	0.00	3.40	0.00	0.00	0.00	0.00
WP 22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.00	0.00	0.00	0.00

## Project Effort by Activity type per Beneficiary

1. RTD/Innovation activities														
WP 23	0.00	0.00	7.00	0.00	3.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 24	0.00	0.00	73.10	29.00	23.00	0.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 25	0.00	0.00	17.00	55.00	0.00	0.00	17.00	0.00	0.00	1.10	0.00	0.00	0.00	0.00
WP 26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.16	0.00	0.00	0.00	0.00	0.00
WP 27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.00	0.00	0.00	0.00	5.50	2.15	9.00
WP 28	0.00	0.00	35.00	0.00	18.25	0.00	0.00	2.85	0.00	0.00	0.00	0.50	2.15	0.00
WP 29	0.00	0.00	7.00	0.00	8.00	0.00	0.00	12.50	0.00	0.00	0.00	0.00	21.88	0.00
WP 30	0.00	0.00	0.00	0.00	3.00	0.00	0.00	12.83	0.00	0.00	0.00	21.00	7.82	8.14
<b>Total Research</b>	0.00	0.00	270.00	251.00	94.65	72.00	37.00	35.18	27.16	30.30	32.00	27.00	34.00	17.14

2. Demonstration activities														
<b>Total Demo</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

3. Consortium Management activities														
WP 1	0.00	0.00	58.00	5.00	1.35	2.00	1.00	2.00	0.50	0.50	1.00	0.50	0.50	0.61
<b>Total Management</b>	0.00	0.00	58.00	5.00	1.35	2.00	1.00	2.00	0.50	0.50	1.00	0.50	0.50	0.61

4. Other activities														
WP 31	0.00	0.00	8.00	0.00	1.00	0.00	0.00	0.00	1.00	0.70	1.00	0.00	0.00	2.74
<b>Total other</b>	0.00	0.00	8.00	0.00	1.00	0.00	0.00	0.00	1.00	0.70	1.00	0.00	0.00	2.74

<b>Total</b>	0.00	0.00	336.00	256.00	97.00	74.00	38.00	37.18	28.66	31.50	34.00	27.50	34.50	20.49
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## Project Effort by Activity type per Beneficiary

Activity type	Part. 13 UNIBA	Part. 14 IFREMER	Part. 15 ULL	Part. 16 FUNDP	Part. 17 IMR/NIF	Part. 18 CTAQUA	Part. 19 CMRM	Part. 20 SARC	Part. 21 DTU	Part. 22 SWH	Part. 23 ARGO	Part. 24 ITTICAL	Part. 25 DOR	Part. 26 GEI
1. RTD/Innovation activities														
WP 2	0.00	2.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 3	30.00	2.50	3.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	80.00	9.00	0.00	0.00
WP 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 5	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	11.00	0.00	0.00	0.00	0.00
WP 6	0.00	3.00	0.90	0.00	0.00	0.00	11.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 7	3.70	0.50	3.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.20	3.00	0.00
WP 8	0.00	0.00	4.50	0.00	0.00	0.00	0.00	1.50	1.00	0.00	0.00	0.00	0.00	0.00
WP 9	0.00	0.00	5.50	0.00	0.00	0.00	0.00	2.50	0.00	0.00	0.00	0.00	0.00	0.00
WP 10	0.00	0.00	4.00	11.00	0.00	0.00	0.00	0.00	6.50	0.00	0.00	0.00	0.00	0.00
WP 11	0.00	0.00	4.50	0.00	6.00	0.00	0.00	1.70	0.00	0.00	0.00	0.00	0.00	0.00
WP 12	0.00	0.00	0.00	0.00	0.00	0.00	3.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 13	5.30	0.00	0.00	0.00	0.00	3.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 14	0.00	0.00	1.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 15	0.00	0.00	8.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.50	0.00	0.00	0.00	0.00	0.00
WP 17	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	6.00	0.00	0.00	0.00	0.00
WP 18	0.00	0.00	0.00	0.00	0.00	0.00	3.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.00
WP 20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.00	0.00	0.00	0.00
WP 21	0.00	0.00	4.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 22	0.00	0.00	0.00	26.00	0.00	0.00	0.00	0.00	7.00	0.00	0.00	0.00	0.00	0.00
WP 23	0.00	0.00	0.00	0.00	0.00	5.80	0.00	0.00	0.00	0.00	0.00	0.00	10.00	12.00
WP 24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.30	0.00	0.00	0.00	0.00	0.00	0.00

## Project Effort by Activity type per Beneficiary

1. RTD/Innovation activities														
WP 25	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 28	0.00	0.00	6.50	0.00	0.00	7.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 29	0.00	0.00	0.00	0.00	0.00	8.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 30	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.10	0.00	0.10	0.00
<b>Total Research</b>	<b>39.00</b>	<b>8.75</b>	<b>52.40</b>	<b>37.00</b>	<b>8.00</b>	<b>26.05</b>	<b>18.30</b>	<b>9.00</b>	<b>19.00</b>	<b>17.00</b>	<b>110.10</b>	<b>12.20</b>	<b>16.10</b>	<b>12.00</b>
2. Demonstration activities														
Total Demo	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3. Consortium Management activities														
WP 1	0.50	0.25	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.29	0.10	0.50
<b>Total Management</b>	<b>0.50</b>	<b>0.25</b>	<b>0.50</b>	<b>0.50</b>	<b>0.50</b>	<b>0.50</b>	<b>0.50</b>	<b>0.50</b>	<b>0.50</b>	<b>0.50</b>	<b>0.50</b>	<b>0.29</b>	<b>0.10</b>	<b>0.50</b>
4. Other activities														
WP 31	1.50	0.00	0.00	0.00	0.00	13.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Total other</b>	<b>1.50</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>13.10</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
<b>Total</b>	<b>41.00</b>	<b>9.00</b>	<b>52.90</b>	<b>37.50</b>	<b>8.50</b>	<b>39.65</b>	<b>18.80</b>	<b>9.50</b>	<b>19.50</b>	<b>17.50</b>	<b>110.60</b>	<b>12.49</b>	<b>16.20</b>	<b>12.50</b>

## Project Effort by Activity type per Beneficiary

Activity type	Part. 27 FORKYS	Part. 28 CANEXMA	Part. 29 ASIALOR	Part. 30 CULMARE	Part. 31 IRIDA	Part. 32 MC2	Part. 33 FGM	Part. 34 BVi	Part. 35 MAHAL	Part. 36 ANF	Part. 37 EUFIC	Part. 38 HRH	Part. 39 F2B	Part. 40 GMF	Total
1. RTD/Innovation activities															
WP 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	40.65
WP 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.50	197.40
WP 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00
WP 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.50
WP 6	0.00	0.00	0.00	0.00	0.00	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	34.16
WP 7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.30
WP 8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.00
WP 9	0.00	12.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	46.80
WP 10	0.00	0.00	4.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.00	0.00	38.00
WP 11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.20
WP 12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.60
WP 13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	68.15
WP 14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.70
WP 15	15.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	79.50
WP 16	0.00	0.00	7.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.70	0.00	34.70
WP 17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.00
WP 18	0.00	0.00	0.00	0.00	0.00	1.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.81
WP 19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.00
WP 20	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	91.50
WP 21	15.00	45.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	111.40
WP 22	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00	0.00	61.00
WP 23	0.00	0.00	0.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	43.80
WP 24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	148.40

## Project Effort by Activity type per Beneficiary

1. RTD/Innovation activities															
WP 25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	95.10
WP 26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.16
WP 27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.65
WP 28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.30	0.00	0.00	77.85
WP 29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.90	0.00	0.00	75.48
WP 30	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.10	0.00	55.19
<b>Total Research</b>	<b>30.00</b>	<b>57.10</b>	<b>17.00</b>	<b>1.00</b>	<b>4.00</b>	<b>3.07</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>24.20</b>	<b>19.80</b>	<b>9.50</b>	<b>1,478.00</b>
2. Demonstration activities															
Total Demo	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3. Consortium Management activities															
WP 1	0.50	0.50	0.25	0.10	0.25	0.25	0.25	0.16	0.25	0.75	0.25	0.60	0.30	0.50	84.01
Total Management	0.50	0.50	0.25	0.10	0.25	0.25	0.25	0.16	0.25	0.75	0.25	0.60	0.30	0.50	84.01
4. Other activities															
WP 31	0.00	0.00	0.00	0.00	0.00	0.00	2.50	0.42	3.00	2.25	3.00	0.00	0.00	0.00	40.21
Total other	0.00	0.00	0.00	0.00	0.00	0.00	2.50	0.42	3.00	2.25	3.00	0.00	0.00	0.00	40.21
<b>Total</b>	<b>30.50</b>	<b>57.60</b>	<b>17.25</b>	<b>1.10</b>	<b>4.25</b>	<b>3.32</b>	<b>2.75</b>	<b>0.58</b>	<b>3.25</b>	<b>3.00</b>	<b>3.25</b>	<b>24.80</b>	<b>20.10</b>	<b>10.00</b>	<b>1,602.22</b>

# WT8: Project Effort and costs

Project Number <sup>1</sup>	603121	Project Acronym <sup>2</sup>	DIVERSIFY
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## Project efforts and costs

Beneficiary number	Beneficiary short name	Estimated eligible costs (whole duration of the project)						Requested EU contribution (€)
		Effort (PM)	Personnel costs (€)	Subcontracting (€)	Other Direct costs (€)	Indirect costs OR lump sum, flat-rate or scale-of-unit (€)	Total costs	
-35 (UTRO)	MASZ	0.00	6,500.00	0.00	10,000.00	3,300.00	19,800.00	19,800.00
-17 (UTRO)	NIFES	0.00	81,815.00	0.00	36,183.00	82,435.00	200,433.00	152,424.00
1	HCMR	336.00	698,803.00	18,000.00	475,679.00	566,031.00	1,758,513.00	1,401,588.00
2	FCPCT	256.00	353,344.00	2,000.00	197,325.00	330,401.40	883,070.40	665,212.00
3	IRTA	97.00	404,443.00	2,000.00	397,424.00	192,522.00	996,389.00	760,653.00
4	IOLR	74.00	166,311.00	2,200.00	188,125.00	212,661.60	569,297.60	430,061.00
5	UNIABDN	38.00	91,152.00	0.00	87,668.00	107,292.00	286,112.00	217,008.00
6	DLO	37.18	248,140.00	0.00	21,734.00	124,070.00	393,944.00	299,958.00
7	IMR	28.66	183,553.00	4,851.00	266,928.00	322,015.00	777,347.00	592,260.00
8	IEO	31.50	139,867.00	0.00	214,677.00	212,726.40	567,270.40	432,702.00
9	UL	34.00	115,558.00	0.00	71,915.00	112,483.80	299,956.80	233,942.00
10	TU/e	27.50	189,525.00	0.00	24,526.00	138,004.00	352,055.00	267,879.00
11	AU	34.50	233,811.00	2,500.00	10,500.00	146,586.60	393,397.60	298,113.00
12	APROMAR	20.49	92,762.00	0.00	21,757.00	22,903.80	137,422.80	87,397.00
13	UNIBA	41.00	85,205.00	0.00	65,367.00	90,343.20	240,915.20	188,389.00
14	IFREMER	9.00	69,409.00	0.00	22,000.00	43,726.00	135,135.00	103,351.00
15	ULL	52.90	161,462.00	0.00	36,347.00	118,685.40	316,494.40	240,021.00
16	FUNDP	37.50	137,480.00	0.00	49,740.00	112,332.00	299,552.00	226,716.00
17	IMR/NIFES	8.50	0.00	0.00	0.00	0.00	0.00	0.00
18	CTAQUA	39.65	137,940.00	0.00	52,956.00	114,537.60	305,433.60	253,619.00

# WT8: Project Effort and costs

Beneficiary number	Beneficiary short name	Estimated eligible costs (whole duration of the project)						Requested EU contribution (€)
		Effort (PM)	Personnel costs (€)	Subcontracting (€)	Other Direct costs (€)	Indirect costs OR lump sum, flat-rate or scale-of-unit (€)	Total costs	
19	CMRM	18.80	55,000.00	0.00	65,979.00	72,587.40	193,566.40	147,299.00
20	SARC	9.50	93,950.00	0.00	39,000.00	22,400.00	155,350.00	85,125.00
21	DTU	19.50	130,717.00	0.00	30,327.00	135,717.00	296,761.00	220,997.00
22	SWH	17.50	96,047.00	0.00	95,055.00	53,786.00	244,888.00	127,252.00
23	ARGO	110.60	149,000.00	0.00	153,959.00	181,775.40	484,734.40	365,800.00
24 (TERMINATED)	ITTICAL	12.49	31,251.00	0.00	30,746.00	37,198.20	99,195.20	75,087.00
25	DOR	16.20	28,400.00	0.00	19,875.00	28,965.00	77,240.00	58,490.00
26	GEI	12.50	20,000.00	0.00	8,500.00	17,100.00	45,600.00	35,000.00
27	FORKYS	30.50	17,100.00	0.00	28,130.00	27,138.00	72,368.00	55,608.00
28	CANEXMAR	57.60	72,000.00	0.00	48,150.00	72,090.00	192,240.00	146,440.00
29 (TERMINATED)	ASIALOR	17.25	54,128.00	0.00	22,173.00	43,303.00	119,604.00	90,372.00
30 (TERMINATED)	CULMAREX	1.10	8,453.00	0.00	1,484.00	8,645.00	18,582.00	10,040.00
31	IRIDA	4.25	14,995.00	0.00	30,600.00	27,357.00	72,952.00	56,954.00
32	MC2	3.32	13,504.00	0.00	18,716.00	19,332.00	51,552.00	40,784.00
33	FGM	2.75	6,900.00	0.00	9,100.00	3,200.00	19,200.00	19,200.00
34	BVFi	0.58	5,214.00	0.00	14,450.00	11,798.40	31,462.40	31,461.00
35	MAHAL	3.25	0.00	0.00	0.00	0.00	0.00	0.00
36	ANF	3.00	8,516.00	0.00	8,700.00	3,443.20	20,659.20	20,658.00
37	EUFIC	3.25	19,500.00	8,700.00	6,150.00	5,130.00	39,480.00	39,480.00
38	HRH	24.80	122,034.00	54,852.00	51,666.00	104,220.00	332,772.00	251,579.00
39	F2B	20.10	70,050.00	15,000.00	28,800.00	59,310.00	173,160.00	130,350.00
40	GMF	10.00	57,400.00	0.00	39,600.00	11,648.00	108,648.00	81,936.00



# WT8: Project Effort and costs

Beneficiary number	Beneficiary short name	Estimated eligible costs (whole duration of the project)						Requested EU contribution (€)
		Effort (PM)	Personnel costs (€)	Subcontracting (€)	Other Direct costs (€)	Indirect costs OR lump sum, flat-rate or scale-of-unit (€)	Total costs	
Total		1,602.22	4,671,239.00	110,103.00	3,002,011.00	3,999,200.40	11,782,553.40	8,961,005.00

### 1. Project number

The project number has been assigned by the Commission as the unique identifier for your project. It cannot be changed. The project number **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

### 2. Project acronym

Use the project acronym as given in the submitted proposal. It cannot be changed unless agreed so during the negotiations. The same acronym **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

### 53. Work Package number

Work package number: WP1, WP2, WP3, ..., WPn

### 54. Type of activity

For all FP7 projects each work package must relate to one (and only one) of the following possible types of activity (only if applicable for the chosen funding scheme – must correspond to the GPF Form Ax.v):

- **RTD/INNO** = Research and technological development including scientific coordination - applicable for Collaborative Projects and Networks of Excellence
- **DEM** = Demonstration - applicable for collaborative projects and Research for the Benefit of Specific Groups
- **MGT** = Management of the consortium - applicable for all funding schemes
- **OTHER** = Other specific activities, applicable for all funding schemes
- **COORD** = Coordination activities – applicable only for CAs
- **SUPP** = Support activities – applicable only for SAs

### 55. Lead beneficiary number

Number of the beneficiary leading the work in this work package.

### 56. Person-months per work package

The total number of person-months allocated to each work package.

### 57. Start month

Relative start date for the work in the specific work packages, month 1 marking the start date of the project, and all other start dates being relative to this start date.

### 58. End month

Relative end date, month 1 marking the start date of the project, and all end dates being relative to this start date.

### 59. Milestone number

Milestone number: MS1, MS2, ..., MSn

### 60. Delivery date for Milestone

Month in which the milestone will be achieved. Month 1 marking the start date of the project, and all delivery dates being relative to this start date.

### 61. Deliverable number

Deliverable numbers in order of delivery dates: D1 – Dn

### 62. Nature

Please indicate the nature of the deliverable using one of the following codes

**R** = Report, **P** = Prototype, **D** = Demonstrator, **O** = Other

### 63. Dissemination level

Please indicate the dissemination level using one of the following codes:

- **PU** = Public
- **PP** = Restricted to other programme participants (including the Commission Services)
- **RE** = Restricted to a group specified by the consortium (including the Commission Services)
- **CO** = Confidential, only for members of the consortium (including the Commission Services)

- **Restreint UE** = Classified with the classification level "Restreint UE" according to Commission Decision 2001/844 and amendments
- **Confidentiel UE** = Classified with the mention of the classification level "Confidentiel UE" according to Commission Decision 2001/844 and amendments
- **Secret UE** = Classified with the mention of the classification level "Secret UE" according to Commission Decision 2001/844 and amendments

**64. Delivery date for Deliverable**

Month in which the deliverables will be available. Month 1 marking the start date of the project, and all delivery dates being relative to this start date

**65. Review number**

Review number: RV1, RV2, ..., RVn

**66. Tentative timing of reviews**

Month after which the review will take place. Month 1 marking the start date of the project, and all delivery dates being relative to this start date.

**67. Person-months per Deliverable**

The total number of person-month allocated to each deliverable.



**PART B (amendment 4, 2018)**

**Proposal full title:**

Exploring the biological and socio-economic potential of new/emerging candidate fish species for the expansion of the European aquaculture industry.

**Proposal acronym:**

DIVERSIFY



**New species for EU aquaculture**



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## **B1. Concept and objectives, progress beyond state-of-the-art, S/T methodology and work plan**

### ***B1.1 Concept and objectives***

*Describe the objectives of the project in detail, in particular in a measurable and verifiable form.*

*The objectives should be those achievable within the project (not through subsequent development) and be specific and timed (e.g., by which date and milestone the objective will be achieved), well in line with the milestones that will be indicated under section 1.3 below.*

The European aquaculture sector is a modern industry employing 190,000 people (directly or indirectly), with a €7 billion ex-farm value (EATIP, 2012). Many world-class researchers and facilities exist in various research centres and universities throughout Europe, while the private sector employs highly skilled and educated personnel, with modern production facilities. This sector is well situated to be among world leaders in the **efficient and sustainable production of safe seafood of the highest quality and nutritional value**, taking into account consumer preferences and lifestyles, and the immense diversity of aquatic products from the wild, to which the consumer is accustomed (EATIP, 2012).

Aquaculture is undertaken in all EU and EEA member states, and plays an important role in the supply of high quality seafood to the European consumer (EATIP, 2012). Europe has an increasing demand for a diverse range of fish products especially for **fish fillets or processed products** (Failler, 2007; FAO, 2012). However, while the worldwide contribution of aquaculture towards fish consumption (63.6 million t) is just shy of 50% (131 million t in total) (FAO, 2012), in the EU only 10% of the seafood consumption originated from EU aquaculture (Bianci, 2012) and the consumption of imported seafood is currently at 65% today (EATIP, 2012). This situation can be attributed partially to a lack of diversity of aquaculture products and, perhaps more importantly, **a lack of processed aquaculture products**.

Even though some 35 aquatic species are cultured in Europe, **finfish aquaculture production is dominated both in volume and value by a handful of species** --such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*), European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*)-- that in turn limit the number of aquaculture products available in the market. All these species have experienced periods of pricing problems when production had surpassed demand resulting in price decreases, often close to or below the cost of production. Combined with less expensive imports, these pricing problems have slowed or even decreased the growth of aquaculture in the EU. At the same time, imports have been increasing steadily (EATIP, 2012). **An efficient, sustainable and market-oriented expansion of the EU aquaculture sector based on new species and products will reduce the dependence of the EU consumer on imports** from countries of questionable, often, production, health, environmental and social standards, **and reduce the pressure on over-exploited fisheries in the EU**.

Periods of pricing problems are common for perishable products that have high production. Common solutions to this situation are to (a) reduce production costs to offer a lower market price, (b) increase marketing to expand the market or (c) transform the product into new products that are demanded by the market. Atlantic salmon has been one of success stories of European aquaculture, probably because it has used these three solutions to surpass many periods of pricing problems and continues to increase production and market share. A significant part of the success story of Atlantic salmon is its relatively **fast growth and large size (~3 kg), which provides excellent opportunities for processing and development of value added products**. This has enabled Atlantic salmon to provide new products, which cannot be obtained from many of the current aquaculture species. Most of these are marketed whole at a small size, since filleting has a very low yield (30-40%). Following the objectives of this Call, DIVERSIFY identified a number of new/emerging, large and/or fast growing finfish species, which are believed to be excellent candidates for the expansion of the aquaculture industry of Europe. The emphasis is on the **Mediterranean or warm-water cage culture industry**, but also addressed is **pond/extensive culture, fresh water recirculation systems and cold-water species**. **These new/emerging species are marketed at a large size** and can be processed easily into a range of products to provide the consumer with both a greater diversity of fish species and new processed products. In collaboration with a number of SMEs, DIVERSIFY **will build on recent/current**



**national initiatives for species diversification in aquaculture**, in order to **overcome the documented bottlenecks in the aquaculture production** of these selected species.

**DIVERSIFY will provide the knowledge needed to repeat** the success story of Atlantic salmon aquaculture **with these new/emerging species**. Atlantic salmon aquaculture technologies, particularly production of good quality juveniles and cage-based grow-out, developed first in the 70s. Pricing problems starting in the 90s put strong pressure to cut production costs and improve marketing. Production costs were cut first with dietary improvements (reducing feed costs and improving growth). Then, selective breeding resulted in additional improvement in growth. Improved marketing development of processed products, fillets, steaks, smoked, battered “fish finger” type products and various other value added products contributed decisively to ameliorate pricing problems. Today, health is the major issue that affects salmon production. **DIVERSIFY will provide knowledge where needed to solve bottlenecks in juvenile production, grow-out, nutrition and feeding husbandry, new product development and marketing**. The programme will also provide tools for genetic improvement and disease control. This will provide improved efficiency in production and reduced costs, and identify markets for the new products. The expertise in the consortium and lessons learned, could provide in a 5 year period what took the Atlantic salmon industry 20 years of development

A **strong socioeconomic component** is also included in DIVERSIFY, in order to address issues that are presently important bottlenecks in **aquaculture consumption and diversification** --which are beyond biological/production issues. The socioeconomic part of the project has an applied market development approach. In this component the perception of aquaculture products in general and products specifically, market demand, consumer and professional buyer preferences, new product development, value adding to raw products and market development have a central role. An important bottleneck in **aquaculture consumption** is that in many countries and/or segments of the EU market, aquaculture fish have a weaker image than wild fish. This threat to the expansion of the aquaculture sector must be recognized and addressed in parallel to any technological improvement of production methods or the addition of new fish species or products by the aquaculture industry.

The combination of biological, technological and socioeconomic research activities planned in DIVERSIFY are expected to **support the diversification of the aquaculture industry and help in expanding production, increasing aquaculture products and development of new markets**.

### *Species selection*

DIVERSIFY focuses on **meagre** (*Argyrosomus regius*) and **greater amberjack** (*Seriola dumerili*) for marine warm-water cage culture, **wreckfish** (*Polyprion americanus*) for warm- and cool-water marine cage culture, **Atlantic halibut** (*Hippoglossus hippoglossus*) for marine cold-water culture, **grey mullet** (*Mugil cephalus*) a euryhaline herbivore for warm-water pond, extensive and integrated culture, and **pikeperch** (*Sanders lucioperca*) for freshwater intensive culture using Recirculation Aquaculture Systems (RAS). These species were selected based both on their **biological and economical potential**, and to **cover the entire European geographic area and stimulate different aquaculture types**.

Firstly, given their **large size and/or fast growth**, they provide for **high dress-out and fillet yield, short time to market and suitability for product diversification and development of value-added products**. This is the reason why species such as Senegalese sole (*Solea senegalensis*) or members of the Sparidae family that have been investigated by the industry over the past decade were not considered for this proposal. Another criterion included the potential of the fish to be reared in sea cages --especially offshore, where the future expansion of marine aquaculture may lie.

Secondly, since **aquaculture is of interest to European countries**, where different aquaculture methods are employed in diverse environmental and climatic conditions, species selection included **a freshwater fish** of high demand for RAS culture (pikeperch), **a cold-water species** of very high demand for production in the sub-arctic northern Europe (Atlantic halibut) and **a euryhaline warm-water fish suitable for extensive aquaculture** in earthen ponds, coastal lagoons, "valli" or "Salinas" (grey mullet).

Finally, all selected species are either **cosmopolitan species** found --and cultured in some cases-- throughout the world or their **very similar congeners are fished or cultured around the world**. As a result, these





species or their congeners **have existing markets** and the potential exist for the EU aquaculture production of these species to **reduce imports to the EU, as well as supply global markets**.

These six species are at **different stages of "inclusion" in the Aquaculture industry**, with meagre and Atlantic halibut being the most developed followed by pikeperch, the greater amberjack, and the wreckfish with no production and very limited research. The grey mullet is produced by capture-based aquaculture, using wild fry. In all, DIVERSIFY includes species that are already cultured to some degree and for which there is a need to **improve production technologies, diversify products and enhance marketing aspects to boost the growth of the aquaculture industry**, as well as other species that have a **high biological and economical potential** (Quemener et al., 2002; Teletchea and Fontaine, 2012), but as **new biological models**, require more work to enter commercial production.

Following the topics raised by the Call, work in DIVERSIFY will address in a species-specific way aspects related to reproduction and/or larval rearing and/or nutrition and/or husbandry technologies and/or fish health and/or product development-evaluation; a strong emphasis is also given on the evaluation of the socioeconomic potential of the selected species. DIVERSIFY will address the **main documented species-specific bottlenecks in the production** of the selected species, in order to **develop adequate husbandry practices and technologies for the industry** to enable production (greater amberjack and wreckfish) or to optimize production (meagre, pikeperch, Atlantic halibut and grey mullet).

The overall aim of this proposal is to **diversify and increase the fish aquaculture production** in Europe and **stimulate demand for EU-cultured fish in Europe** by overcoming particular bottlenecks in the selected species, enhancing the value added along the value chain by initiating product innovation, improving consumer acceptance, and developing the knowledge to expand current and create new markets in Europe and beyond by identifying and capturing local and international market opportunities.

Using specific questionnaires to the European aquaculture industry, the input of the participating SMEs and the extensive experience in finfish domestication of the participating RTD institutions, DIVERSIFY has **identified and prioritized the essential bottlenecks in the production of each of the selected species, and designed the work to address these bottlenecks**. The following sections provide a **justification for the selection of each species** and a description of the **major bottlenecks** in their production.

### *Meagre*

The meagre is found in the Mediterranean and Black Sea, and along the eastern Atlantic coast (Haffray et al., 2012). It has attractive attributes for the market that include **large size, good processing yield, low fat content, excellent taste and firm texture** (Monfort, 2010). The species also has the biological characteristics required for commercial aquaculture using well-established culture technologies (Papadakis et al., 2013). These characteristics include a **fast growth of ~1 Kg per year** (Duncan et al., 2013), a **low feed conversion ratio of 0.9-1.2** (Monfort, 2010; Duncan et al., 2013) –which is similar to the Atlantic salmon-, relatively easy larval rearing (Roo et al., 2010; Vallés & Estévez, 2012) and established induced spawning protocols for the production of viable eggs (Duncan et al., 2012). Meagre was first produced in 1997 in a commercial hatchery in France and since then it has exhibited annual production increases as high as 7 fold (FAO, 2012). In 2010, European meagre aquaculture production was 2,387 t, mainly in Spain, with smaller quantities from France, Portugal, Italy, Greece and Croatia (FAO, 2012).



The survey of meagre producers identified four principal bottlenecks to the expansion of the industry. Firstly, **variable growth rates** --whose exact cause is not obvious-- are reducing greatly yield (Duncan et al., 2013). A multidisciplinary approach is required in order to examine the role of genetics, nutrition --particularly dietary requirements during weaning, pre-ongrowing and in cage culture-- feeding behaviour and grow out husbandry. Secondly, the distribution of this fish only in specific areas in the Mediterranean region has resulted in the acquisition of broodstocks from a limited number of sources (mainly a hatchery in France), resulting perhaps in a **limited genetic variation of the available broodstocks**. This will have significant negative implications for the future initiation of breeding selection programs, which are necessary



to move the industry to the next level of efficiency and production. Thirdly, the industry must address issues in **fish health**, emerging diseases, parasites (Toksen et al., 2007; Merella et al., 2009; Ternengo et al., 2010; Koyuncu et al., 2012) and the wide occurrence of Systemic Granulomas (Elkesh et al., 2012), which may stem from the fact that no diets have been developed for this fish. Finally, **socioeconomic factors have been identified as bottlenecks**, including the need for a more expanded market and diversification of provided products (Monfort, 2010) beyond the whole fresh fish. **National initiatives for meagre domestication are underway in Spain and Greece, coordinated by Partners of the consortium, and DIVERSIFY will build on the acquired information by targeting specific issues recognized as bottlenecks for further production.**

### *Greater amberjack*



This is a cosmopolitan species (Andaloro & Pipitone, 1997; Cummings et al., 1999; Thompson et al., 1999) of great interest to the aquaculture sector due to its **excellent flesh quality, worldwide market availability** and high **consumer acceptability** (Nakada, 2000). Its rapid growth (*i.e.*, **short time to market size**) and large size makes this species **very suitable for product diversification and**

**development of value added products.** In the Mediterranean (Lovatelli & Holthus, 2008), farming started with capture-based activities using wild juveniles (Crespo et al., 1994). Fish of ~90 g reached ~1 kg in a year, and 6 kg in a period of 2.5 years (Jover et al., 1999; Mazzola et al., 2000). The high growth rate of cultured greater amberjack and its feeding on fish of low commercial value made the activity profitable. More recently, using standard dry feeds, wild caught individuals of 50-100 g exhibited great growth performance of 1.8, 4 and 7.5 kg body weight in 1, 2, and 3 years, respectively (Jover et al., 1999; Mazzola et al., 2000). Still, the Mediterranean production in 2012 was only ~2 t, while market price –mainly for capture fisheries catches-- reached values >14 € kg<sup>-1</sup>. Today, **a very limited commercial activity with hatchery-produced individuals exists in Malta**, though interest exists and efforts have been made by various aquaculture companies in Spain, Greece, Italy and Cyprus.

The **major bottlenecks for the incorporation of greater amberjack in the EU aquaculture industry** include lack of (a) **reliable reproduction** and (b) **production of adequate numbers of juveniles**. In captivity, reproduction has been problematic (Kozul et al., 2001), but **recently captive-reared broodstocks have reproduced after hormonal treatments** (Mylonas et al., 2004), and in some cases also spontaneously (Jerez et al., 2006). Also, some knowledge has been acquired on the nutritional requirements of reproduction (Rodríguez-Barreto et al., 2012). **DIVERSIFY will study the reproduction in captivity and in the wild, and develop spawning induction methods, as well as appropriate broodstock diets.**

**Larval rearing of greater amberjack** was done initially using semi-intensive methods (Papandroulakis et al., 2005). Survival was limited (3%), but recently it has been improved with adaptations in feeding regime and diet quality (Anonymous, 2008; Roo, et al., 2012). Since both the greater amberjack (Matsunari et al., 2012) and its congeners the yellowtail (*S. quinquerediata*) (Nakada, 2000), yellowtail kingfish (*S. lalandi*) (Ma et al., 2012) and almaco jack (*S. rivoliana*) (Roo et al., 2012) have been produced in hatcheries in other areas of the world, once the bottleneck of egg availability is surpassed, the available information on these congeners can hasten the **development of larval rearing protocols for the greater amberjack.**

Another area of concern for the commercial production of greater amberjack is **fish health**. Bacterial pathogens cited in the literature as potential threats include *Photobacterium damsella* (Crespo et al., 1994) and epitheliocystis (Rigos and Katharios, 2010) and *Cryptocaryon irritans* has caused severe losses in broodstock (Rigos et al., 2001). During grow out, monogenean parasites cause occasional mass mortalities in farmed fish (Grau et al., 2003; Montero et al., 2004), while *Neobenedenia* spp was identified in a major outbreak causing losses in both in juveniles and broodstock (P2. FPCPT). Therefore, DIVERSIFY will study the potential pathologies that will occur in the course of the project in an effort to develop **early diagnosis tools, veterinary solutions and preventive veterinary protocols** that will be available and will support the sustainable rearing of the species.



### ***Pikeperch***

This freshwater fish is considered to have the highest potential for inland aquaculture diversification in Europe (Wang et al., 2008). Based on EU projects LUCIOPERCA and LUCIOPERCIMPROVE, reproductive control (Kucharczyk et al., 2007) and bio-economic feasibility of pikeperch intensive rearing (Steenfeldt & Lund, 2008; Steenfeldt et al., 2010) have been demonstrated. **Pikeperch demand has been strengthened** by the strong decline of wild catches from Russia, Estonia and Finland from 50.000 t in 1950 to 20.000 t currently (FAO, 2009). Over the last decade, 10 new farms have been built in Europe to produce pikeperch using (RAS) (Fontaine et al., 2012), producing an estimated 300-400 t (1<sup>st</sup> Workshop of the European Percid Fish Culture Group, 1 Sept 2012, Prague). Numerous more commercial operations have been designed and/or are under construction in Belgium, Czech Republic, Denmark, France, Germany, Hungary, Italy, Poland, Portugal and the Netherlands. Year-round production of pikeperch requires constant high temperatures (24-26°C), which is only feasible in RAS to ensure relatively high growth rates (*i.e.*, **production of 1.2 kg fish in 15 -18 months** from non-selected strains). These RAS also allow high densities of 80-100 kg m<sup>-3</sup> (Dalsgaard et al., in press). Pikeperch flesh quality has a neutral taste, thus lending itself to different forms of preparation, and the filets are without bones --unlike carp, which competes on the same market segment. At present, pikeperch is sold either as whole fish at a weight of 600-3000 g or as filets of 100-800 g to markets in Europe (mainly Western, Eastern and Northern areas) and North-America, showing strong demand. The market value is high at 8-11 € kg<sup>-1</sup> at farm gate, whole fish.



Identified by a survey addressed to fish farmers in preparation for DIVERSIFY, the **major bottlenecks for further expansion of pikeperch** culture today include (a) **high sensitivity to stressors, handling and husbandry practices** that result in high and sudden mortalities, (b) **low larval survival** (typical 5-10%) and **high incidence of deformities**, (c) **lack of knowledge of the genetic variability of the used broodstocks**. Identification of genetic relationships among different broodstocks, inbreeding phenomena and loss of heterozygosity is important in aquaculture, since it may result in subsequent reproductive and productive failure (reduced progeny survival, growth, food conversion efficiency and increased frequency of deformities). It is also important to know how the domesticated stocks differ from their wild counterparts, which could potentially be a future source of fish to implement in effective breeding programs. Overcoming the above bottlenecks is very important to reduce production costs and, therefore, expand the aquaculture production of this species in the EU, and will be the objective of DIVERSIFY.

### ***Atlantic Halibut***

The **Atlantic halibut is the world's largest flatfish** and can attain a weight of over 300 Kg. It is **highly prized at markets worldwide**, but availability of wild Atlantic halibut is decreasing and the fish is classified as **endangered on the IUCN red list**. Last year a complete ban was imposed on Icelandic fisheries, and stocks along the Norwegian coast are declining and under strict regulation. This has led to a **higher market demand for Atlantic halibut than cannot be met by fisheries alone**. Cultured Atlantic halibut has an excellent reputation, but is rarely available outside specialty restaurants due to low annual production. The Atlantic halibut is a semi-fat fish rich in omega-3 fatty acids, with a characteristic flaky white meat with few bones. In terms of product diversification, Atlantic halibut is traditionally marketed as large fish steaks or cutlets. It can be smoked or marinated in the typical Scandinavian style. These characteristics led to the







## **inclusion of Atlantic halibut in DIVERSIFY, as a great candidate for fish species and product diversification in European aquaculture.**

Research and cultivation efforts of Atlantic halibut started in the 1980's, but the **total annual production of cultured Atlantic halibut is still only ~1600 t** (Norwegian Directorate of Fisheries). In Europe, Atlantic halibut farms exist in Norway and Scotland. The desired market size is 5-10 kg and production time is currently 4-5 yrs. Despite a significant research effort between 1985 and 2000, the complicated life cycle of Atlantic halibut made aquaculture progress slow, and very little research funding has been allocated thereafter. However, during this time slow but steady progress has been made by the farmers in order to improve production stability, and **interest in cage culture is growing**. The remaining **bottlenecks for increased and stable production are related to a steady supply of fry and a need to decrease the production time**. The latter may be achieved with the recent establishment of "all female" juvenile production (Hendry et al., 2003; Babiak et al., 2012). This is expected to have a major impact on production time as females grow faster and mature later –80% of slaughtered fish <5 kg are mature males (P22 SWH, production statistics). DIVERSIFY will address these important bottlenecks with a coordinated **research effort in reproduction, and larval nutrition and husbandry**.

### ***Wreckfish***

Wreckfish is one of the largest Serranid species, **reaching a size of 100 Kg**. It is a deep-water fish found **almost throughout the world** and is characterized by an extended pelagic juvenile phase (Sedberry et al., 1999; Ball et al., 2000; Deudero et al., 2000). Wreckfish is one of the most interesting new species for aquaculture, due to its **fast growth** (Suquet & La Pomélie, 2002; Rodriguez-Villanueva et al., 2011), **late reproductive maturation** (Sedberry et al., 1999), **high market price and limited fisheries landings** --quotas have been reduced by 90% in 2012 in the U.S.A. (NOAA, [www.fishwatch.com](http://www.fishwatch.com))-- and ease of manipulation in captivity (Papandroulakis et al., 2008; Rodriguez-Villanueva et al., 2011). Its large size lends itself to **processing and development of value added products**, and its **cosmopolitan distribution may enable EU exports**.



Wreckfish acclimatizes easily to captivity and, despite its large size, no mortalities have been reported due to handling. It accepts inert food easily, being a very voracious carnivore. In a recent study of wild-caught individuals it was shown that fish **grew from 1 kg to 5 kg in a period of 10 months** (Rodriguez-Villanueva et al., 2011). The slow reproductive maturation of wreckfish, which occurs at an age of 5-10 y in captivity, may be a problem for broodstock development and management. On the contrary, its **long juvenile stage is a great advantage from the aquaculture viewpoint**, allowing for commercialization before sexual maturity, and thus avoiding problems linked to maturation, such as reduction in growth, or loss of flesh quality and organoleptic properties.

**Lack of reproduction control and of established larval rearing protocols are considered major bottlenecks** preventing wreckfish aquaculture. Limited egg collection has been achieved from captive spawners using hormonal induction (Papandroulakis et al., 2008) or stripping of naturally maturing fish (Peleteiro et al., 2011). Embryonic development and the early life stages have been described (Papandroulakis et al., 2008, Peleteiro et al., 2011), indicating that the **large egg size of this fish (~2 mm in diameter) may offer significant advantages for its larval rearing**. Reproduction and larval rearing of a very close relative, the hapuku (*Polyprion oxygeneios*) has been achieved recently in New Zealand (Anderson et al., 2012). The **scarcity of broodstock is a disadvantage for this fish**, but the **clear biological and economical potential of this species justifies allocation of part of the effort of DIVERSIFY** in bringing together almost all partners involved so far in Europe in wreckfish domestication, to **overcome its documented bottlenecks --i.e., reproduction and larval rearing--** in order to produce appropriate numbers of juveniles to launch commercial production.



### **Grey mullet**

Farming of grey mullet has been practiced for centuries, but production of this potentially invaluable source of animal protein in Europe has been small and non-intensive (Nash & Koningsberg, 1981; Pillay, 1993). It is a **euryhaline species, found throughout the world** (Oren, 1981) and is a **rapid-growing, herbivorous species** that can be **reared over the wide geographical and temperature range of the Mediterranean basin**.



As it is detritivorous in the wild, it has been stocked in fish ponds to improve sediment quality and avoid oxygen depletion (Milstein et al., 1991). Therefore, it can be an **excellent candidate for the enhancement of aquaculture in earthen ponds, coastal lagoons, "valli" and deserted Salinas** that exist throughout the EU Mediterranean countries. Hatchery produced juvenile females have been **grown to 1.9 kg in 2 years** on a fishmeal-containing pelleted feed (P4. HCMR). The development of fishmeal-free feed will reduce the cost of fish production, and will be **more sustainable and environmentally friendly**. In this way, grey mullet would be more acceptable to an increasingly aware consumer public that demands sustainability and lower environmental impact. Moreover, grey mullet aquaculture has the advantage of providing not only affordable whole fish and fillets, but also **fish roe ("bottarga" in Italian), a high value product (>100 € kg<sup>-1</sup>)**, whose market is expanding around the Mediterranean. Therefore, grey mullet has a great **biological and economical potential for fish species and product diversification, and development of value added products**.

**A market for grey mullet is well established**, though a niche one, in the Mediterranean. Even without any marketing effort by the aquaculture industry, the European market demand for grey mullet is likely to increase in the coming years, due to the demand from established and newly immigrant families originating from North Africa, Middle East and Asia. Currently, **the industry is a capture-based aquaculture, relying exclusively on capture of wild fry** (ca 1,000,000,000) that are subsequently grown out to market weight (600-1200 g) in captivity, in lagoons or earthen ponds. The sustainability of such an activity is, of course, questionable, and the **future growth of the grey mullet aquaculture is limited by a number of bottlenecks**, which will be addressed in DIVERSIFY. Firstly, **controlling the reproductive cycle and improving egg quality** via broodstock management and nutrition is necessary not only for the production of robust larvae, but also for producing high value bottarga. Secondly, **development of a larval rearing protocol** is necessary to reduce early mortalities, size dispersion as well as increasing metamorphic synchrony, which will lead to a supply of high quality juveniles. Finally, development of a sustainable, economical, **fishmeal-free grow out feed** is needed, which would perform well under different environmental conditions of temperature, pond type, and water quality, thus broadening the geographical range of grey mullet aquaculture in Europe.

### **Socioeconomics (including new product development)**

Based on the development of the EU market and the demand characteristics, the following socioeconomic bottlenecks were identified:

- **Demand for seafood in the EU is increasing.** While the EU fisheries are stable or decreasing, the total EU demand for seafood is increasing. This increasing demand is currently fulfilled with imports from third countries. However, in order not to become overly dependent on seafood that is sourced in an increasingly competitive local and international market, it is important to introduce locally produced, sustainable and safe seafood to meet the demands of EU consumers.
- **EU consumer's negative attitude towards aquaculture fish and products.** This means that effective communication strategies have to be developed for the existing and newly developed products (new fish species and their value added products). This requires changing consumer perceptions and attitudes towards the entire aquaculture industry and range of products.
- **Demand for new aquaculture products in the EU market and subsequently in the world has to be developed.** New quality products for new markets have to be developed and targeted to potential market segments, in order to increase demand in the EU and world markets. New species have to be introduced in the market to diversify the aquaculture assortment, so that the risk of image loss of a specific species has lower market consequences for the whole sector.



- **Demand for European aquaculture products in the world markets has to be created.** Rising global consumption of aquaculture fish constitutes a great challenge and opportunity for the EU aquaculture industry. The proposed new species, cultured with sustainable methods and leading to high added-value products can be a driver for growth of the market share of EU aquaculture in local and global markets.
- The range and **added value of the aquaculture products has to increase.** According to the EU-funded project SEAFOODPLUS, consumers ask for more convenient products in the seafood market. In addition, the added value and cost price of the products have to be positioned in relation to other protein sources. This requires that the price elasticity of fish must be related to the price elasticity of other protein sources. In addition, additional value of European aquaculture products has to be implemented in chain revenue models that lead to a better livelihood for aquaculturists.
- The **sustainability of the aquaculture sector has to be improved further**, as sustainable fish products are requested more and more by EU and global consumer segments, industrial buyers and regulators; at the same time, investing on a sustainable image of the EU aquaculture will create a competitive advantage for the EU aquaculture industry. This requires that technological innovations have to be achieved, which are driven by market demand (consumers and retailers) and sustainability demands of NGO's.

All the above aspects underline that the image of the aquaculture sector has to be improved. New, sustainable --and high added value-- products with a longer shelf life have to be developed and SME's have to be more innovative for the introduction and market development of these new species.

As mentioned above, each of the selected species has the potential to grow in the market and to be perceived as an added value product, and their **biological and economical potential is expected to stimulate the growth of the European aquaculture sector.** Below, we reiterate the economic potential of each species in relation to the socioeconomic bottlenecks, and the actions planned in DIVERSIFY to overcome them:

**Meagre is a large fish with excellent taste.** As it is rather rare in fishery captures in the Mediterranean, it is not well known by consumers and the European market is still a niche product. Market development and consumer acceptance of relative species is done successfully in Japan, Australia and the USA. **Market development is imperative for the EU** and should focus mainly on consumer and retail awareness, and a better positioning with regard to gilthead sea bream and European sea bass. **New product development could support market development.**

The **greater amberjack is a large fish with high flesh quality and market value.** In addition to its economic potential in the EU market, cultured greater amberjack has a significant potential for exports, as it is distributed worldwide, and congener species are produced commercially elsewhere. This cultured fish **has proven its potential in other markets.** In Europe, there has recently been an intense interest from the aquaculture sector for this species, but production levels are miniscule. Therefore, **a consumer oriented market introduction of cultured amberjack is necessary.** Also, **market development is necessary for growth** with preservation of the added value and price, once production increases.

**Pikeperch is a medium size freshwater fish, with a good taste and a high market value.** There is already a market in Europe and North America, showing strong demand. The production capacity of this fish is expected to grow fast in the coming years. To keep up the high market value, **product development and market development is necessary** for coordinated growth. Therefore, potential markets and consumer segments have to be identified to maintain or increase the added value.

**Atlantic Halibut is a large fish with a very good reputation** in the north European market and a high market value. Demand exceeds the current production capacity. Market and product development is not necessary for the short run, but a **market development strategy** for the long run is necessary, because new entrants in the market can be expected given the added value of Atlantic halibut.

**Wreckfish is a large fish with excellent flesh,** but not available as a cultured fish. It is distributed throughout the world and products from the capture fishery are highly regarded. A very close relative is produced experimentally in New Zealand, where it is considered one of the best marine fishes. Because of this potential excellence, wreckfish could be interesting for the European market. For this species technical bottlenecks have to be solved first. So, only **market positioning in relation to other species is necessary** for the short run, and for the long run the **market potential will be identified.**

**Grey mullet is a medium size herbivorous fish, cultured extensively throughout the world, but often not well regarded by consumers.** It has a niche market in the Mediterranean for its flesh and high priced roe. Due to its good taste and low cost of rearing, grey mullet could have large potential market



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all over Europe, especially within segments of population of North African, Middle Eastern or Asian origin. **Market and new product development** are necessary for growth in the middle-long run in the native European market and the immigrant market.

To cover these market bottlenecks, DIVERSIFY gives a central role to positioning of species, and market and product development. In the first year a competitive market and environment analysis will be done and a study on consumer preferences with regard to cultured fish will be undertaken. Both studies are the basis for the development of new product prototypes, which will be developed in this project. These prototypes will be tested on food safety, preservation and market acceptance by consumers. Communication research will find out ways to overcome the negative image of aquaculture fish. The outcomes of all these studies will be the basis for the business plans per species, which will be developed together with the involved SMEs.



## **B 1.2 Progress beyond the state-of-the-art**

*Describe briefly the state-of-the-art in the area concerned, and the advance that the project would bring about. If applicable, refer to the results of any patent search you might have carried out.*

*Include also a description of the “baseline” of the project in terms of “where does the project start”, and the “baseline data” against which the project will measure its progress and the results the project aims to achieve (e.g. advances over the state of the art, increase of innovation/exploitation potential, etc.)*

*The Consortium should in particular include the definition of criteria and “performance/research indicators” for the project along which results, progress and impact of the project will be measured in later reviews and assessments.*

To facilitate the work in DIVERSIFY, the research tasks designed to address the identified bottlenecks in each selected species have been separated by scientific discipline, so separate WPs address work in a specific discipline and species. The WPs were then **organized in Groups of WP (GWP) according to research area, and not according to species**. This was done in order to **bring together researchers with similar expertise** (e.g., reproduction, nutrition, larval rearing, etc.), but working in different species, thus increasing the potential for problem solving in each area of research.

### **Reproduction and Genetics**

Reproductive control has been identified as the basis of sustainable aquaculture production. Inadequate reproductive control limits the production of market-sized fish, which may also depend on the unsustainable capture of wild organisms. For example, *Pangasius* spp catfish did not complete maturation in captivity and the development of hormone induced spawning (Cacot et al., 2002) enabled unlimited production of eggs, larvae and juveniles removing this bottleneck to provide the conditions for Vietnam to increase *Pangasius* spp aquaculture production from 40,000 tonnes in 1997 to 376,000 tonnes in 2005 (FAO, 2012). A reproductive bottleneck to the production of a species new to aquaculture is principally caused by two types of reproductive dysfunctions, related to failure of final oocyte maturation (FOM) and ovulation, and/or failure to spawn after ovulation (Mylonas et al., 2010a). The exact cause of these dysfunctions is unclear, but is probably related to inadequate culture environment (physical and/or social) and nutrition, which do not provide the optimal conditions that enable maturation to be completed (Mylonas et al., 2010a; Duncan et al., 2013). The lack of maturation is controlled by the endocrine brain-pituitary-gonad axis and Zohar et al. (1995) demonstrated that gilthead sea bream arrested in late vitellogenesis produced gonadotropins in the pituitary, but gonadotropin releasing hormone (GnRH) was not produced by the hypothalamus to release the gonadotropins and stimulate oocyte maturation and ovulation. Broodstock nutrition clearly plays a role in the progress of maturation and work within the consortium (Norambuena et al., 2012; 2013) suggested that inadequate dietary arachidonic acid (ARA) and cholesterol affected negatively the production of reproductively important prostaglandins and steroids. **DIVERSIFY will study the underlying endocrine and nutritional status of reproductive dysfunctions in some of the proposed species for Aquaculture diversification, which have been demonstrated to present significant reproductive dysfunctions, preventing the reliable production of eggs and, thus, juveniles for grow out.** This approach has proven results in other species, such as European sea bass (Mylonas et al., 2003) and Atlantic bluefin tuna (*Thunnus thynnus*) (Mylonas et al., 2007, 2010b). The work in **DIVERSIFY, will advance the state-of-the-art in the understanding of the mechanisms that control reproductive dysfunctions in these new species, and produce reproduction control protocols for the EU aquaculture industry.**

Despite of the incomplete understanding of the causes of reproductive dysfunctions, solutions, hormone therapies and strip spawning have been developed by consortium members of DIVERSIFY and used successfully by the industry in a wide range of species (reviewed by Mylonas et al., 2010a; Duncan et al., 2013). The hormone therapies used to induce FOM, ovulation and spawning in aquaculture include the application of exogenous gonadotropins (GtH) or agonists of gonadotropin releasing hormone (GnRH<sub>a</sub>) to fish at the correct stage of maturity. The recent development of recombinant fish GtHs offers a source of





exogenous, but homologous hormone for induction therapies that may be targeted to specific receptors, are species specific and without many of the disadvantages of other sources of heterologous GtHs, such as immune reactions and spread of pathogens (Yaron et al., 2009; Rosenfeld et al., 2011; Chauvigné et al., 2012). In the aquaculture industry, egg production from species that fail to spawn after ovulation is based on stripping eggs that are ovulated spontaneously or by hormonal induction (Mylonas et al., 2010a). Strip spawning protocols identify the hormonal therapy to induce ovulation (if required), when ova must be stripped to avoid over-ripening and how sufficient fresh, cooled or cryopreserved sperm is obtained (Duncan et al., 2013a). Combinations of these two solutions will be used in DIVERSIFY to solve the reproductive bottlenecks documented in the chosen species.

In the **greater amberjack**, oogenesis often did not proceed in a predictable and reliable way in captivity (Micale et al., 1999, Mylonas et al., 2004). Work within the consortium has reported spontaneous spawning in a single female held in captivity in the eastern Atlantic (Jerez et al., 2006) and in the Mediterranean Sea, spawning of good quality eggs from a single female with GnRHa implants (Mylonas et al., 2004). Kozul et al. (2001) induced spawning with heterologous GtH (hCG), but all eggs died in the gastrula stage. Recent comparisons between the nutritional status of wild and cultured amberjack broodstock have indicated how diets could be improved (Rodríguez-Barreto et al., 2012). With collaboration between Reproduction and Genetics, and Nutrition on the development of an appropriate amberjack broodstock diet and the application of the most recent knowledge on spawning induction methods (Mylonas et al., 2010a), **DIVERSIFY will develop GnRHa-based spawning protocols for the greater amberjack.**

In excess of 100,000 t of **grey mullet** (FAO, 2012) are produced from wild juveniles, due to lack of egg availability from cultured broodstocks (Aizen et al. 2005). Spermiating males are rarely observed and in most cases the produced milt is highly viscous and fails to fertilize the eggs (Yashouv, 1969; De Monbrison et al., 1997). Females are often arrested in the early stages of vitellogenesis, or fail to undergo oocyte maturation and ovulation (De Monbrison et al., 1997). In a previous study, DIVERSIFY Partners described dopamine inhibition of GnRH (Aizen et al. 2005) and induced spawning with controlled-release GnRHa implants combined with dopamine antagonist and 17 $\alpha$ -methyl-testosterone (Aizan et al 2005). Work also demonstrated that recombinant tuna FSH induced spermiation in grey mullet (Rosenfeld et al., 2011). Building on these advances, **DIVERSIFY will establish an optimized hormonal therapy for grey mullet in captivity**, leading to mass-scale egg production. Furthermore, DIVERSIFY will evaluate the effects of captivity on first sexual maturity to establish strategies for **production of an additional high-value product (mullet roe) with minimal extra risks and/or costs.** Mullet roe (known also as "bottarga" or "Karasumi") is a high-priced product in Mediterranean and Asian markets, but fully developed vitellogenic roe (10-20% GSI) in nature occurs first in 3 year-old fish. A reduction of age of maturity by a year will enhance the cost-efficiency of grow out production by the Aquaculture industry.

The availability of **wreckfish** broodstocks is limited throughout the world (Brick & Klippel, 2003; Cergole et al., 2005) and consequentially few reproductive studies have been completed. DIVERSIFY Partners have maintained fish in captivity for some years and obtained important information on reproduction, including gametogenesis (Papandroulakis et al., 2004), strip spawning of an ovulated female (Peleteiro et al., 2011) and GnRHa induced spawning and *in vitro* fertilization (Fauvel et al., 2008; Papandroulakis et al., 2008). These studies indicate that wreckfish may require induction of FOM and ovulation combined with strip spawning. **DIVERSIFY will build on these advances to enhance our knowledge of wreckfish reproduction and broodstock management**, by describing gamete characteristics and develop hormone induction and *in vitro* fertilization protocols.

Wild **Atlantic halibut** broodstock mature in captivity and are strip-spawned in relation to the natural ovulatory rhythms of individual fish (Norberg et al., 1991). Until recently, a bottleneck preventing the expansion of the industry was the slow growth of males. Artificial sex reversal of females (Hendry et al., 2003; Babiak et al., 2012) and breeding of the produced phenotypic males (that are genotypic females) has established all female production in the industry in Norway and North America, and has greatly ameliorated this bottleneck. However, F1/F2 females produced in a hatchery display a high degree of reproductive dysfunction, with irregular spawning cycles, low and unstable fertilisation rates, low survival of gametes, and lower fecundity than wild females. **DIVERSIFY will examine controls of fecundity and apply hormone induction technologies to develop solutions to poor F1/F2 Atlantic halibut spawning performance.**



Control of reproduction of **meagre** and **pikeperch** is not considered as a bottleneck. DIVERSIFY Partners have developed spawning induction protocols for meagre (Fernández-Palacios et al., 2009, 2011; Mylonas et al., 2011; Duncan et al., 2012) and pikeperch (Kestemont & Melard, 2000; Zarski et al., 2012). However, the industry identified two genetically related bottlenecks, which are **unknown genetic variability in captive broodstocks and unpredictable growth and performance during grow-out**. A solution to both of these bottlenecks in other species has been achieved with genetic breeding programs that control rates of inbreeding and improve growth with every generation to form an important part in maintaining the competitiveness of an aquaculture business (Gjedrem, 2012; Duncan et al., 2013a). Microsatellite-based studies on meagre (Haffray et al., 2012) and pikeperch (Saisa et al., 2010; Saliminen et al., 2011) have characterised wild populations. Within the consortium, microsatellites have been identified from the meagre genome (Andree et al., 2010) and used for paternity analysis and to characterise broodstocks (Duncan et al., 2012; 2013b). To date, no studies have been published that genetically characterise captive pikeperch broodstocks or analyze the genetic basis of phenotypic traits such as growth in either species. **DIVERSIFY will characterise genetically available cultured broodstocks of both species, and fast/slow growers for meagre. DIVERSIFY will also provide tools that the industry can use to establish genetic breeding programs, improving on and developing new genetic tools for meagre and pikeperch.** These include protocols to produce planned family crosses for meagre using paired tank spawning, *in vitro* fertilization, and characterisation of sperm and sperm management procedures. Protocols for pikeperch planned family crosses have been developed already (Kestemont & Melard, 2000; Zarski et al., 2012).

### ***Nutrition***

Four types of feeds are used in aquaculture, differing in nutritional and physical characteristics, following important changes in nutritional requirements during development: enrichment products for live prey, and dry feeds for weaning, grow out and broodstock, all differing in their formulation and production technology. DIVERSIFY will (a) **develop adequate first feeding regimes**, (b) identify the **optimum dietary nutrient levels required for weaning** and (c) build the knowledge on nutritional requirements to develop **sustainable and cost-effective grow out diets** for the candidate species. DIVERSIFY will also study the **requirements or feeding regimes to optimize reproductive success** in some of the species.

Feed constitutes the largest running cost for European aquaculture, reaching up to 70% in cage-based facilities. Moreover, suboptimal commercial feeds and feeding protocols result in direct economic losses through feed waste, poor growth and water quality deterioration (Kaushik, 1998). Also, feed composition affects fish health and welfare (Kiron et al., 2010; Montero and Izquierdo, 2010) and affects markedly fillet quality (Izquierdo et al., 2003) and consumer acceptance (Rosenlund et al., 2010). Species-specific formulations for aquaculture fish can improve markedly reproductive performance and progeny quality (Izquierdo et al., 2001; Fernández-Palacios et al., 2011), larval development and survival (Hamre et al., in press) and fry quality (Izquierdo et al., 2010, 2012). **Considering the specific bottlenecks defined for the proposed species and the nutritional knowledge available, particular tasks will be addressed in this proposal to provide specific solutions for the species considered.**

In **meagre**, DIVERSIFY Partners have developed successful first feeding protocols (Roo et al., 2009a, 2010a; Fernández-Palacios et al., 2009; Hernández Cruz et al., 2007; Scabini et al., 2008) and used different commercial enrichment products for larval rearing (Fernández-Palacios et al., 2007; Roo et al., 2007; Scabini et al., 2008; Abreu et al., 2009, Vallés & Estévez, 2011). However, feeding meagre during weaning and grow out with diets developed for other marine fishes (*i.e.*, gilthead sea bream and European sea bass) restricts the fast growing potential of this species (Robaina et al., in press), affects negatively the fish appearance and fillet quality (Gines et al., in press; Poli et al., 2003) and may be the cause of Systemic Granulomatosis (Ghittino et al., 2004). Lack of specific diets for meagre is due mainly to the very limited information regarding its nutritional requirements (Chatzifotis et al., 2010, 2012). Early studies denote that this species has high protein requirements, while dietary lipids are restricted around 17% (Chatzifotis et al., 2010, 2012). However, digestible energy (DE) and digestible protein (DP) requirements are not yet determined. Requirements for essential fatty acids (EFA), such as docosahexaenoic (DHA), eicosapentaenoic (EPA) and arachidonic (ARA) acids, which play important roles in fish growth (Izquierdo & Koven, 2011), health (Montero & Izquierdo, 2010) and fillet quality (Rosenlund et al., 2011), have not



been studied in juvenile meagre. Finally, there is an almost complete lack of information on the requirements for essential amino acids (EAA, particularly in combination with plant protein sources), vitamins and minerals (Robaina et al., in press). DIVERSIFY Partners, **building up on already existing knowledge, have selected the most relevant nutritional aspects** (DE, DP, EFA, EAA) for dose-response studies, whereas vitamin and mineral requirements will be studied in multifactorial or whole micronutrient package approaches. **The lack of meagre-specific weaning and grow out diets** will be addressed in DIVERSIFY, in order to maximize growth potential, enhance fry quality and promote health of this species. The WP on Nutrition – meagre will determine requirements of specific nutrients generally important for growth, welfare and health to address two of the main specific **bottlenecks** identified for meagre: variable growth rates during the pre-growing phase and variable growth during on-growing phase. **Variable growth rates** constrain production planning and may be related to different causes including unsuitable feed formulations. In meagre, despite its great growth potential, still both growth and feed utilization rates may be poor (Chatzifotis et al., 2010; Estévez et al., 2011) and feeds must be improved to consistently obtain high growth rates. Indeed, diets specially formulated to satisfy nutritional requirements of related species such as the mullet (*Argyrosomus japonicus*, Woolley et al., 2010) and brown meagre (*Sciaena umbra*, Chatzifotis et al., 2006) produce better growth and feed utilization rates in these related species than those obtained until present in meagre. Despite specific diets for meagre would produce more consistent growth rates (Martinez-Llorens et al., 2011), **there is not enough information on the specific nutritional requirements** of this species (Chatzifotis et al., 2010, 2012).

**Building up on already existing knowledge** available for other Sciaenids such as mullet, brown meagre and, particularly, the abundant information on the requirements of the red drum (*Sciaenops ocellatus*), DIVERSIFY **selected the most relevant nutritional aspects to promote meagre growth and welfare**. According to previous studies in several Sciaenids (Daniels and Robinson, 1986; Gatlin, 1995; Pirozzi, et al., 2010a; Kaushik, unpublished data), meagre seems to have higher protein and lower lipid requirements than seabream and seabass, whose commercial diets are used by European farmers to produce up to 2,387 t of meagre. Optimum crude protein and crude lipid contents in diets for meagre have been estimated up to now to be around 50% (Chatzifotis et al., 2012) and 17% (Panagiotidou et al., 2007; Chatzifotis et al., 2010; Chatzifotis et al., 2012), respectively. However, protein requirements could be even higher and lipid requirements lower, if this species, as it occurs in mullet, has a limited capacity to spare dietary protein (Pirozzi et al., 2010b). Thus, in a closely related species, utilization efficiencies for DP and DE are independent of fish size, ration level or temperature (Pirozzi and Booth, 2009; Pirozzi et al., 2010), whereas the red drum shows lower protein efficiency ratios (McGoogan and Gatlin III, 1999; Peng et al., 2008).

Despite **essential fatty acids** such as docosahexaenoic (DHA), eicosapentaenoic (EPA) and arachidonic (ARA) acids have been found to be essential for growth and welfare of several Sciaenids (Gatlin III, D.M., 2009), the requirements for these essential nutrients have not been yet studied in meagre. Another essential nutrient for fish growth is vitamin E (Hamre and Lie, 1995; Kocabas and Gatlin, 1999; Montero et al., 1998; Tocher et al., 2003; Lin and Shiau, 2005; Abdel-Hameid et al., 2012) and despite its optimum dietary levels have been determined in other Sciaenids (Peng et al., 2008; Peng, and Gatlin, 2009), its requirements are yet unknown in meagre. Finally, antioxidant nutrients such as vit C and selenium have been also found to be essential for growth of other species of the same family (Sealy and Gatlin, 2002), despite their importance for meagre has not been yet study. Among aminoacids, **lysine** is considered as an indispensable amino acid for proper growth being the first limiting EAA in protein sources commonly used in fish feeds based on plant feedstuffs (Wilson, 2003), but its requirement has not been yet determined in meagre. Lysine plays an essential role as precursor of carnitine, which carries long chain fatty acids into the mitochondria for  $\beta$ -oxidations of lipids to produce energy (Walton et al., 1984).

**Health problems** in meagre may be related to the use of inadequate feeds, as it occurs in other aquaculture produced species (Cooke and Sneddon, 2007, Montero and Izquierdo, 2010). Farmers are also aware of the harmful consequences of nutritionally-unbalanced diets on fish welfare (Conte, 2004). In turn, poor welfare conditions could not only markedly reduce meagre growth as it has been seen in a closely related species (Pirozzi et al., 2009), but also negatively affect immune system and disease resistance. Imbalances in nutrition negatively affect fish welfare and health in many fish species (Lall, 2000). The importance of nutrition on welfare and health involve almost all physiological functions related to health, including a direct effect on modulation of the immune system, stress response, mechanisms of defense against infection or



tissue integrity (Waagbø, 2006). Among different nutrients, deficiency in **essential fatty acids** or **antioxidants** including **vitamin E** and **vitamin C**, negatively affect fish welfare, increasing plasma cortisol levels and affecting fish behaviour and stress responses (Montero et al., 1998; Montero et al., 1999). Besides unbalanced levels of these nutrients cause immune deficiencies, pathological features in several tissues and reduce disease resistance in several fish species (Montero and Izquierdo, 2010; Betancor et al., 2012a, 2012b) including some Sciaenids (Sealey and Gatlin, 2002). **Amino acids** and their metabolites have been characterized as important regulators of main physiological pathways that are required for fish maintenance, growth performance, feed utilization, protection from oxidative stress and resistance to environmental stressors and pathogenic organisms (Wilson and Halver, 1986; Li et al., 2009). Among them, **lysine** has a role in calcium absorption and formation of collagen, a substance important for bones and connective tissues including skin and cartilage (Civitelli et al., 1992), being essential for the health status of these tissues. Despite the importance of those nutrients on fish health, the nutritional requirements of meagre have not been yet defined particularly in relation to welfare and health issues.

Being a carnivorous species, meagre should have a high requirement for digestible protein (DP), which may be affected by fish size and temperature. Previous work has been carried out in a closely related species, the mullet in Australia by Pirozzi et al (2010), but data obtained from mullet cannot be directly transferred to meagre since both species are cultured under greatly different temperature conditions. In this sense, mullet may be considered as a warm water species with an optimal temperature range comprised between 25 to 26.4°C (Bernatzeder & Britz, 2007), whereas meagre is a temperate species inhabiting the Mediterranean and Black Sea, and along the eastern Atlantic coast, where the best temperature for rearing meagre lies between 17 and 21°C. As nutrient utilization efficiencies have been shown to be influenced by many different factors such as species effects, fish size and temperature (Bendiksen et al. 2003; Moreira et al. 2008), it is clear that optimal DP:DE data obtained for mullet cannot be transferred to the meagre industry without previous experimental validation. Thus, a species-specific research on the nutrition of this species (DP:DE) is needed in order to optimize actual feeding practices and reduce **one of the main bottlenecks identified by the industry** during the on-growing phase of this species, which is the **highly variable growth rates** that may be linked to **unsuitable feed formulation** (of which DP:DE is the key aspect determining growth), among other potential reasons that will also be investigated (mainly potential genetic effects).

Contrary to meagre, the **greater amberjack** exhibits high mortalities during larval development, thus juvenile availability is a major bottleneck to its industrial production, in addition to reproduction control. Previous studies conducted by the Partners on ontogenic development (Abreu et al., 2009) and larval rearing techniques (Roo et al., 2010b, Grossi et al., 2009) have shown that larval greater amberjack perform poorly when fed the available commercial enrichment products. However, the elevation of certain nutrients in experimental enrichment products (Fernández-Palacios, unpublished data) increased up to 5 fold larval survival (Yamamoto et al., 2008). A recent review on larval nutrition (Hamre et al., in press), as well as preliminary studies on greater amberjack (Yamamoto et al., 2009) point to the importance of EFA, vitamins E and C, carotenoids and taurine (Tau) as essential nutrients during fish development. DIVERSIFY Partners will **study these nutrients through dose-response and multifactorial approaches, in order to develop specific live food enrichments and improved weaning diets**. Regarding grow out, information on greater amberjack nutritional requirements is scarce (Aly et al., 1999 (lipid sources); Talbot et al., 2000 (Lipid levels); Takakuwa et al., 2006 (DP/DE); Vidal et al., 2008 (DP/DE); Uyan et al., 2009 (phospholipid levels)). However, the close congener yellowtail (*S. quinqueradiata*) has been studied extensively (*i.e.*, Furutani et al., 2012 (alternative ingredients); Matsunari et al., 2005 (Tau); Ren et al., 2008 (Vit C)) and high requirements in protein, DHA, Tau and antioxidants are foreseen for greater amberjack. Therefore, DIVERSIFY partners **will build up on already existing knowledge available for this and other *Seriola* species** (Watanabe and Kiron, 1994; Sakai et al., 1998; Takeuchi, 2001; Liu, 2001; Uyan et al., 2008; Kolkovski et al., 2009, 2010; Miki et al., 2011), to specifically address lack of knowledge on nutrients that are particularly important for larval development, juvenile performance and reproduction.

A main bottleneck for the mass production of high quality greater amberjack juveniles is the low survival and growth obtained during larval development and metamorphosis (Yamamoto et al., 2009). However, larval greater amberjack perform very poorly when fed the available commercial enrichment products for live preys. The partners are familiar with the abundant information produced in Japan and Australia





regarding larval rearing of greater amberjack (Yamamoto et al., 2009), but very few studies have determined the specific nutritional requirements of this species testing a sufficient number of diets varying only the target nutrient. Thus, despite Yamamoto et al., (2009) pointed out the importance of essential fatty acids, antioxidants such as vitamins E and C, **carotenoids** and **taurine** as essential nutrients for greater amberjack larval development, the specific requirements for these nutrients have not been yet determined. For instance, Matsunari and coworkers (2012), studied the effect of four enrichment products for rotifers, such as *Chlorella* or a commercial emulsion that besides having different **docosahexaenoic acid (DHA)** content also differ in many other nutrients and other compounds that also may affect larval performance such as fat-soluble vitamins, pigments, antioxidants, minerals, etc. Moreover, despite they tested a range of DHA (0.0-1.9 mg g), DHA requirements for other *Seriola* species (i.e., yellowtail, *S. quinqueradiata*) are known to be the highest among all studied species (Takeuchi, 2001), well above the levels studied by Matsunari and coworkers (2012). Indeed, essential fatty acid requirements in rotifers may be as high as to 3.9% in the fast growing larvae of yellowtail (Kolkovsky et al., 2010). The relevance of DHA in diets for marine fish larvae has been well documented (Watanabe et al., 1989; Izquierdo, 1996; Sargent et al., 1999) and its positive effect on survival has been related to its important role in stress control (Watanabe et al., 1993; Izquierdo, 2005; Ganga et al., 2006), immune system development (Montero et al., 2003) and improvement of health and bacterial resistance in fish larvae (Brandsden et al., 2003). Moreover, DHA has been found to increase eye diameter and density of photoreceptors in gilthead seabream larvae (Izquierdo *et al.*, 2000) and, in agreement, visual capacity was found to be reduced in yellowtail fed DHA-deficient diets (Masuda *et al.*, 1999). Although both arachidonic and eicosapentaenoic acids are also considered essential and play important roles in fish metabolism, not only their absolute amounts but also their relative proportions are determinant of larval growth and survival as it has been seen in other species (Izquierdo and Koven, 2010). For instance in gilthead seabream, dietary ARA is more efficiently incorporated into larval tissues than EPA (Atalah et al., 2011) and therefore **EPA/ARA ratios** become lower in larval tissues than in diets. Similarly, increased dietary EPA has been found to reduce dietary DHA incorporation into larval tissues, higher **EPA/DHA ratios** decreasing larval growth (Rodríguez et al., 1997, 1998). Up to date, the optimum EPA/ARA and EPA/DHA ratios have not been yet determined in enrichment products for greater amberjack, despite their importance for larval performance. The high polyunsaturated fatty acid requirements foreseen in greater amberjack increase the risk of lipid peroxidation in this species. For instance, in yellowtail lipid peroxidation as a consequence of an imbalance between polyunsaturated fatty acids and antioxidants damages the biomembranes, producing several pathological conditions (Sakai et al. 1998) and causing irreversible changes in the developing tissues of marine fish larvae. Therefore, the enrichments must contain high levels of antioxidants, such as vit E (Betancor et al., 2011; Robaina et al., in press) and **carotenoids**. Despite **taurine** requirements have not been determined in greater amberjack larvae, the requirement of this aminoacid for very young juveniles of yellowtail (Matsunari et al., 2005) suggest its importance in early larval stages of *Seriola* spp as proposed by Yamamoto et al. (2009).

Regarding on-growing, despite no specific diets are produced in Europe for this species, commercial diets for other *Seriola* spp are available in the Pacific region, although they are far from satisfying the requirements of this species as the legal demand of Australian farmers has pointed out. There are some published studies on greater amberjack nutrition during on-growing periods regarding dietary lipid sources (Aly et al., 1999) optimum lipid levels (Talbot et al., 2000), digestible protein/ energy ratios (Takakuwa et al., 2006; Vidal et al., 2008 (DP/DE) or phospholipid levels (Uyan et al., 2008) but a closely related species (yellowtail) has been extensively studied. For instance, in yellowtail the effect of alternative ingredients (Furutani et al., 2012), taurine (Matsunari et al., 2005), vit C (Kanazawa et al., 1992; Ren et al., 2008), vit E (Shimeno et al., 1991; Sakai et al., 1998) or P (Sarker et al., 2009). As the greater amberjack is a highly carnivorous species, high protein requirements are expected. Poor sustainability of fishmeal is encouraging the use of alternative plant proteins. When high plant protein diets are fed the first limiting essential aminoacid is frequently **lysine** (Wilson, 2003), but its requirement has not been yet determined in greater amberjack.

Reproduction success in terms of gonad development, fecundity, fertilization or hatching rates is markedly affected by broodstock diets in many fish species including *Seriola* spp. (Watanabe and Kiron, 1994; Fernandez-Palacios et al., 2011). Previous studies in this (Roo et al., in press) and other *Seriola* spp suggested that high protein, DHA and carotenoids are required for the reproductive success, but precise levels have not been investigated yet (Rodríguez-Barreto et al., 2012; Roo et al., in press). For instance, dietary **protein** and **essential fatty acids** markedly affect gamete quality in yellowtail (Verakunpiriya et al., 1997a, Watanabe et



al., 2000). **Taurine** has been also identified as an essential component in broodstock diets for yellowtail necessary to improve fecundity, percentage of viable eggs and fertilization rates (Matsunari *et al.*, 2006). Finally, carotenoids also play an important role in yellowtail reproduction (Verakunpiriya *et al.*, 1997a, 1997b; Vassallo-Agius *et al.*, 2001a). For instance, dietary astaxanthin increased fecundity but did not improve the egg quality in the yellowtail (Verakunpiriya *et al.*, 1997b). Despite the importance of these nutrients for reliable reproduction of other *Seriola* species, **their optimum levels and ratios among them in diets for greater amberjack broodstock have not been yet studied.**

Regarding **pikeperch**, studies on percid larvae suggest that supplementation of diets by phospholipids or specific vitamins may decrease scoliosis and lordosis rates and increase larval resistance to osmotic stress (Kestemont *et al.*, 1996; Hamza *et al.*, 2008; Henrotte *et al.*, 2010), but the optimal levels for major essential nutrients are still unknown for pikeperch and thus very important to increase quality of the produced larvae. Besides pike perch eggs have a high DHA content, which could be related to its strict carnivorous behavior or reflect the evolution of this species from marine water fish. Recent studies have suggested (Lund & Steinfeldt, 2011; Lund *et al.*, 2012), that lack of LC-PUFAs especially DHA during live feed first feeding (i.e. within 25 days post hatch) both may have immediate and long term negative consequences on stress sensitivity and mortality in pikeperch larvae and in juveniles.

Interestingly, despite pikeperch is generally considered a freshwater fish, this species inhabits brackish waters (Baltic Sea  $\leq 10$  ppt.) and estuaries, sharing several characteristics with marine fish. Thus, pikeperch egg/larvae tissue LC PUFA composition and requirements resemble those of marine fish (Lund *et al.*, 2011). Also in common with marine fish, pikeperch has the ability to hypo-osmoregulate keeping their body fluid osmolality below that of the environment (Scott *et al.*, 2008). Laboratory studies revealed a great tolerance to saline waters, tolerating direct transfer to 16 ppt salinity and simulated tidal cycles of 33 ppt even though in both cases it induced increased cortisol levels (Brown *et al.*, 2001). Thus during exposure to low salinity between 6- and 12 ppt pike perch are able to manipulate their nitrogen metabolism (Sadok *et al.*, 2004). Despite previous studies have demonstrated a strong effect of salinity on fatty acid requirements and metabolism in other species such as Atlantic salmon (Tocher *et al.* 1995), the salmoniform fish *Galaxias maculatus* (Dantagnan *et al.*, 2007) or the Mexican silverside, *Chirostoma estor* (Fonseca- Madrigal *et al.*, 2012), the ability of pikeperch to regulate its fatty acid metabolism and LC- PUFA synthesis in combination with salinity has not been investigated. Nevertheless, this species has the ability to elongate and desaturate precursor fatty acids for n-3 PUFA synthesis (Schulz *et al.*, 2005). Commercial production of pikeperch is practiced in freshwater, but low salinity initiate physiological changes that could affect growth rate and development, and therefore it is interesting to better understand the interactive effect of salinity and nutrition on stress resistance in pikeperch. Therefore, **DIVERSIFY will study the effect of selected dietary nutrients on pike perch larval development and performance, and particularly of EFA on long-term stress sensitivity.**

Despite the fact that **Atlantic halibut** commercial rearing has started many years ago, early weaning still constitutes a main bottleneck. Feeding on-grown *Artemia* may improve halibut weaning, contributing to complete larval metamorphosis and pigmentation (Olsen *et al.*, 1999) and leading to stronger juveniles. There is great interest in Atlantic halibut larval rearing using RAS technologies, where a different microbiota might have a positive effect on intestinal health (Nayak, 2010) and contribute with essential nutrients such as DHA, EPA or certain vitamins (Ray *et al.*, 2012). However there is a lack of specific studies to determine their importance in Atlantic halibut productive systems. Among minerals, the importance of iodine for larval rearing has been emphasized (Morris *et al.*, 2011; Ribeiro *et al.*, 2011). The slow growth in late larval stages could be overcome by early weaning. Most often, weaning of Atlantic halibut occurs only at 70 days post first-feeding (dpff), but attempts have been made to introduce formulated diets from 20 and 50 dpff, with varying results. The first problem arising is that the larvae refuse to eat formulated feed (Harboe, Hamre and Erstad, unpublished results). It has frequently been observed, however, that they ingest inert particles such as *Artemia* cysts and pollen from pinewood, the main similarity being that both particles have neutral buoyancy and a bright color. Previous experiments have also shown better feed ingestion with floating compared to sinking feed particles. Furthermore, the structure of the visual system of halibut larvae indicates that they hunt prey in the horizontal plane (Helvik pers. com.), favoring feed intake when particles stay in the same position in the water column for some time. Additionally the type of feed could also affect digestive capacity



determined as proteases, carbohydrases and lipases activities (Caruso et al., 2009) or even ATPase activity, which in gut is essential to ensure the ion gradient necessary for nutrient uptake.

Another strategy to alleviate the slow growth in the later larval stages is to use on-grown *Artemia*. Ongrown *Artemia* are larger, contain more protein and phospholipids and have a different micronutrient status from *Artemia* nauplii (Hamre and Harboe, NIFES, preliminary results). They also have a lower shell to nutrient content. This may explain why Atlantic halibut larvae fed on-grown *Artemia* develop into juveniles with better pigmentation and eye migration than Atlantic halibut fed *Artemia* nauplii (Olsen et al., 1999; Hamre and Harboe, NIFES, preliminary results). The industry is considering implementing this knowledge in the production line, but will need further documentation.

Atlantic halibut larvae kept in a RAS system will encounter matured water, which will affect their gut flora (Nayak, 2010) in a way that probably has a positive effect on intestinal health. Gnotobiotic and conventional studies indicate the involvement of gut microbiota in nutrition and epithelial development (Nayak, 2010). Gastrointestinal bacteria may also produce essential nutrients such as vitamins and polyunsaturated fatty acids, and enzymes that can aid digestion (Ray et al., 2012). These considerations favor the hypothesis that the general nutrient absorption and retention in the fish is affected by RAS. Iodine retention must have an extra focus, since  $\text{NO}_3^-$  at levels found commonly in recirculation systems block iodide uptake by the sodium iodide symporter and may cause goiter in the fish (Morris et al., 2011; Ribeiro et al., 2011).

The third important bottleneck in halibut production is slow growth after weaning. One possible reason for this is a suboptimal diet. We have shown that juvenile Ballan wrasse increase the growth rate by up to 40% when lipids are added as phospholipids (PL) in stead of triacylglycerols (TAG, Sæle et al., unpublished), while requirements for PL in *A. halibut* juveniles are not known. DIVERSIFY will develop a new production strategy for on-growing *Artemia* and subsequently test them to **improve weaning performance of Atlantic halibut juveniles. In addition, new information will be gathered on the effects of RAS vs Flow Through Systems (FTS) in Atlantic halibut larval development.**

Studies on **wreckfish** nutritional requirements and optimum diets are missing, since control of reproduction and reliable supply of eggs has not been achieved yet (Fauvel et al., 2008; Papandroulakis et al., 2004). Nevertheless, some information is available from studies on feeding habits of wild populations, biochemical composition of eggs, larvae and juveniles, or results obtained in other relative species (Anderson et al., 2012). Therefore, studies on nutrition of this species will focus mainly on **broodstock feeds for enhancing fecundity and spawn quality, and the development of adequate live prey enrichments for wreckfish larvae**, as first steps for the development of proper nutrition and culture of this serranid species.

Preliminary studies suggest that rotifer enrichment with EPA and DHA improve **grey mullet** larvae performance (Eda et al., 1990; Tamaru et al., 1992), although the optimum levels and ratios have not been determined yet. Requirements of EFA in fish seem to be dependent on salinity conditions (Dantagnan et al., 2010). Interestingly, older grey mullet juveniles seek out less saline coastal environments and show best weight gain in low salinity or freshwater lakes and ponds. Another nutrient that may be closely related is Tau, which may improve bile salt-assisted lipid transport and metabolic regulation (Hansen & Mortensen, 2012). Importantly, the larvae of many marine species lack a key enzyme in Tau synthesis (Yokoyama et al., 2001), whereas this amino acid is present only in trace levels in rotifers (Van der Meeren et al., 2008). Moreover, fishmeal replacement by plant protein in grow-out diets reduces markedly Tau levels and its supplementation improves greatly juvenile growth performance (Lunger et al., 2007; Chatzifotis et al., 2008). Having this in mind, **DIVERSIFY will focus on the requirements of grey mullet for EFA and Tau, to improve enrichment products, weaning, grow out and broodstock diets.**

### ***Larval Husbandry***

The successful larval husbandry of any fish species determines the number of juveniles that will be available for grow out to market weight, but also the quality of these fry, in terms of future growth performance, metamorphic success, incidence of morphological deformities and disease resistance (Koven, 2003; Georgakopoulou et al., 2007; Sandel et al., 2010). In order to improve juvenile quality, research must be carried out on environmental (*e.g.* salinity, temperature, light, microalgae type and concentration) and husbandry (type and concentration of zooplankton prey) requirements that can pose bottlenecks to a



successful industry. The results of these studies form the basis for species- specific protocols for enhanced production of high quality juveniles.

**Meagre** has the biological characteristics to be an excellent candidate for commercial aquaculture and has adapted well to modified gilthead sea bream and European sea bass culture protocols (FAO, 2005-2011), particularly those based on rotifer and *Artemia* feeding methodologies. This has led to meagre production, which increased 7 fold since 1997. Although the precise requirements for essential amino and fatty acids are not completely known, the larvae show very good growth and survival rates using commercially available enrichment products for live prey. Therefore, producers do not consider larval rearing in general to be a major bottleneck for the expansion of meagre culture, although cannibalism and variable size distribution in larvae and juveniles are causing increasing concern. This may be due to a protracted dependence on feeding on *Artemia*, which have lower protein levels and are less dense in nutrients than dry feeds, possibly resulting in asynchronous growth. **Consequently, advancing the early weaning of larvae on dry feeds is a priority and the major focus of the larval work on meagre.**

Early larval rearing techniques applied for **greater amberjack** based on a variety of semi-intensive approaches (Papandroulakis et al., 2005) have shown promising results. Nevertheless, substantial gaps remain in these protocols as inadequate live food quality and quantity continue to cause significant mortality. Growth performance during early life is rapid, with 40 dph individuals reaching 0.5 g and having completed weaning to artificial diets. However, survival is about 3%, while it is argued that modifying the feeding regime and developing specific diets could improve survival to 20% during the first month (Anonymous, 2008; Hamasaki et al., 2009; Roo et al., 2012). Further studies are essential for the definition of nutritionally sensitive periods during larval rearing and the development of appropriate feeding regimes adapted to the development of the digestive system of the larvae (Papadakis et al., 2009). Furthermore, environmental factors such as the intensity and duration of light, as well as rearing tank volume and hydrodynamics may have a significant effect on larval performance (Carton, 2005; Stuart & Drawbridge, 2011). In the proposed research, these factors will be studied, to develop intensive rearing protocols that will improve fry production and quality.

In percids such as **pikeperch**, survival is 5-20% during larval rearing while cannibalism represents 30-50% of all mortality (Babiak et al., 2004; Mandiki et al., 2007; Ledoré et al., 2010) and it is caused primarily by size heterogeneity (Kestemont et al., 2003; Mandiki et al., 2007). Cannibalistic behaviour is observed first around 7 dph (Babiak et al., 2004) and progresses to become very severe from 18 to 39 dph representing up to 80% of all mortality at weaning time (19 to 23 dph) in pikeperch (Kestemont et al., 2007; Szkudlarek and Zakeś, 2011). Presently, there is no effective larval rearing protocol to reduce this very high mortality during larval rearing. One approach to reduce cannibalism is to sort out the larger larvae from their slower growing cohorts. However, this method is weakly effective as a new group of faster growing individuals emerges shortly afterwards (Kestemont et al., 2003, 2007; Mandiki et al., 2007), suggesting a social interaction factor may be involved. Consequently, successful culture of pikeperch requires development of effective larval rearing and weaning protocols that reduce cannibalism and mortality while improving growth. These protocols will be based on knowledge gathered from multifactorial experiments that integrate the combined effects of numerous environmental, social, genetic and nutritional factors, as well as their many interactions (Folkvord and Otterå, 1993; Baras et al., 2003, 2011; Kestemont et al., 2003; Teletchea et al., 2011; Trabelsi et al., 2011). Moreover, **practical protocols can also be evaluated by their effect on the ontogeny of the digestive and sensory systems, larval behaviour and sensitivity to stress** (Kestemont et al., 2003, Ostaszewska et al. 2005; Sabate et al., 2009; Baras et al., 2010; Trabelsi et al., 2011).

Commercial production of **Atlantic halibut** fry currently is carried out in FTS. However, there is growing agreement that RAS would offer more stable environmental and chemical water conditions that would lead to improved larval performance. Larval feeding is based on the intensive use of *Artemia* nauplii and metanauplii as the sole food, which is provided during a long photo-phase (17L:7D) until weaning (60 days or more after first feeding) onto a dry feed (Hamre & Harboe, 2008a,b). However, the protracted use of feeding *Artemia* nauplii and metanauplii in discrete meals until weaning (Harboe et al., 2009) appears to reduce prey intake and digestion efficiency. Antibiotics have been found to ease this situation (Roiha Sunde et al., 2011) although larval growth still decreases during the second half of the live prey period. A practice that is currently in use in order to reduce bacterial load as well as production costs is the addition of clay instead of algae to the rearing tank, which creates the necessary turbidity (Harboe & Reitan, 2005). Having





this in mind, the objectives in the proposed project are to **(1) compare the use of antibiotics in RAS with FTS during yolk sac and early feeding on larval performance, (2) optimise use of probiotics during early development and (3) develop a production protocol for on-growing *Artemia* and compare the effects of this live food with *Artemia* nauplii on larval performance, development and behaviour at different developmental stages.**

Embryonic development and early stages of larval life until yolk sac consumption have been described in **wreckfish** (Papandroulakis et al., 2008, Peleteiro et al., 2011). These studies show that larvae at mouth opening are large (5 mm) and, therefore, are expected to adapt easily to commonly cultured live food such as rotifers and *Artemia*. Nevertheless, there is a need to investigate the factors influencing the larval rearing environment of the species such as light, temperature and hydrodynamics of larval rearing tanks, as well as the type and concentration of zooplankton prey to be fed to the larvae. To accomplish these aims, it is essential to identify nutritionally sensitive periods by relating the type and concentration of live prey with the development of the larval digestive tract (Papadakis et al., 2009). In addition, it is expected that the application of knowledge from the rearing of related species such as *Polyprion oxygenius* in New Zealand (Anderson et al., 2012) and dusky grouper (*Epinephelus marginatus*) (Bruzón, 2007) will facilitate the domestication of wreckfish. In the proposed project **the objective is to evaluate larvae** in terms of growth, survival as well as biochemical and biometric analyses from three culture systems --**mesocosm, RAS and flow-through-- in order to develop a larval rearing protocol for this species.**

There is a general consensus that a major factor limiting the commercial rearing of **grey mullet** is the high mortality occurring during early larval development (Murashige et al., 1991; Yoshimatsu et al., 1995; Harel et al., 1998). Harel et al. (1998) found that the “greening” of the rearing tanks with *Isochrysis galbana*, which has characteristically high levels of DHA (22:6n-3), contributed significantly more to larval survival than adding *Nannochloropsis oculata*, a microalgae relatively rich in EPA (20:5n-3). On the other hand, a number of researchers have claimed that ceramic clay is a viable alternative to adding microalgae to the rearing tanks (Attramadal et al., 2012), suggesting that the role of “greening” in rearing tanks is mostly to provide turbidity and background lighting to facilitate the larval hunting of zooplankton prey. Taken one step further, this suggests that adding concentrated algal pastes, frozen or freeze-dried, might be an economical alternative. On the other hand, the addition of live algae to the larval rearing tanks may be imparting other biochemical and stimulatory benefits to the larvae that would outweigh the advantage of freeze dried or concentrated algal pastes. Therefore, studies are necessary to **compare the effect of microalgae species and concentration on larval rotifer ingestion, biochemical composition, digestive enzyme ontogeny and metamorphic synchrony.** In addition, there is a need to investigate if the **benefit of algal addition is due to its effect on facilitating larval hunting. Finally the effectiveness and cost-benefit of using dried or frozen algal pastes must be examined.**

### **Grow out Husbandry**

Grow out represents the longest production phase in aquaculture and the husbandry procedures applied affect significantly the overall performance. Depending on the applied production system different methods are employed, in regards to feeding, stocking, handling, etc. Several production systems have been employed for grow out of fish including floating cages, RAS, lagoons and earthen ponds (FAO, 2003-2012). In Europe, the intensive culture of trout is successfully performed in earth ponds for several years but intensive rearing of marine species in the Mediterranean and the Atlantic is performed almost exclusively using floating cages that are anchored in protected or semi-protected areas (<http://www.feap.info>). Although the intensification achieved in land-based RAS systems during the 1980's allowed commercial grow out activities --especially for species such as the turbot, *Scophthalmus maximus* and later Senegalese sole-- the higher production costs compared to cage culture have reduced the fraction of land-based grow out systems in operation today (Mozes et al., 2011). However, in areas like the Mediterranean, for aquaculture to grow sustainably, some basic problems related to the lack of available coastal areas for farm locations due to a high site competition with other human activities should be solved. Following technological development, offshore aquaculture may provide a solution to overcome restrictions (environmental, geographical, political) regarding the present use of near shore coastal space (Ryan, 2004). To this end, the development of both appropriate technologies and use of appropriate species (large and fast growing) represent a continuous effort



of the sector in recent years (EATIP, 2012). **DIVERSIFY will advance already applied and develop new methodologies necessary to provide the required tools for grow out husbandry of the meagre, greater amberjack, pikeperch and mullet.**

The technologies and practices used currently for **meagre** grow out are the same as those used for gilthead sea bream and European sea bass, although this fish presents significant differences in growth rates, feeding and spatial behaviour in the cage. Commercial diets are not available for meagre, so gilthead sea bream diets have been employed although not completely adequate. Further to the development of appropriate feeds for meagre (W8 Nutrition - meagre and WP24 Fish health - meagre) species-specific husbandry practices and methods can improve the performance during rearing. At the initial period after transfer in the cages (up to 15 g), cannibalism can be significant and increased feeding frequency is used to ameliorate the problem. As meagre grow, farmers often reduce feeding frequency to once a day, but this may not be appropriate for the species resulting in large size dispersion. Meagre presents a distinct feeding behavior and has a tendency to stay in the bottom of the cage, feed low in the water column and take time to rise towards the surface to feed. As fish are not very visible to the farmer, feeding may often not be adequate for maximum growth, resulting in large size dispersions. Meagre require approximately double the ration used for gilthead sea bream and can be fed 1–2% body weight day<sup>-1</sup>. For grow out, FCRs of 1.7 (FAO, 2005–2011b), 0.9–1.2 (Monfort, 2010) and 1.8 (Duncan et al., 2013a) have been obtained. The objectives in this WP for meagre are **(i) the development of an appropriate feeding method that respects the specific behaviours of meagre and (ii) the modification of existing methodologies for cage culture related to volume and light conditions, in order to maximize the performance.**

Preliminary data for grow out of **greater amberjack** with standard feeds, suggested that growth performance is high and wild caught individuals reached 1.8 kg body weight after 1 year (Jover et al., 1999; Mazzola et al., 2000). Hatchery produced fish reached 4 kg after 2 years and almost 7.5 after 3 years of rearing (HCMR, unpublished data), while in land-based trials fish reached 1.1 kg body weight at 1 year and increased 9 times their weight in 4 years (Jerez et al., 2007). Studies on the effects of feeding frequency, feed rations, and food type during grow out have been carried out in tanks, so far, in order to improve growth and health of fish (Jerez et al., 2009a,b; 2011), but **further studies, performed in the frame DIVERSIFY, are required to define proper feeding strategies.** As the applied methodologies for grow out are adaptations from other species (as for meagre) that have a final size of only 20% of the greater amberjack, it is essential that **DIVERSIFY will develop appropriate techniques for cage rearing** with the main objectives being (i) to identify the **adequate volume** for the rearing, based on the experience from other *Seriola spp* (FAO, 2012) and (ii) to **test the application of submersible cages** as this technology is targeted for offshore locations and can also serve as a method to prevent parasitic infestations during rearing. Finally, **DIVERSIFY** will address the development of appropriate management practices related to the **specific thermal ranges** for optimal growth and health, for appropriate site selection for cage culture and the optimum **rearing density**, a parameter that affects the performance of the reared populations.

One of the bottlenecks identified by **pikeperch** SMEs is the unpredictable depression of growth observed sometimes during fish grow out. This increases production costs, while management manipulations are often followed by high mortalities with unknown causes. Reduced growth and/or high mortality can be related to high stress responsiveness to intensive culture conditions and to the use of pikeperch broodstock of various domestication levels, including wild populations (Teletchea & Fontaine, 2012). Depending on the stressor and species sensitivity, allostatic load may turn into allostatic overload and stress response may shift from adaptive to maladaptive, resulting in a continued loss of homeostasis (Shreck et al., 2001; Barton, 2002; Pruett, 2003; Segner et al., 2011). Moreover, due to the associated allostatic costs, less energy would be available for other energy-demanding biological functions, such as growth and resistance to disease. In the Eurasian perch (*Perca fluviatilis*) a stress-induced growth reduction (by 35%) has been reported (Jentoft et al., 2005). Although it is suggested that stress can impact negatively the fish immune system, this cannot be generalized and currently no information exists on stress sensitivity and subsequent immune effects in pikeperch, necessitating further investigation. Also, reduction of stress responsiveness may be an important part of domestication, because of the positive selection of stress-resistant animals (thus displaying improved fitness) along generations (Douxflis et al., 2011, 2012) and studying the influence of domestication level and geographical origin is important. Likewise, the levels of stress indicators following exposure to chronic stressors have been shown to be heritable in salmonids and European sea bass (Pottinger & Carrick, 1999a,



b). Geographic origin may also influence significantly the growth potential of percid fish (Mandiki et al., 2004), and their physiological response to environmental conditions. The **DIVERSIFY** objectives for grow out of pikeperch are to study the effect of **(i) husbandry practices and environmental factors on growth, immune and physiological status and (ii) of domestication level and geographical origin on growth and stress sensitivity and immune performances.**

Presently, most **grey mullet** are reared extensively in polyculture systems (Whitfield et al. 2012) and to a lesser degree as a bio-remedial approach to improve the anoxic environment under marine fish cages (Katz et al., 2002; Lupatsch et al., 2003). However, in order for EU countries to supply an established market in North Africa and the growing demand in the Mediterranean, the intensive monoculture of grey mullet has to be developed. One of the major obstacles to achieve this is the lack of a suitable and economical grow-out feed. This issue will be addressed in the Nutrition GWP, which will focus on improving a non-fishmeal grow-out grey mullet diet through determining taurine and essential fatty acid requirements. Recently, the IOLR in Eilat has grown in monoculture F1 grey mullet in 40 m<sup>3</sup> tanks in an open seawater (40 psu) system. The females reached 1.9 kg in 30 months and were fed a commercial fish-protein based diet for bass. In addition, over 85% of these females demonstrated ripe ovaries during the spawning season. Importantly, the fact that F1 fish were used implies that grey mullet grown in captivity may perform better under grow-out conditions than wild caught juveniles. On the other hand, the grow-out period from this study was too long for commercial consideration and the FCR was very high (approximately 3.0) while the stocking density and nitrogen retention of fish in these ponds were low (about 15 kg m<sup>-3</sup> and 15%, respectively). Moreover, maximum growth, feed conversion efficiency and intestinal enzyme activity has been shown to take place at 10 psu (Barman et al., 2005). Bakeer (2006) found that increasing stocking densities from 1-3 fish m<sup>-2</sup> in 12 psu saline water ponds increased fish yield from 213 kg per 1000 m<sup>2</sup> to 459 kg per 1000 m<sup>2</sup>, respectively, after 8 months. Nevertheless, there was no benefit to overall profitability with increasing density as growth rate was insufficient to offset the rising operating costs of fingerling and food purchase. The Israeli SME DOR, using a commercial carp feed and stocking 0.5 and 1 fish m<sup>-2</sup> in 6000 m<sup>2</sup> ponds showed yields of about 500 and 1000 kg per 1000 m<sup>2</sup>, respectively after about 1 year. Taken together, these results suggest the importance of an effective feed in grey mullet culture in order to improve higher density performance. To this end, the aims of the grey mullet studies in this GWP are to **(1) test and compare the performance of an improved grow out feed**, in terms of FCR, PER, SGR and survival, on F1 and wild caught fish at two stocking densities (0.5 and 1 juvenile/m<sup>2</sup>) and **(2) compare the performance of this feed under the different environmental conditions of commercial farms in Israel, Greece and Spain, which span the Mediterranean Sea.**

### ***Fish Health***

This GWP will address issues surrounding the health and welfare of **greater amberjack and meagre**. For both species health issues have been identified as a bottleneck for future production, and several disease issues are already considered problematic. There is very little known about the immune systems of these species and virtually no immune genes have been cloned. In this GWP we will **(1) improve the ability to diagnose and treat these known diseases**, and **(2) make an exponential increase in knowledge on the immune system of these fish in terms of cloning key marker genes of innate and adaptive responses.** This will allow examination of when the immune system matures during development (*i.e.*, when might be an optimal time to vaccinate) and what responses occur post-vaccination. The latter will take advantage of the tremendous increase in information on immune genes in other cultured species (salmonids, gadoids, tetraodontidae) and model species (stickleback, zebrafish, medaka), and the unparalleled expertise available at the UNIABD, the Partner that leads on this GWP.

The most important, but also interesting disease of **meagre** is a condition called Systemic Granulomatosis, which affects almost 100% of cultured populations. The disease is characterized by multiple systemic visceral granulomas that manifest progressively as calcified and necrotic organs (Katharios et al., 2011a). The aetiology is unknown, but there is evidence it may be a metabolic disorder, and similar to systemic granulomas observed in cultured fish species such as gilthead sea bream (Paperna, 1987), rainbow trout (Dunbar & Herman, 1971) and turbot (Messenger et al., 1986). In all cases the development of the disease has been associated with a nutritional imbalance (phosphorus levels, vitamin C deficiency, etc) or inadequacy



(plant ingredients, tyrosine, etc). Systemic Granulomatosis is not associated with high mortalities, however, both prevalence and intensity are so high that this disease ranks as one of the major bottlenecks of further expansion of meagre production. The disease may lead to reduced growth and physiological performance during grow-out and, in addition, it affects the final product, making it unacceptable to the consumer. DIVERSIFY will study the **development of the disease under different feeding/nutritional regimes**, in order to investigate the underlying causes and to reduce its incidence in aquaculture. Since there is evidence that the cause is related to nutrition, the variety of feeding trials foreseen in these studies will provide a thorough insight on the aetiology of the disease.

Chronic Ulcerative Dermatopathy (CUD) is a newly described disease affecting the lateral line organ of freshwater and marine cultured fish, including **meagre** (Baily et al., 2005; Katharios et al., 2011b). The disease is directly associated with the use of borehole water, although the aetiological factor is still unknown. High mortalities are not associated with CUD, although it results in severe disfigurement of the fish, especially around the head where the lateral line canals are located, making the fish unacceptable for marketing. The lesions resolve when fish are transferred to surface water - fresh and marine depending on the species (Baily et al., 2005; Katharios et al., 2011b)-- however complete recovery is observed only in the cases where fish are transferred early during development. Chronic Ulcerative Dermatopathy has been reported in several marine fish (Katharios et al., 2011b), amongst which meagre seems to be one of the most vulnerable (Rigos & Katharios, 2010). Here, we propose the **study of the development of the disease using two parallel rearing systems with different water sources; natural seawater and borehole water**. Lesion resolution will be studied following transfer of CUD-affected fish in natural seawater. The study will involve advanced histological techniques and measurements of the abiotic factors in order to identify the causes of the disease.

A number of parasite infections have also been seen during **meagre** rearing, including *Sciaenacotyle panceri*, a monogenean found on the gills (Merella et al., 2009). These diseases are also known to cause mortality in fish farmed in the Mediterranean and require development of appropriate treatments. Here we will use a **range finding test to evaluate the tolerance of fish to a variety of chemical treatments, with a view to optimize treatment via a medicated feed or medicated bath, as outlined in the call**.

Very little is known about the **meagre** immune system and responses. Currently there are some 85 nucleotide and 19 protein sequences in NCBI for meagre, and none of these entries are for immune genes (they are mostly relatively conserved genes used for evolutionary analysis and/or microsatellites). Hence there have been no studies on the development of the immune system in this species, or on responses post-vaccination. DIVERSIFY will use information from related farmed fish species to **target key immune genes for cloning, for use as markers of immunity**. Anticipating that future vaccines will be required, initially the focus will be on molecules of adaptive immunity, and development of antisera to the main immunoglobulins present. Expression of these molecules will be monitored during development, and post-vaccination with a common bacterial vaccine. In addition, **key antimicrobial and antiviral genes will be cloned and studied** in the context of how they are regulated and whether they can be used as markers for increased resistance by dietary (*e.g.*, immunostimulants) or other means.

In **greater amberjack**, the most important disease during early stages (post-larva until juvenile) is epitheliocystis, caused by intracellular chlamydia or clamymydia-like microorganisms. Mortalities up to 100% have been observed in mesocosm rearings (Katharios et al., 2008). The disease has been described as acute in the literature causing mass mortalities in young cultured greater amberjack (Crespo et al., 1990) or as a chronic, non-pathogenic condition in larger wild greater amberjack (Grau & Crespo, 1991). Since treatment of the disease (oxytetracycline) is based on early diagnosis, new methods for detecting the pathogen in water are required. Chlamydia-like organisms are obligate intracellular parasites and it is speculated that they enter rearing water using an invertebrate host as a vehicle. Since the production of greater amberjack will be based on mesocosm technologies where water at certain stages is not treated or disinfected, epitheliocystis will constitute a major bottleneck for juvenile production. Here we propose the **development of molecular probes for the detection and early diagnosis of pathogenic Chlamydia and Chlamydia-like organisms in the rearing water**.

A variety of other bacterial diseases has also been noted during **greater amberjack** larval culture and may also be considered potential bottlenecks for production. For example, high mortality of *Seriola* spp. has been





reported to be associated with bacterial pathogens in Australia (Stephen & Savage, 2010), and different bacterial species such as *Streptococcus dysgalactiae* (Nishiki et al., 2010) or *Lactococcus garvieae* (Kawanishi et al., 2005) have been isolated from greater amberjack and yellowtail (*Seriola quinqueradiata*) in Japan. To better characterise this problem, **larvae will be reared under different dietary regimes and the incidence of different bacterial diseases will be determined.** The most important diseases will be typed and cultures stored. Diets (from WP 9 Nutrition – greater amberjack and WP 21 Grow out husbandry – greater amberjack) that promote better larval health will be identified.

**Greater amberjack** juveniles suffer from a number of parasitic diseases during grow out, which require the development of treatments. Here, we will **modulate juvenile resistance to parasite infection through dietary means**, where fish will be given diets enriched with nucleotides, vitamins or immunostimulants. The impact on mucosal defences and on parasite resistance will be determined, by biomarker analysis and morphological studies of gills, skin and intestine.

In relation to the development of treatments for increased disease resistance, it is essential to have some immune markers that can aid selection of the best treatments and to optimise their delivery. As with meagre, there are very few known immune markers currently in greater amberjack, although a partial sequence for RAG-1 exists and in yellowtail nearly 4,000 ESTs are in GenBank, with many used in a recent microarray experiment by Darawiroj et al. (2008). The latter include some immune genes, such as TcR $\alpha$ , Ig heavy and light chain, MHC class II beta chain, and two cytokines (IL-1 $\beta$  and a CC chemokine), which will aid in cloning the equivalent greater amberjack genes. **DIVERSIFY will expand the immune gene markers to include those useful for mucosal immunity and study their expression at mucosal sites following the different antiparasite and antibacterial treatments, at different developmental stages.**

### ***Socioeconomics (including new product development)***

For boosting the competitiveness of the European aquaculture sector, emphasis on adding value (given the potential of each selected species) along the seafood chain from the farm to the consumer is crucial. Bostock et al. (2009) defined market orientation, sustainability and access to resources, the structure of the industry, innovation and industry support and the image of the industry as factors to improve the competitiveness of the EU aquaculture sector. Ernst & Young (2008) also mentioned innovation as driver for competitiveness, but stress that the competitiveness in the EU aquaculture sector is largely affected by many regulatory and socioeconomic aspects. STECF (2012) stresses that environmental regulations are difficult to integrate in business models of aquaculture companies. The above information guided the Socioeconomics WPs to concentrate on market-oriented diversification in close cooperation with the involved SME's.

Macro- and micro-environmental (competitive) factors influence supply and demand in the aquaculture production chains in general, and the chains of the considered species that are currently in production (meagre, halibut, pikeperch and grey mullet) or are supplied by the capture fishery (wreckfish and greater amberjack). Much is known already on the market for fish products from fisheries and cultured fish (Nielsen et al., 2009; AIPCE-CEP), but this is analysed mainly in relation to each other, instead of in relation to meat. DIVERSIFY will analyse the competitive factors within fish consumption and fish buying processes, but also in relation to meat and meat products, as direct competitors of seafood. For this analysis the PESTEL-model (Gillespie, 2011) and the Porter five forces model (Porter, 1985, 1998) will be used, since these are approved practical models in giving direction for product development.

The development of new products from the selected species by:

- incorporating consumer, market and professional buying criteria of buyers from retail organisations and catering organisations, and
- monitoring the quality of these new products in terms of organoleptic characteristics and nutrition-rearing history

is important because this gives direction to potential success of products. Furthermore, the understanding of the overall value perceptions of consumers with regard to cultured fish in general and the value as well as the sensory perceptions towards the newly developed fish products in particular are crucial. This is necessary to



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optimize the newly developed products in terms of ideal extrinsic product attribute combinations that have the potential to generate ideal consumer value perceptions.

Once the new products have been developed, effectiveness of market communication is important. It should help to create awareness for the new product and stimulate customer interest. However, for aquaculture fish also achieving a change in consumer behaviour in relation to the fish species considered and the new raw and value added products developed may be required to overcome possible negative sentiment. An extensive body of literature has pointed out the need of proper communication strategies in order to escalate one of the major impediments of innovation acceptance, namely consumers' lack of knowledge about and familiarity with new food products (Rollin, 2011; Sonne et al., 2011; Bruhn, 2007; Ronteltap et al., 2007). Effective communication regarding details of the new product development methods, the resulting new products and their consumer-perceived benefits becomes essential for loosening reluctance and enhance acceptance and successful marketing of these products (Lee and O'Connor, 2003; Deliza et al., 2003; Cardello et al., 2007).

However, the marketing of new products comprises more than just the communication challenges. An overarching marketing strategy for the newly developed products should be developed to ensure the development of markets that are as large and profitable as possible. As Leslie and Holloway (2006) note, "the biggest risk for most companies has shifted from getting the product to work, to getting it to market," making the commercialization of new products a strategic imperative. While companies have invested more in improving their New Product Development sections, research shows that attention for commercialization, in particular developing appropriate marketing strategies, lags behind and is the main explanation why new product failure rates have not improved and still range from 33 to over 60% (Barczak et al. 2009). The marketing strategy should address both strategic issues such as targeting, positioning and timing, as well as tactical ones such as communications, distribution and pricing/rebate issues (Guiltinan 1999).

Appropriate business models for sustainable profitability and improved competitiveness are necessary, because they explain how firms along the value chain will be able to be profitable. A business model explains who the customer is, why they value and buy the products delivered, and also demonstrates the economic logic of how the suppliers can deliver value to customers at an appropriate cost (Margetta, 2002). Good business models can stand both a narrative and number test, that is, "the story" of the new business needs to make sense, but also the numbers should "add up" and thus show a profit. The Business Model Canvas is a strategic management template for developing new or documenting existing business models, and offers clear descriptions and a visual chart with elements describing a firm's value proposition, infrastructure, customers and finances (Osterwalder and Pigneur, 2010).

Therefore, the Socioeconomics WPs will concentrate on consumer market analysis, product development ending with physical prototypes, accompanying marketing and communication strategies for these products, and market and business models development for the introduction of these products in the market.



### ***B 1.3 S/T methodology and associated work plan***

*A detailed work plan should be presented, broken down into work packages (WPs), which should follow the logical phases of the project implementation.*

*It must include consortium management and assessment of progress and results.*

*Overall, the workplan should be sufficiently detailed to justify the proposed effort and allow progress monitoring by the commission.*

#### ***B 1.3.1 Overall strategy of the work plan.***

The six new/emerging fish species selected in DIVERSIFY are at different stages of "inclusion" in the EU Aquaculture industry (available research and aquaculture production). Three species (meagre, pikeperch and Atlantic halibut) are already produced commercially, but there is a need for **improving production technologies, diversifying products and enhancing the marketing strategies to stimulate the growth of both the aquaculture industry and market**. The other three species have a **high biological and economical potential** (greater amberjack, wreckfish and grey mullet), but as **new biological models** require more work and new approaches to enter into commercial production.

DIVERSIFY has **identified and prioritized the essential bottlenecks** that exist in the production and marketing of each of the selected species, and designed tasks to address them. These bottlenecks were selected based on specific questionnaires to the European aquaculture industry, the input from the participating SMEs and the extensive experience in finfish domestication and aquaculture research of the participating RTD institutions. Therefore, the work plan of DIVERSIFY focuses on the **main documented species-specific bottlenecks in the production** of the selected species, in order to **develop adequate husbandry practices and technologies** for optimized industrial production.

In the area of **Socioeconomics**, DIVERSIFY will research the aquaculture market in general and for the new/emerging species in particular, and create new products for these species --including added value products. The research will develop strategies and tactics to **increase consumer appreciation and consumption of aquaculture products** in general and for the new/emerging species in particular. Finally, DIVERSIFY will develop plans together with SME Partners to exploit and maximise the potential of the new species and to **increase demand within the EU and worldwide for themselves and the aquaculture sector in general**.

The planned work for all six species has been organized in Discipline-Species WPs, which are then organized in six RTD Groups of WPs (GWP) according to research area, namely GWP Reproduction and genetics, GWP Nutrition, GWP Larval husbandry, GWP Grow out husbandry, GWP Fish health and GWP Socioeconomics. This was done in order to bring together scientists with similar expertise from different species, thus increasing interactions and the potential for problem solving in each research area. The appointed **GWP Leaders (GWPL)** or **Discipline leaders (DL)** will **coordinate the WP leaders working with different species in their respective GWP**, and have drawn from their expertise and encouraged the maximum of interactions among Partners during the preparation of the proposal --which will continue during the programme-- in order to ensure the **successful implementation of the tasks and the achievement of the proposed deliverables**.

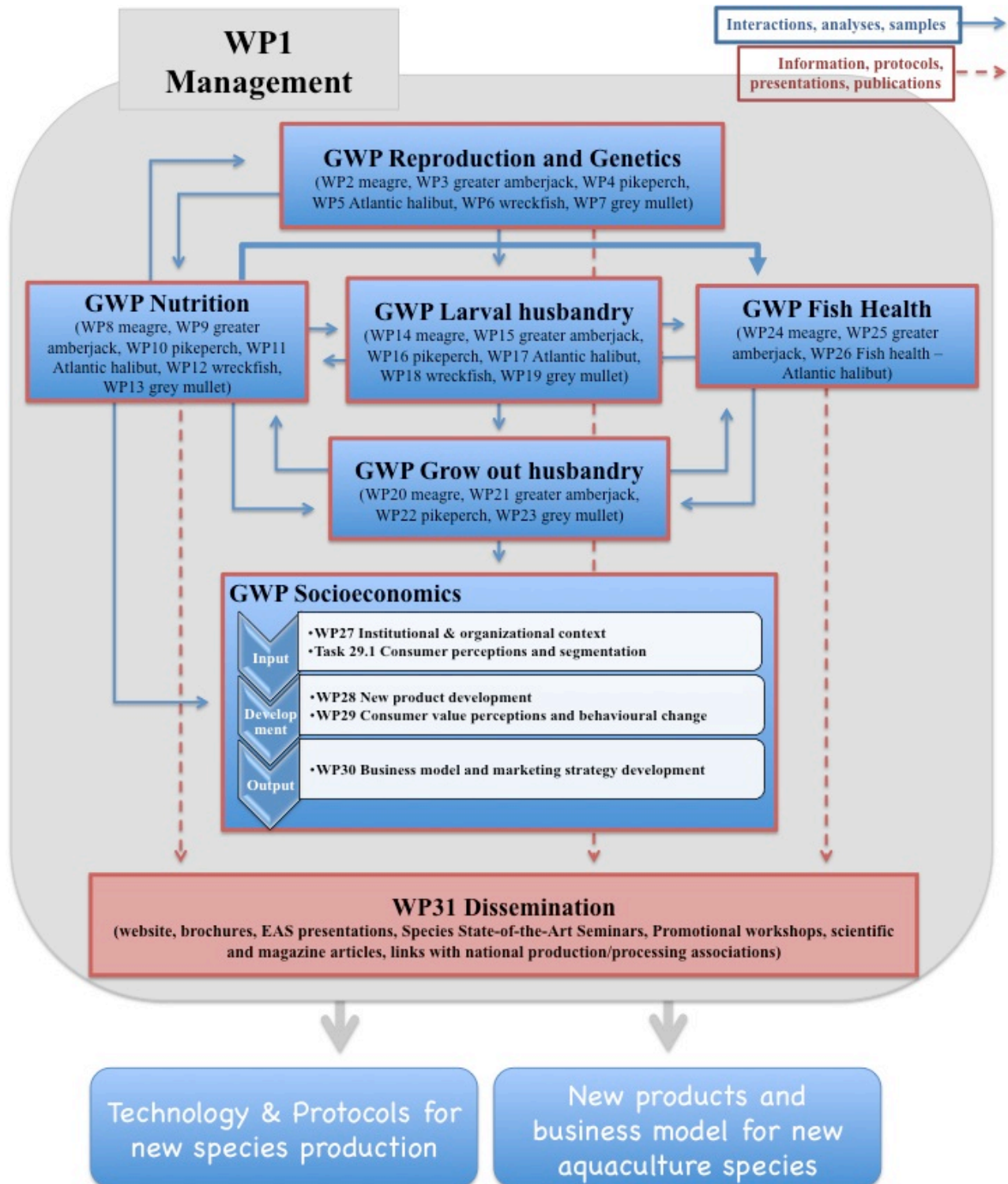
Six **Species leaders** (one for each of the selected species) were appointed during the proposal preparation phase and have been responsible for the **identification of the production bottlenecks of each species**, which was carried as described above. During the implementation of DIVERSIFY, their role will be to **oversee, compile and disseminate the work done for each species** in the different RTD WPs.

The **Dissemination Leader (WP31)** will interact with the **Programme Coordinator (WP1)**, the WP Leaders and the Species Leaders in preparing all the dissemination material for DIVERSIFY, as well as in preparing six **species-specific State-of-the-Art Seminars**, which are planned for the last year of the project to disseminate the acquired knowledge to the aquaculture industry. The Dissemination Leader will be responsible also for the **liaisons and dissemination of relevant project information to other relevant stakeholders**, such as food processors, retailers, caterers, food scientists, consumer organizations, as well as



for the preparation of the **Promotional Workshops to be carried out in 4 strategic seafood consumer countries** (Spain, Greece, UK and Italy) for the promotion of the project's activities and results.

**Graphical presentation of the components showing their interdependencies (Pert diagram or similar)**







v) ***Describe any significant risks, and associated contingency plans***

As in every project there are two types of risks, systematic risks (macro in nature) that are not controllable and unsystematic risks (micro in nature) that are controllable. **Because the project deals with new species to aquaculture, systematic risks are inherently higher compared to other projects on well-established species.** To minimize systematic risks, the project has been built on State-of-the-art knowledge on the selected species, in many cases produced by DIVERSIFY Partners. In addition the projects focuses on specific bottlenecks that can be properly addressed by the DIVERSIFY Partners, who have species-specific expertise in all stages of production. However, despite the recognized expertise of the different Partners, a special effort has been made in each WP to identify those risks and prepare contingency plans (Table B 1.3.1a).

Specifically, for any new species the main risk is the absence of sufficient spawning and, consequently, the lack of eggs (GWP Reproduction and genetics). To minimize this risk, the contingency plans include the incorporation of more than two Partners that possess broodstock and expertise for each of the selected species --except in Atlantic halibut where two broodstocks are available. This allows some redundancy in capacity and increases the potential for the rapid acquisition of results. Also, the project timing has been planned so in case a broodstock does not spawn in one year, the reproduction experiments could be repeated the following year, without risking any of the deliverables.

The risks associated to the control of reproduction to achieve the proposed studies are different for each species and, therefore, each species should be considered separately (Table B 1.3.1a). Meagre, pikeperch and Atlantic halibut have relatively well-controlled reproduction and should be considered a low risk. Consortium partners have been involved in the development of control of reproduction of meagre (Fernández-Palacios et al. 2009, 2011; Mylonas et al., 2011; Duncan et al., 2012) and three partners (HCMR, FCPCT and IRTA) have expertise and broodstock, each of which could be used to complete the studies and provide the biological material required in the proposal. Consortium partners have also been involved in the development of control of reproduction of pikeperch (Kestemont & Melard, 2000; Zarski et al., 2012) and three partners (UL, FUNDP and ASIALOR) have expertise and eggs, larvae or juveniles can be supplied by ASIALOR or bought from established commercial suppliers, to be used to complete the studies and provide the biological material required in the proposal. Consortium partners have a long history in the control of reproduction of halibut (Norberg et al., 1991) and three partners (IMR, NIFES and SWH) have expertise and two commercial broodstocks that have an established history that ensures the broodstock can be used to complete the studies and provide the biological material required in the proposal. Partners in the consortium have regularly obtained spawns from greater amberjack and grey mullet and these species should be considered a medium risk. Consortium partners have obtained spawns from the greater amberjack (Mylonas et al., 2004; Jerez et al., 2006) and five partners (HCMR, FCPCT, IEO, ARGO and ITICAL) have or will obtain broodstock. In addition two partners (FCPCT and IEO) have obtained regular spawning in recent years (in the process of publication) and this combined with published studies (Stewart 2013) and collaborations with Australian researchers working with yellowtail amberjack (*Seriola lalandi*) will place the consortium in a good position to complete the studies and provide the biological material required in the proposal. Consortium partners have also obtained spawns from the grey mullet (Aizen et al. 2005) and four partners (IRTA, IOLR, ITICAL and DOR) have or will obtain broodstock to complete the studies on broodstock. Wild juveniles can also be bought from established commercial suppliers, to be used to complete the studies and provide the biological material required in the proposal. Lastly wreckfish can be considered medium to high risk to obtain spawning of good quality eggs. The partners in the consortium have obtained spawns (Fauvel et al., 2008; Papandroulakis et al., 2008; Peleteiro et al., 2011) and three partners hold all broodstock held in European research centres. In addition to these broodstock and expertise, a new partner (P32. MC2) has been now included in the consortium. This Partner is an aquarium that has this year (2013) collaborating with P8. IEO in obtaining successfully spontaneous spawning of good quality wreckfish eggs. This Partner maintains a very large broodstock (n = 24), which is well acclimated to captivity, and will ensure that the proposed research will be successful in providing eggs for the larval rearing tasks. Therefore, the proposal has four broodstock, one with a recent history of spontaneous spawning of good quality eggs and three with a history of fish maturing to late stages of maturity from which the consortium has considerable experience to successfully induce spawning. Despite of the medium to high risk of this species,



the experience in the consortium, the four established broodstock and the five year project gives 20 (4 broodstock x 5 years) opportunities to solve the identified bottlenecks and high confidence that the deliverables will be achieved for wreckfish.

Also, the project design allows for Partners to be provided with biological material from another Partner (e.g., eggs or larvae) and the costs of production and shipment will be covered by the receiving Partner. In this way, planned deliverables will not be compromised. In fact, this contingency exists in the EU project AQUAEXCEL where some of the DIVERSIFY Partners (HCMR, FCPCT, IRTA, IMR, IFREMER) are designated as Aquaculture Facilities of Excellence and have developed protocols for the safe transfer of biological material between them, that satisfy national, European and international legislation. Finally, if a Partner involved in a trial with live fish suffers any problem that does not allow a trial to be completed, contingency plans have been made to relocate the trial to be conducted by another Partner, with the necessary reallocation of funds.

In GWP Nutrition, various nutrients and ingredients have been selected to improve the performance of enrichment products and diets in larviculture and juvenile rearing. These are relatively short-term studies that allow the option of repeating them within the season, if high mortality or technical problems prohibit their completion. This would decrease the risk of producing incomplete results. In addition, feed production will be carried out by commercial feed companies (SARC and IRIDA), whose experience and expertise will provide feed consistency in quality and supply, thus minimizing the risk of failure to implement the proposed nutrition studies. Also, the use of common standards in feed production by all Partners facilitates comparing results among Partners. Furthermore, the participation of feed manufacturers eliminates the risk of testing diet formulations that do not meet market criteria.

In GWP Fish Health, a risk related with Task 24.3 is that there may be a lack of fish with parasites with which to perform this task. Our contingency plan includes the participation of more than one Partner maintaining meagre and the inclusion of an SME that will perform grow out trials where fish are likely to become infested with monogenean parasites. Additionally, within Task 24.4 the isolation of gene fragments for development of expression assays of immune functions is currently planned to include tissue culture. This would be simpler for the stimulation using PAMPs rather than from whole tissues/animals. However, as a contingency in case cell culture performs poorly, isolation of the intended target genes can be performed from intact animals stimulated with PAMP injections to boost expression of target genes to facilitate isolation of gene fragments.

The methodologies of GWP Socioeconomics are approved, validated and used in several other international studies, reducing the methodological/operational risk. However, the market analysis in WP 27 is dependent on availability of reliable and comparable data of the fish market in various geographical areas. This means that comparing data between countries might be problematic. In this case the countries can be handled as cases. A problem might appear in relation to task 29.2, where physical product prototypes (i.e. raw fish) are needed for the sensory analyses. It is expected that the issue of availability of those physical products coming from all species under study is treated properly through the measures taken by preceding WPs, as explained above. Finally, since the cross-cultural consumer surveys that test acceptance of optimized final products in task 29.3 need to run within a specific, albeit limited period of time (M28-38) due to practical (i.e., time and budget) considerations, it is anticipated that product mockups will be used instead of physical prototypes, to avoid the danger of not having enough and/or all of the species needed for the cross-cultural surveys. The use of mockups is again a standard practice in international consumer surveys, and is also in line with the aim of the research at that phase of the project where extrinsic (labeling-related) quality characteristics are tested for perceived product quality optimization purposes.

The remaining activities do not involve any considerable risks and any issues that arise will be solved either by the Partners and GWPLs involved or by the mediation of the PC and/or SC. To conclude, **the project carries the type of risks inherent to all projects dealing with new species**, but the DIVERSIFY consortium through its expertise is in the best position to mitigate them.



**Table B 1.3.1a** Identification of the level of risk per activity, and associated contingency plans.

Type of risk		Risk level	Contingency plan
<b>GWP Reproduction and genetics.</b> Failure of spawning of broodstocks	<b>General</b>	Low to High	Multiple broodstocks will be available for each species, so that if one broodstock fails to spawn, another will be able to provide eggs for the larval rearing activities (WP4) of the various Partners. For all species, at least one of the broodstocks will be maintained by a commercial Partner. Partners with existing broodstocks have good records in spawning the species selected. For the reproduction experiments (WP2), given the duration of the project, it is possible to repeat experiments in following reproductive seasons, without affecting any of the deliverables
	<b>Meagre</b>	Low	Partners have history of successful spawning. Duplication of broodstock (total of 3 broodstock). Chronogram allows experiments to be repeated. Exist established commercial suppliers of eggs and juveniles. Transport of material between partners.
	<b>Pickperch</b>	Low	Partners have history of successful spawning. Chronogram allows experiments to be repeated. Established commercial suppliers of eggs and juveniles are available. Transport of material between partners.
	<b>Halibut</b>	Low	Partners have history of successful spawning Duplication of broodstock (total of 2 broodstock held at separate locations). Chronogram allows experiments to be repeated. Exist established commercial suppliers of eggs and juveniles Well established routines for transport of material between partners .
	<b>Greater amberjack</b>	Moderate	Partners have recent history of successful spawning. Duplication of broodstock (total of 5 broodstock). Chronogram allows experiments to be repeated. Transport of material between partners.
	<b>Grey mullet</b>	Moderate	Partners have history of successful spawning. Duplication of broodstock (total of 4 broodstock). Chronogram allows experiments to be repeated. Exist established commercial suppliers of juveniles. Transport of material between partners.
	<b>Wreckfish</b>	Moderate to High	Partners have history of obtaining advanced maturation from which spawning induction can and has been achieved. A new (P32. MC2) has been added to the consortium, maintaining a large broodstock (24 individuals) that is well acclimitized to captivity (sizes up to 28 kg) and has been spawning spontaneously in 2013. One broodstock has one season of spontaneous spawning. Duplication of broodstock (total of 4 broodstock). Chronogram allows experiments to be repeated. Transport of material between partners.
<b>GWP Nutrition.</b> Mortalities during nutrition studies		Low	As most studies will be conducted on larvae and young juveniles, acquisition of results will be rapid, allowing a repetition of a trial if hampered by a sudden mortality or technical problems. Furthermore, if a Partner encounters a problem that does not allow a trial to be completed,



		contingency plans have been made to relocate the trial and be conducted by another Partner.
<b>GWP Fish health.</b> Lack of fish with parasites (Task 6.1.3)	Low	More than one Partner maintaining meagre and inclusion of an SME that will perform grow out trials where fish are likely to become infested with monogenean parasites.
<b>GWP Fish health.</b> Failure to isolate gene fragments for development of expression assays of immune function using cell culture (Task 6.1.4)	Moderate	Isolation of the intended target genes can be performed from intact animals stimulated with PAMP injections to boost expression of target genes to facilitate isolation of gene fragments.
<b>GWP Socioeconomics.</b> Lack of comparable data of the fish market among different countries (WP27.1)	Moderate	Countries can be handled as cases.
<b>GWP Socioeconomics.</b> Not having physical new product prototypes for the cross-cultural surveys	Low	Product mockups will be used instead of physical prototypes.



**B1.3.2 Timing of work packages and their components (Gantt chart or similar)**

		Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
		Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De
		3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
*continues in following pages (1/3)																					
	<b>Task 1.1</b> Establishment of management bodies																				
	<b>Task 1.2</b> Kick off meeting																				
	<b>Task 1.3</b> Annual Coordination Meeting																				
	<b>Task 1.4</b> Communication with the European Commission (EC)																				
	<b>Task 1.5</b> Mid-term evaluation of progress																				
	<b>Task 1.6</b> Interactions with other projects																				
<b>GWP</b>	<b>Reproduction &amp; genetics-meagre</b>																				
<b>WP2</b>	<b>Reproduction &amp; genetics-meagre</b>																				
	<b>Task 2.1</b> Evaluation of the genetic variation in captive meagre broodstocks																				
	<b>Task 2.2</b> Development of protocols for paired crossing in spontaneous spawning																				
	<b>Task 2.3</b> Description of sperm characteristics and cryopreservation methods																				
	<b>Task 2.4</b> Development of in vitro fertilization methods for planned crosses																				
	<b>Task 2.5</b> Development of Single Nucleotide Polymorphisms (SNP) marker tools for the genetic characterization of fast and slow growers																				
<b>WP3</b>	<b>Reproduction &amp; genetics-greater amberjack</b>																				
	<b>Task 3.1</b> Description of the reproductive cycle of greater amberjack																				
	<b>Task 3.2</b> Development of an optimized spawning induction protocol for captive greater amberjack in the Mediterranean																				
	<b>Task 3.3</b> Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic																				
	<b>Task 3.4</b> Development of an optimized spawning induction protocols for F1 greater amberjack in the eastern Atlantic																				
	<b>Task 3.5</b> Spawning induction of greater amberjack and egg collection in cages																				
<b>WP4</b>	<b>Reproduction &amp; genetics-pike perch</b>																				
	<b>Task 4.1</b> Evaluation of the genetic variation in available domesticated broodstocks of pike perch																				
	<b>Task 4.2</b> Evaluation of the genetic variation in non-domesticated broodstocks of pike perch																				
<b>WP5</b>	<b>Reproduction &amp; genetics-Atlantic halibut</b>																				
	<b>Task 5.1</b> Documentation of reproductive performance in wild-captured vs cultured female Atlantic halibut																				
	<b>Task 5.2</b> GnRH implant therapy as a means to improve spawning performance																				
	<b>Task 5.3</b> Fecundity regulation																				
<b>WP6</b>	<b>Reproduction &amp; genetics-wreckfish</b>																				
	<b>Task 6.1</b> Collect wild fish to establish new broodstocks																				
	<b>Task 6.2</b> Describe reproductive cycle																				
	<b>Task 6.3</b> Development of spawning induction procedures																				
	<b>Task 6.4</b> Evaluation of sperm characteristics and cryopreservation protocols																				
<b>WP7</b>	<b>Reproduction &amp; genetics-grey mullet</b>																				
	<b>Task 7.1</b> Evaluation of the effectiveness of hormone-based treatments on synchronizing gonadal development																				
	<b>Task 7.2</b> Development of hormone-based treatments for inducing spawning																				
	<b>Task 7.3</b> Optimization and scale-up of a breeding protocol for grey mullet in captivity																				
	<b>Task 7.4</b> Assessment of the effects of captivity on first sexual maturity of wild-caught and hatchery-produced fish																				
	<b>Task 7.5</b> Establish a shipping protocol for grey mullet eggs																				
<b>GWP</b>	<b>Nutrition</b>																				
<b>WP8</b>	<b>Nutrition-meagre</b>																				
	<b>Task 8.1</b> Improvement of larval weaning feeds																				
	<b>Task 8.2</b> Determination of nutritional requirements to promote feed utilization, consistent growth rates and fish welfare																				
<b>WP9</b>	<b>Nutrition-greater amberjack</b>																				
	<b>Task 9.1</b> Improve larval enrichment products to enhance production of larvae and juveniles																				
	<b>Task 9.2</b> Development of diets for grow-out of amberjack to maximize growth																				
	<b>Task 9.3</b> Design adequate feeding regimes for broodstock to optimize reproduction																				
<b>WP10</b>	<b>Nutrition-pike perch</b>																				
	<b>Task 10.1</b> Effect of selected dietary nutrients on pikeperch larval development and performance																				
	<b>Task 10.2</b> Effects of pikeperch early fatty acid nutrition on long-term stress sensitivity																				



\*continues from previous page (2/3)

	Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
	Ma 3	Ju 6	Se 9	De 12	Ma 15	Ju 18	Se 21	De 24	Ma 27	Ju 30	Se 33	De 36	Ma 39	Ju 42	Se 45	De 48	Ma 51	Ju 54	Se 57	De 60
<b>WP11 Nutrition-Atlantic halibut</b>																				
Task 11.1 Early weaning of Atlantic halibut																				
Task 11.2 Development of a production strategy for ongrown <i>Artemia</i>																				
Task 11.3 Nutrient retention and digestive physiology of Atlantic halibut juveniles fed <i>Artemia</i> nauplii or on-grown <i>Artemia</i>																				
Task 11.4 Comparison of nutrient retention in Atlantic halibut larvae reared in RAS vs FTS																				
Task 11.5 Effect of dietary PL on digestion, absorption and metabolism of lipids in Atlantic halibut juveniles																				
<b>WP12 Nutrition-wreckfish</b>																				
Task 12.1 Live preys and enrichments for wreckfish larvae																				
Task 12.2 Influence of broodstock feeds for fecundity and spawn quality																				
<b>WP13 Nutrition-grey mullet</b>																				
Task 13.1 Improvement of larval performance through adequate first feeding regimes																				
Task 13.2 Determining grey mullet nutritional needs for improved weaning to a dry diet																				
Task 13.3 Determining grey mullet nutritional needs for a more cost-effective production																				
Task 13.4 Design adequate feeding regimes for brood stock to optimize reproduction success																				
<b>GWP Larval husbandry</b>																				
<b>WP14 Larval husbandry-meagre</b>																				
Task 14.1 Determining the earliest and most cost effective weaning period																				
<b>WP15 Larval husbandry-greater amberjack</b>																				
Task 15.1 Effect of feeding regime and probiotics																				
Task 15.2 Comparison of semi-intensive and intensive rearing																				
Task 15.3 Effect of environmental parameters during rearing																				
Task 15.4 Development of industrial protocol																				
<b>WP16 Larval husbandry-pike perch</b>																				
Task 16.1 Optimal combinations of factors to improve larval rearing																				
Task 16.2 Development of an industrial protocol																				
<b>WP17 Larval husbandry-Atlantic halibut</b>																				
Task 17.1 Recirculation (RAS) vs Flow through (FT) systems during yolk sac and first feeding stages and the effects on larval survival, quality and growth																				
Task 17.2 The effect of probiotics on larval microbiota and survival																				
Task 17.3 Production of on-grown <i>Artemia</i>																				
Task 17.4 Comparison of feeding on-grown <i>Artemia</i> versus <i>Artemia</i> nauplii on larval performance																				
<b>WP18 Larval husbandry-wreckfish</b>																				
Task 18.1 Development of feeding methodology																				
Task 18.2 Defining optimum conditions for larval rearing																				
<b>WP19 Larval husbandry-grey mullet</b>																				
Task 19.1 Effect of algal type and concentration on larval performance																				
Task 19.2 Comparing the selected microalgae type and protocol with lyophilized substitute																				
Task 19.3 Determine the effect of co-feeding ciliates and rotifers on digestive tract maturation and enzyme production																				
Task 19.4 Determine when to wean larvae and to feed weaning diet type according to DT maturation and the shift from carnivorous to omnivorous feeding																				
Task 19.5 Testing the improved grey mullet larval rearing protocol in a commercial hatchery																				
<b>GWP Grow out husbandry</b>																				
<b>WP20 Grow out husbandry-meagre</b>																				
Task 20.1 Size variability at juveniles																				
Task 20.2 Effect of rearing environment																				
Task 20.3 Development of feeding methodology																				
<b>WP21 Grow out husbandry-greater amberjack</b>																				
Task 21.1 Development of rearing method in cages																				
Task 21.2 Development of feeding methods																				
Task 21.3 Development of appropriate husbandry practise																				
<b>WP22 Grow out husbandry-pike perch</b>																				
Task 22.1 Effect of husbandry practices and environmental factors on pikeperch growth, immune and physiological status																				
Task 22.2 Characterization of pikeperch growth, immune and physiological status in farm conditions																				
Task 22.3 Effect of pikeperch domestication level and geographical origin on growth and stress sensitivity																				
<b>WP23 Grow out husbandry-grey mullet</b>																				
Task 23.1 Determine the cost-benefit of different weaning diets on the performance and health status of wild juvenile grey mullet																				
Task 23.2 Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture of F1 juveniles stocked at two different densities in cement and earthen ponds																				
Task 23.3 Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture of wild caught juveniles stocked at two different densities in cement ponds in Greece																				
Task 23.4 Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture of wild caught juveniles stocked at two different densities in cement ponds in Spain																				





\*continues from previous page (3/3)

	Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
	Ma 3	Ju 6	Se 9	De 12	Ma 15	Ju 18	Se 21	De 24	Ma 27	Ju 30	Se 33	De 36	Ma 39	Ju 42	Se 45	De 48	Ma 51	Ju 54	Se 57	De 60
<b>GWP</b>	<b>Fish Health</b>																			
<b>WP24</b>	<b>Fish health-meagre</b>																			
Task 24.1	Systematic Granulomatosis																			
Task 24.2	Chronic Ulcerative Dermatopathy																			
Task 24.3	Anti-parasitic treatments																			
Task 24.4	<i>Nocardia</i> infection in meagre																			
Task 24.5	First characterisation of the immune system																			
Task 24.6	Monitor specific immune responses																			
Task 24.7	Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in meagre																			
Task 24.8	Diagnostic-recommendation manual for meager health																			
<b>WP25</b>	<b>Fish-health-greater amberjack</b>																			
Task 25.1	Study of Epitheliocystis during larval rearing																			
Task 25.2	Promoting resistance to parasitic incidence on greater amberjack																			
Task 25.3	Identification of immune markers																			
Task 25.4	Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites																			
Task 25.5	Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in greater amberjack																			
Task 25.6	Diagnostic-recommendation manual for greater amberjack health																			
<b>WP26</b>	<b>Fish-health-Atlantic halibut</b>																			
Task 26.1	Production of VNN capsid protein																			
Task 26.2	Monitor and assess immune response and protection																			
<b>GWP</b>	<b>Socioeconomics</b>																			
<b>WP27</b>	<b>Socioeconomics-Institutional and organizational context</b>																			
Task 27.1	External environmental analysis																			
Task 27.2	Competitive analysis																			
Task 27.3	Opportunities and barriers for growth																			
<b>WP28</b>	<b>Socioeconomics-New product development</b>																			
Task 28.1	Product concept development: technical and consumer-driven																			
Task 28.2	New Product Development																			
Task 28.3	Monitoring technical quality of the products																			
<b>WP29</b>	<b>Socioeconomics-Consumer value perceptions and behavioural change</b>																			
Task 29.1	Consumer value perceptions and segmentation																			
Task 29.2	Consumer sensory perceptions																			
Task 29.3	Optimization of intrinsic-extrinsic attribute combinations																			
Task 29.4	Communication effectiveness in behavioural change																			
<b>WP30</b>	<b>Socioeconomics-Business model and marketing strategy development</b>																			
Task 30.1	Business models																			
Task 30.2	New product marketing strategy development																			
Task 30.3	Recommendations of industry development and international market expansion																			
<b>WP31</b>	<b>Dissemination</b>																			
Task 31.1	Project website and brochure																			
Task 31.2	Annual coordination meetings																			
Task 31.3	Presentation of DIVERSIFY at the AQUA EUROPE meetings																			
Task 31.4	Scientific presentations and submission of manuscripts																			
Task 31.5	Full-day seminar on "Know-how Transfer" of the aquaculture for each of the studies species																			
Task 31.6	Promotional workshops																			
Task 31.7	Dissemination to the food industry and consumers																			



## B2. Implementation

### B 2.1 Management structure and procedures

*Describe the organisational structure and decision-making mechanisms of the project. Show how they are matched to the complexity and scale of the project.*

DIVERSIFY is characterized by (a) a long duration (5 years), (b) the involvement of 20 RTD organizations, 2 large commercial companies and 9 SMEs from 10 European countries and (c) work on six different new/emerging species and seven scientific disciplines. Given the nature of this large-scale project, particular attention needs to be given to project management and coordination. **This effort has been initiated from the very early stage of forming the consortium and preparing the proposal.**

#### Proposal preparation

The species to be studied were chosen tentatively via email and personal one-on-one interactions of some of the main Partners in the consortium during Spring 2012. DIVERSIFY Partners include well-known researchers that are **pioneers in the study of the selected species** (see Section 2.1 Individual Partners and 2.3 Consortium as a whole), in various scientific disciplines --including reproduction, nutrition, larval rearing, grow out, fish health and final product quality-- with a number of scientific publications already in these species. In some cases, the participating Partners maintain the major available broodstocks of the selected species (*e.g.*, MC2, IEO, HCMR and CMRM for wreckfish; FCPCT, IEO and HCMR for greater amberjack; IMR for Atlantic halibut and IOLR for grey mullet).

The species evaluation followed a Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis, which was presented to the consortium at a 2-day organizational meeting (Spain, July 2012). At this time, the final selection of the six species was made. A **Species Leader (SL)** was appointed for each selected species, with the responsibility of **coordinating the identification of the existing industrial bottlenecks** in the production of each species. The SLs were selected from among the consortium based on their involvement, expertise and excellence in research with the particular species. The SLs are:

Dr. Alicia Estevez (IRTA, Spain) for meagre,

Dr. Nikos Papandroulakis (HCMR, Greece) for greater amberjack,

Dr. Pascal Fontaine (UL, France) for pikeperch,

Dr. Birgitta Norberg (IMR, Norway) for Atlantic halibut,

Dr. Blanca Alvarez is replacing Dr. Jose Benito (Tito) Peleteiro Alonso (IEO, Spain) for wreckfish, due to his recent retirement, and

Dr. Bill Koven (IOLR, Israel) for grey mullet.

In addition to using the State-of-the-Art and the experience possessed by the scientific Partners of DIVERSIFY, identification of bottlenecks was done through the use of questionnaires sent to and/or personal communications with production managers of aquaculture companies involved with the production of each species (currently or attempted in the past). Once the bottlenecks were identified, a list was produced and Partners and aquaculture companies were asked to prioritize the importance of the bottlenecks, given limitations in the budget. Once the top bottlenecks were identified and selected for DIVERSIFY, the work required to solve each bottleneck was allocated to a specific RTD Work package (WP).

At this time, the RTD budget was allocated to species and scientific disciplines and **the people leading the RTD Groups of WPs (GWPL or DL) were selected, again given their expertise and excellence in research** in the scientific discipline. The GWPLs and identified scientific disciplines are:

Dr. Neil Duncan (IRTA, Spain) for GWP Reproduction & Genetics,

Dr. Daniel Montero Vitores (FCPCT, Spain) for GWP Nutrition,

Dr. Bill Koven (IOLR, Israel) for GWP Larval husbandry,





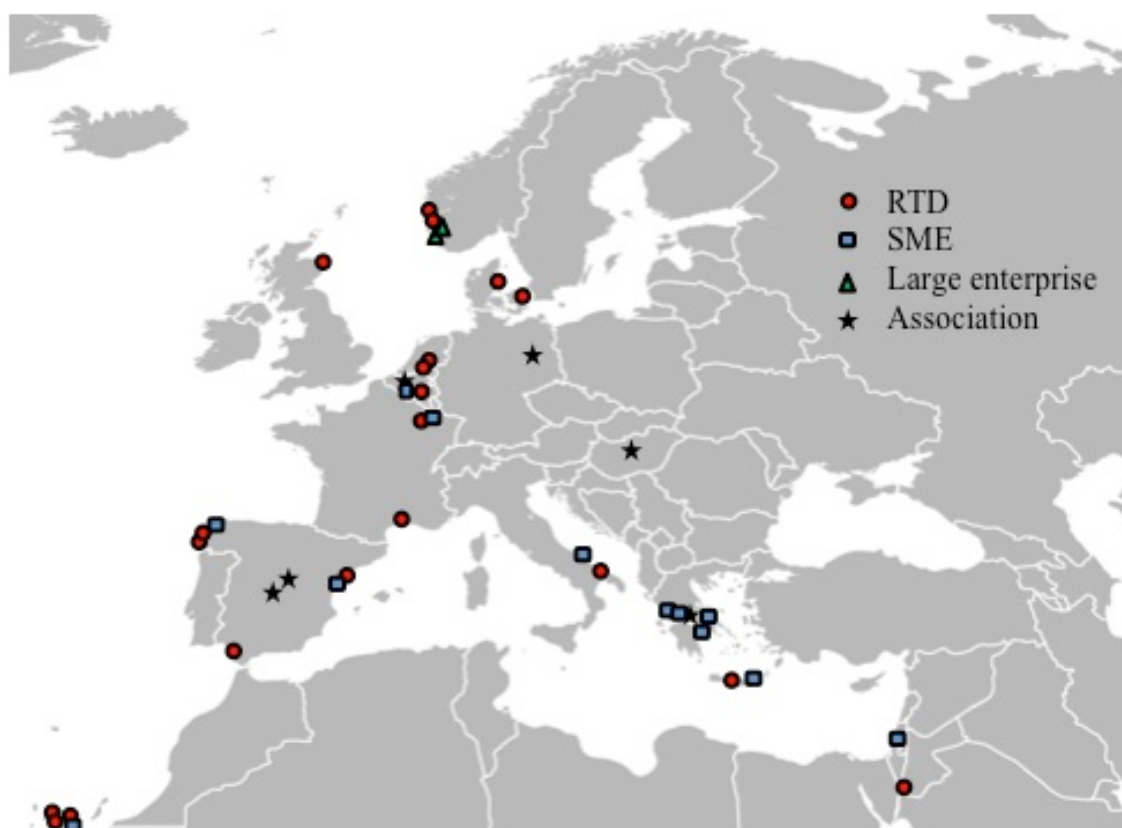
Dr. Nikos Papandroulakis (HCMR, Greece) for GWP Grow out husbandry,

Dr. Chris Secombes (UNIABDN, UK) for GWP Fish health, and

Drs. Gemma Tacken (SWR, the Netherlands) for GWP Socioeconomics.

The responsibility of the GWPLs at this stage was to interact with all Partners involved in their scientific discipline and decide on which activities were to be included in DIVERSIFY, based on the priority of the bottleneck they are addressing and the available budget. A leader for WP31 Dissemination was also selected at this time (Mrs Rocio Robles, CTAQUA, Spain), and was in charge of interacting with the relevant Partners for the design of the dissemination activities of DIVERSIFY.

The structure of the consortium with the various management bodies and the flow of responsibilities and information is shown below.



### **Establishment of management bodies**

As mentioned already, given the nature of this large-scale project particular attention needs to be given to the management and coordination of DIVERSIFY at the stage of implementation. Coordination and management of DIVERSIFY will be achieved through the following bodies:

#### **1. Project coordinator (PC)**

The PC is Dr. Constantinos (Dinos) C. Mylonas (HCMR, Greece) and is responsible for the overall management of the project and communications with the EC, and will be assisted by an Executive Secretary



to be hired for the duration of the project, the Administration and Financial Manager Mr. Stelios Kastrinakis, the Financial officer Mr Sifis Marinakis and the Communications officer Mrs Maria Papadaki. The Executive Secretary will also be responsible for the web-based project management tool (see later). The PC with the support of the management team will have all the responsibilities assigned by the Grant Agreement and Consortium Agreement, including the preparation of the progress, financial reports and deliverables to the DG RTD.

Dr. Mylonas is a Director of Research (the highest level of researcher) at the Hellenic Center for Marine Research, Institute of Marine Biology, Biotechnology and Aquaculture. He is a reproductive endocrinologist with a B.Sc. in Aquaculture (Texas A&M University, USA), M.Sc. in Zoology (North Carolina State University, USA) and Ph.D. in Marine-Estuarine and Environmental Sciences (University of Maryland, USA), and has been a researcher in HCMR since 1999. His area of expertise is endocrinology of fish reproduction and the development of pharmacological methods for the control of reproduction in captivity, and worked with many aquaculture species including greater amberjack, meagre, Atlantic halibut, wreckfish, as well as Atlantic bluefin tuna, Senegalese sole and shi drum (*Umbrina cirrosa*). Dr. Mylonas (H-index = 26) has co-authored 91 scientific articles with an ISI index, which have 1,757 citations and 1,382 non-self citations ([apps.webofknowledge.co](https://apps.webofknowledge.co)).

Since 1999, Dr. Mylonas has participated in many national and international projects as a coordinator or scientific responsible for HCMR, including the EU FP5 projects PROBASS and REPRODOTT and the EU FP7 project SELFDOTT. Currently he is coordinating two national projects on meagre reproduction and culture (ESPA-Cooperation 2009, 512,100€; [kranios.weebly.com](http://kranios.weebly.com)) and Atlantic bluefin tuna reproduction and culture (ESPA-SMEs 2009, 480,000€).

The HCMR is the national research and advisory body for marine aquaculture, fisheries and the environment and the **Institute of Marine Biology, Biotechnology and Aquaculture** is the main research organization in Greece in the subject area, with 18 staff scientists. In the last 10 years, the Institute of Marine Biology, Biotechnology and Aquaculture (previously the Institute of Marine Biology & Genetics and the Institute of Aquaculture) has participated in 35 EU FP projects and was the coordinator in 5 of them. Also HCMR has been the coordinator of FP6 SESAME (15 million € with 59 Partners) and FP7 PERSEUS (17 million € with 53 Partners). The latter project ([www.perseus-net.eu](http://www.perseus-net.eu)) has been described as one of the most significant initiatives within the FP7 and its administration will be run through the PROJECTA management tool, used for reporting and monitoring progress. HCMR has also developed and runs the Financial Management System (FMS), which authorises, tracks and reports on spending at the Workpackage level within the project. Both facilities will be available to DIVERSIFY.

## **2. Group Work package Leaders (GWPL) or Discipline Leaders (DL)**

The GWPLs were selected based on their expertise in research in specific scientific disciplines at the time of the proposal preparation and were responsible for the selection of research activities in DIVERSIFY. A leader for WP31 Dissemination was also selected. During the implementation of DIVERSIFY, the GWPLs will be coordinating the WP leaders of (Lead Beneficiaries) for each WP in their related scientific discipline in order to achieve (a) the timely execution of all planned research activities in their specific WP following the projects time schedule (Table B1.3.2 Gantt chart), (b) the compilation of all results and preparation of the periodic reports and (c) liaising with the PC, WP8 Dissemination leader and the SLs (see below and Section Section 3.2 Dissemination) for the preparation of the dissemination material for the project (e.g., web information, brochures, presentations and articles). Due to the size and complexity of the work in DIVERSIFY, each GWP consists of several Discipline-Species specific WPs. For each of these WP, the Lead Beneficiary (WP leader) will be responsible for the actions, reports and deliverables of its specific WP. Similarly, due to the participation of many Partners in most research activities, for each Task and Action in the subWPs, a Partner has been clearly identified who will be responsible for the implementation of the planned work (See Tables WT3 Work package description).

The above mentioned structure is expected to facilitate both the implementation of the research in DIVERSIFY, but also the compilation of the obtained information, its proper analysis and timely reporting to the DG RTD, as well as to the SPLs and the WP31 Dissemination leader, for the preparation of the



planned dissemination material and activities (See also Section B 3.2 Plan for the use and dissemination of foreground).

### ***3. Species Leaders (SL)***

The SLs were appointed for each selected fish at the time of the proposal preparation and were responsible for the identification of the bottlenecks in the production of each species. As mentioned above, the SLs were selected from among the consortium based on their involvement, expertise and excellence in research with the particular species. During the implementation of DIVERSIFY the SLs will be involved in overseeing, compiling and disseminating all the species-specific work done in the various RTD WPs of the project, and will provide their expertise and advice to the Steering Committee of the project (See below). As such, the SL will be the integration point of all GWPLs in matters related to their species of responsibility, and they will assist each GWPL in aspects related to their species expertise, but the main role of the SLs will be in dissemination, as they will be the ones mainly in charge for the preparation of the planned dissemination material and activities, such as the once-every-two-years presentation at the EAS conference and the organization of the State-of-the-Art seminar for their species (See also Section 3.2 Dissemination).

### ***4. Steering Committee (SC)***

The SC will consist of the PC, the GWPLs and two SME representatives (ARGO and F2B) involved in work with different fish species and one of the aquaculture producers' associations participating in the project (APROMAR). The SC will be the integration unit of the consortium and will be responsible for coordinating the contributions of each Partner to the common effort. The SC will be involved with the overall decision making of the project in matters of Technical Annex implementation and modification, evaluation of project progress, knowledge management and any conflict resolutions. Decisions will be taken by consensus, and if not possible by simple majority vote. Planned meetings of the SC will take place at the onset of the project and at 12-monthly intervals thereafter, just prior to the annual coordination meetings. If the need arises, a budget has been earmarked to allow the SC to convene in order to address issues or events that cannot be dealt during the annual meetings, or with using the other means of communication available to the consortium.

### ***5. Coordination with SME activities***

The research activities of each Large enterprise/SME participating in DIVERSIFY will be coordinated by a specific RTD Partner --one who is located in the same country and/or is collaborating directly with this private Partner-- in order to ensure the effective and successful implementation of all planned activities. These RTDs will be HCMR for ARGO, GEI, FORKYS, GMF and IRIDA (Greece), FCPCT for SARC (Norway) and CANEXMAR (Spain), IMR for SWH (Norway), IOLR for DOR (Israel), UL for F2B (France), and AU for HRH (Greece).

### **Management and coordination activities**

The **Kick-off meeting** will take place during the first month of the project. The meeting will be held in the facilities of the PC (HCMR, Crete, Greece) and attendance will include all Scientific Responsibles of the participating Partners. During this meeting, the consortium will (a) review the signed contract with the European Commission, (b) review the Consortium Agreement and address any conflicts that may arise between the RTD and SME Partners, (c) describe the administration and financial management procedures to all Partners, and (d) plan and organize the research activities for the first year of project. Emphasis will be given on establishing, procedures for sampling acquisition and sharing among Partners, and for exchange of scientific information.



**Annual Coordination Meetings (ACM)** will be held at 12-month intervals. These will be 3-day-long meetings held at different Partner's facilities and have been planned for the end of Y1 at FCPCT (Spain), Y2 at IMR (Norway), Year 3 at IRTA (Spain), Year 4 at UL (France) and at the end of Y5 again at HCMR (Greece). During the first two days of the ACM, the GWPLs will (a) present a summary of the major achievements during the past year, (b) plan and coordinate the activities for the following year and (c) coordinate the preparation of dissemination material (scientific articles, conference presentations, etc.). In addition, selected tasks from each WP will be presented by the responsible scientists, based on the current progress and importance of the findings. On the morning of the 3<sup>rd</sup> day, a **joint session of the SC together with the SLs** will discuss the progress of the research, develop a general outline of the upcoming work, and address conflicts or problems that may arise. In the afternoon, the GWPLs will have separate WP sessions with the attending scientists involved in their WP, in order to (a) further discuss the results obtained in their respective WPs, (b) plan and organize in detail the work to be carried out during the following year and (c) coordinate the preparation of the interim report and dissemination material.

As described in WP31 Dissemination and in Section 3.2 Dissemination, the ACM will serve also a dissemination purpose. The first two days of the meetings will be attended by invited guests from Europe and world-wide, who are experts in the species (or their congeners) and research activities of DIVERSIFY. In addition to providing useful advice from their own experience from outside the consortium, the invited scientists will act in a way as a **Project Advisory Board (PAB)** for the proposal, providing critical assessments of the results and planned tasks for the following period. An official, appointed PAB is not planned for DIVERSIFY, given previous experiences with such a management body. In various previous projects where Partners from this consortium have participated (*e.g.*, LIFECYCLE), it was soon found out that high-level specialists and experts who originally accepted the invitation to act as PAB members, often could not attend the annual coordination meetings, due to conflicts with their own research activities. As a result, these PAB either did not function effectively or at they were abolished. We believe through our experience in previous EU projects such as REPRODOTT and SELFDOTT, that having different people every year attending the coordination meetings, and being exposed to the advice and criticism of people coming from different areas of aquaculture science and production, will benefit the implementation of DIVERSIFY, assist in problem solving and ensure faster exploitation of the results of the project.

**Periodic Reporting.** Periodic progress reports will be compiled by the PC from documents obtained from the GWPLs, and will be submitted 1 mo after the ACM, according to the guidelines provided by DG RTD. The GWPLs will compile the material provided by the leaders of the various subWPs, who in turn will collect the data and information produced by the Partners responsible for each specific Task and Activity. The Partner responsible for each WP, Task and Sub-task is clearly identified in the description of the WPs in the proposal (Tables 1.3d Description of work) and will be so done in the Technical Annex as well. This structure for the preparation of the periodic reports will allow delegation and sharing of the work by the scientists closest to the research activity, ensuring accurate, efficient and rapid reporting to the GWPLs, the PC and eventually the DG RTD. The necessary administrative and financial information (Financial Statements and Use of Resources) will be provided by each Scientific Responsible from the consortium Partners directly to the PC, who will then compile and submit to the DG RTD.

**Mid-term evaluation of progress.** Mid-term progress evaluation of the project will be undertaken at the ACMs in a joined meeting of the SC and SLs. During the meeting, the achieved work will be evaluated vis-à-vis the Technical Annex and any deviations will be examined and addressed. We will examine if there is a need to modify the planned work and take any corrective measures. A report will be produced and be available to the EU.

In the summer of 2018, an Amendment 4 was launched to account for the Universal Take over of two partners. Partner P35. MASZ was taken over by a new Partner (MAHAL), and Partner P17. NIFES was taken over by P7. IMR. Partner MAHAL and P7. IMR, take full responsibility of the activities of P35. MASZ and P17. NIFES, respectively, as regards the project and all activities described in the DOW.



## ***B 2.2 Beneficiaries***

### ***Partner 1. Hellenic Center for Marine Research (HCMR), Greece***

**Description of Organization:** The HCMR is the national research and advisory body for marine aquaculture, fisheries and the environment. It participates with the **Institute of Marine Biology, Biotechnology and Aquaculture**. The HCMR has new land-based and near-shore aquaculture facilities in Crete for studies in broodstock management, larval husbandry, nutrition and grow out husbandry. Well-equipped laboratories undertake research in reproductive endocrinology, spawning induction, larval rearing, developmental biology, nutrition, physiology, feeding behaviour, fish health and biochemical, sensory and chemical final product quality. The HCMR also operates a state-of-the-art genetic lab that includes 10 PCR machines, a real-time PCR and 96-capillary ABI3700 sequencers, for high throughput genotyping.

**Main Tasks:** This Partner is the **Project Coordinator, Species Leader for greater amberjack** and the **Leader of GWP Grow out husbandry**. HCMR's tasks include (1) broodstock maintenance of greater amberjack and wreckfish, study reproductive function and develop spawning induction methods, (2) production of GnRHa delivery systems for controlling reproduction in Atlantic halibut and grey mullet, (3) development of larval rearing methods for greater amberjack and wreckfish, (4) ontogeny of digestive and feed acquisition systems in greater amberjack and wreckfish, (5) ontogenic changes in digestive enzymatic capacities of larvae and juveniles, and oxidative stress and antioxidant enzymes in response to rearing methods, (6) nutrition studies on vitamin and mineral requirements in relation to specific pathologies of meagre and immune system, (7) nutrition and digestibility studies in meagre and greater amberjack, (8) feeding behaviour of meagre, (9) development of grow out methods for meagre and greater amberjack, (10) genetic characterization of meagre and pikeperch broodstocks and fast/slow growing meagre, (11) study of known pathological syndromes of meagre (chronic ulcerative dermatopathy and systemic granulomatosis), (12) development of early diagnosis tools for epitheliocystis for greater amberjack larval rearing, (13) final product quality of all studied species, (14) development of Single Nucleotide Polymorphisms (SNP) marker tools for the genetic characterization of fast and slow growers in meagre, and (15) evaluation of genetic variability of wild and captive broodstock in pikeperch. HCMR also will lead the task 28.3, for monitoring the technical quality of the end products of the produced species. This would mainly focus in analytically describing the sensory qualities of the products (analytic descriptive analysis) and in characterizing their chemical quality.

**Previous Experience:** The expertise of HCMR includes (1) the study of endocrine dysfunctions of cultured fishes and development of methods for the artificial control of spermiation, ovulation and spawning, using exogenous hormones through controlled-release delivery systems, (2) larval rearing of many marine fishes using mesocosm and intensive rearing methods, study of the ontogeny of the digestive and feed acquisition systems, and measurement of digestive enzymes, (3) grow out of marine fishes in sea cages, study of feeding and escape behaviour, (4) nutrient requirements of meagre and weaning of greater amberjack to dry feeds, (5) nutrient requirements of marine fish, including amino acid, protein/energy ratios and digestion physiology, and fatty acids requirements in sea bream, (6) study of fish diseases and emerging pathologies in new aquaculture fish, (7) fish health and welfare, (8) biochemical and sensory quality evaluation in farmed fish, mainly gilthead bream and sea bass, but also meagre and correlation of the quality attributes with extrinsic (feed, environment) or intrinsic (sex, size, life cycle) factors, (9) study the diversity of marine life and phylogeography and (10) development of genomic approaches in fish aquaculture and bioinformatics. In the past 10 years, the HCMR has been the recipient and/or coordinator of many research grants, and is participating in national and EU projects involved with the domestication and nutrition of marine fishes and the use of genetics in aquaculture, including REPRODOTT, BRIDGEMAP, BASSMAP (FP5), AQUAMAX, SELFDOTT, ARRANA, FASTFISH, COPEWELL, AQUAEXCEL, TROPOS (FP7).

**Staff profile:** **Dr C.C. Mylonas** (Scientific Responsible) is the **Project Coordinator** and is a reproductive endocrinologist with extensive expertise in the development of hormone-delivery systems for the control of spawning in fish. He has participated in 3 EU FP programmes as partner scientific responsible and WP leader, and has coordinated many national projects. **Dr N. Papandroulakis** is the **Species Leader for greater amberjack** and the **Leader of GWP Grow out husbandry** and specializes in larval and grow out husbandry of marine species, especially large pelagic fishes such as greater amberjack and Atlantic Bluefin





tuna. Other senior and junior researchers involved in the proposal include **Dr P. Divanach** (larval rearing and live food production), **Drs C. Tsigenopoulos** and **G. Kotoulas** (genetics), **Dr K. Grigorakis** (final product quality) who is the leader in task 28.3 and specializes in food science and in particular in the sensory and chemical characterization of farmed fish quality, **Drs I. Negas** (nutrition), **S. Chatzifotis**, (micronutrients) **E. Fountoulaki** (protein/energy, vitamin, mineral and lipid requirements in marine fish) and **Y. Kotzamanis** (amino acid, protein requirements), **Drs P. Katharios** (pathologies in new aquaculture fish) and **G. Rigos** (fish health), **Dr I. Papadakis** (ontogeny, feeding and fish behaviour) and **Dr E. Cotou** (biochemical markers of metabolism, oxidative stress and digestive enzymes).

In the area of Fish health the research group will consist of 2 staff researchers, 1 research technical scientist and several PhD, and post-grad students. The facilities include 2 fully equipped microbiology labs, histology laboratory and a challenge room with several tanks, which is registered in the National Veterinary authorities for conducting experiments with fish. The staff scientist include:

**Dr. Rigos Georgios** (fish pathology-pharmacology) is a Senior Researcher (level B) with twenty years of experience in aquaculture. He earned his doctoral degree in the University of Kingston, UK (2003) and finished his MSc in the University of Guelph, Canada (1996). He has participated in EU projects as Institute leader and currently coordinating national projects and WPs concerning alternative treatments in aquaculture and parasitic diseases of new farmed species. Dr. Rigos was awarded as International Scientist 2007 & 2008 from the International Biographical Centre, Cambridge, UK. He is a reviewer in 16 scientific journals and 50 publications in peer-reviewed journals are included in his scientific achievements.

**Dr. Pantelis Katharios** is a Researcher (Level C) working since 2004 in HCMR. He has a PhD from the University of Patras and an MSc in Aquatic Pathobiology from the University of Stirling. His main field is the study of fish disease with emphasis on the Mediterranean species. He has published 25 articles in peer reviewed journals and he is the coordinator of several national and EU projects on fish diseases. Recently he was awarded an Excellence grant from the Hellenic General Secretariat of Research and Technology with the aid of which he has upgraded his research laboratory and he has formed a research team consisted of a research technician, a post-doc researcher and several post-graduate students working on the development of phage therapy in aquaculture.

### Relevant Publications:

- Athanassopoulou, F., Ragias, V., Vagianou, St., Cave, D., Rigos, G., Papathanasiou, G., Georgoulakis, J., 2005. Report of *Sparicotyle (Microcotyle) chrysofryi* Van Beneden and Hesse 1863, *Atrispinum seminalis* Euzet and Maillard 1973 and *Polylabris tubicirrus* Paperna and Kohn 1964 (Monogenea) on captive sea bream and sharp snout sea bream in coastal Greece and Italy. *Bulletin of the European Association of Fish Pathologists* 25: 256-262.
- Boulton K., Massault C., Houston R., de Koning D.J., Haley C., Bovenhuis H., Batargias C., Canario A.V.M., Kotoulas G., Tsigenopoulos, C.S., 2011. QTL affecting morphometric traits and stress response in the gilthead seabream (*Sparus aurata*). *Aquaculture* 319(1-2): 58-66.
- Chatzifotis S., Panagiotidou, M., Divanach, P., 2012. Effect of protein and lipid dietary levels on the growth of meagre (*Argyrosomus regius*) juveniles. *Aquaculture International* 20: 91-98.
- Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas Y., Rigos G., Kotzamanis, Y., Venou, B., Alexis, M.N., 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile. Recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. *Aquaculture* 289: 317-326.
- Fountoulaki, E., Alexis, M.N., Nengas, I., 2005. Protein and energy requirements of gilthead bream *Sparus aurata* L.) fingerlings: preliminary results. In : Montero, D., Basurco, B., Nengas, I., Alexis, M., Izquierdo, M. (eds.), *Mediterranean fish nutrition*. Zaragoza: CIHEAM, 2005. p. 19-26. (Cahiers Options Méditerranéennes; n. 63). Workshop on Mediterranean Fish Nutrition, 2002/06/01-02, Rhodes (Greece), <http://om.ciheam.org/om/pdf/c63/05600062.pdf>
- Georgakopoulou, E., Katharios, P., Divanach, P. and Koumoundouros, G 2010. Effect of temperature on the development of skeletal deformities in Gilthead seabream (*Sparus aurata* Linnaeus, 1758). *Aquaculture*. 308. 13-19



- Grigorakis, K., Taylor, K.D.A., Alexis, M.N., 2003. Organoleptic and volatile aroma compounds comparison of wild and cultured gilthead sea bream: sensory differences and possible chemical basis. *Aquaculture* 225: 109-119.
- Grigorakis, K., Fountoulaki, E., Giogios, I., Alexis, M.N., 2009. Volatile compounds and organoleptic qualities of gilthead sea bream fed commercial diets containing different lipid sources. *Aquaculture* 290: 116-121.
- Grigorakis, K., Fountoulaki, E., Vasilak, i A., Mittakos, I., Nathanailides, C., 2011. Lipid quality and filleting yield of reared meagre (*Argyrosomus regius*). *International Journal of Food Science and Technology* 46: 711-716.
- Henry, M.A., Alexis, M.N., Fountoulaki, E., Nengas, I., Rigos, G. 2009. Effects of a natural parasitical infection (*Lernanthropus kroyeri*) on the immune system of European sea bass, *Dicentrarchus labrax* L. *Parasite Immunology* 31: 729-740.
- Kalatzis, PG, Kokkari, C and Katharios, P 2013. Description and relationships of two novel species of *Ceratomyxa* Thelohan, 1892 infecting the gallbladders of Aulopiformes: Atlantic lizardfish *Synodus saurus* Linnaeus, 1758 and royal flagfin *Aulopus filamentosus* Bloch, 1792 from Cretan Sea, Greece. *Parasitology Research*. 112. 2055-2061
- Katharios, P, Papadaki, M, Ternengo, S, Kantham, PK, Zeri, C, Petraki, PE and Divanach, P 2011. Chronic ulcerative dermatopathy in cultured marine fishes. Comparative study in sharpsnout sea bream, *Diplodus puntazzo* (Walbaum). *Journal of Fish Diseases*. 34. 459-474
- Katharios, P, Rigos, G and Divanach, P 2011. *Enteromyxum leei* (Myxozoa), a Lethal Intruder of Tropical Pet Fish: First Case in Humphead Wrasse, *Cheilinus undulatus* (Rüppell, 1835). *Journal of Exotic Pet Medicine*. 20. 138-143
- Katharios, P and Tsigenopoulos, CS 2010. First report of nodavirus outbreak in cultured juvenile shi drum, *Umbrina cirrosa* L., in Greece. *Aquaculture Research*. 42. 147-152
- Katharios, P, Papadaki, M, Papandroulakis, N and Divanach, P 2008. Severe mortality in mesocosm-reared sharpsnout sea bream *Diplodus puntazzo* larvae due to epitheliocystis infection. *Diseases of Aquatic Organisms*. 82. 55-60
- Katharios, P, Agathagelou, A, Paraskevopoulos, S and Mylonas, CC 2007. Comparison of iodine and glutaraldehyde as surface disinfectants for red porgy (*Pagrus pagrus*) and white sea bream (*Diplodus sargus sargus*) eggs. *Aquaculture Research*. 38. 527-536
- Katharios, P, Papandroulakis, N and Divanach, P 2006. Treatment of *Microcotyle* sp. (Monogenea) on the gills of cage-cultured red porgy, *Pagrus pagrus* following baths with formalin and mebendazole. *Aquaculture*. 251. 167-171
- Loukovitis, D., Sarropoulou, E., Tsigenopoulos, C.S., Batargias, C., Magoulas, A., Apostolidis, A.P., Chatziplis, D., Kotoulas, G., 2011. Quantitative Trait Loci involved in sex determination and body growth in the gilthead sea bream (*Sparus aurata* L.) through targeted genome scan. *PLoS ONE* 6(1): e16599.
- Mylonas, C.C., Fostier, A. Zanuy, S., 2010. Broodstock management and hormonal manipulations of reproduction. *General and Comparative Endocrinology* 165: 516-534.
- Mylonas, C.C., Zohar, Y., Pankhurst, N.W., Kagawa, H., 2011. Reproduction and broodstock management. In: Pavlidis, M., Mylonas, C.C. (Eds.), *Sparidae: Biology and Aquaculture of Gilthead Seabream and Related Species*. Blackwell Science Publishers, London, pp. 95-131.
- Rigos, G., Katharios, P., 2010. Pathological obstacles of newly-introduced fish species in Mediterranean mariculture; a review. *Reviews in Fish Biology and Fisheries* 20: 47-70.
- Rigos, G., Grigorakis, K., Christophiligiannis, P., Nengas, I., Alexis, M. 1997. *Ceratomyxa* spp. infections in common dentex from Greece. *Bulletin of the European Association of Fish Pathologists* 17:174-176.
- Rigos, G., Grigorakis, K., Nengas, I., Christophiligiannis, P., Yiagnisi, M., Koutsodimou, M., Andriopoulou, K., Alexis, M. 1998. Stress related pathology seems a significant obstacle for the intensive farming of common dentex, *Dentex dentex* (Linnaeus 1758). *Bulletin of the European Association of Fish Pathologists* 18:15-19.
- Rigos, G., Christophiligiannis, P., Yiagnisi, M., Koutsodimou, M., Andriopoulou, K., Nengas I, Alexis, M. 1998. *Amyloodinium ocellatum* infestation in sharpsnout sea bream, *Puntazzo puntazzo* Cetti. *Bulletin of the European Association of Fish Pathologists* 18: 1-3.
- Rigos, G., Christophiligiannis, P., Yiagnisi, M., Koutsodimou, M., Andriopoulou, K., Nengas I, Alexis, M., 1999. Myxosporean infections in Greek mariculture. *Aquaculture International* 7: 361-364.



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- Sfakianakis, DG, Katharios, P, Tsirigotakis, N, Doxa, CK and Kentouri, M 2013. Lateral line deformities in wild and farmed sea bass (*Dicentrarchus labrax*, L.) and sea bream (*Sparus aurata*, L.). *Journal of Applied Ichthyology*. 29. 1015-1021
- Vogiatzi, E., Lagnel, J., Pakaki, V., Louro, B., Canario, A., Reinhardt, R., Kotoulas, G., Magoulas, A., Tsigenopoulos, C. S., 2011. In silico mining and characterization of simple sequence repeats from gilthead sea bream (*Sparus aurata*) expressed sequence tags (EST-SSRs); PCR amplification, polymorphism evaluation and multiplexing and cross-species assays. *Marine Genomics* 4(2): 83-91.





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**Partner 2. Fundación Canaria Parque Científico Tecnológico de la Universidad de las Palmas de Gran Canaria (FCPCT), Spain**

**Description of Organization:** The FCPCT includes the facilities and research staff of the former Instituto Canario de Ciencias Marinas (ICCM, Canary Islands, Spain) and a new land-based and near-shore aquaculture facility (3000 m<sup>2</sup>, tank volume of 500 m<sup>3</sup>) equipped with (1) a hatchery with spawning broodstock of meagre, greater amberjack and its congeners *Seriola rivoliana* and *Pseudocaranx dentex*, (2) a Feed, Ingredients and Additives Testing Unit (2000 m<sup>2</sup>), (3) a Marine Biosecurity Station for pathogen challenge tests (6 diets and 3 pathogens tested separately in triplicates along the whole life cycle), (4) a nutrition laboratory (GLCs, HPLCs, GLCs/HPLC-MS, etc.), (5) a microbiology and histology laboratory (immune-histology, confocal microscopy, electron microscopy), (6) genetic laboratory (RT-PCRs), (7) ichthyopathology laboratory, including tools for identification of parasites, bacteria and viruses, vaccine and treatments development. All laboratories are completely equipped.

**Main Tasks:** The FCPCT is the **Leader of GWP Nutrition** and it will work on reproduction, nutrition, health, larval rearing and on-growing. The main tasks include (1) broodstock maintenance of meagre and greater amberjack, (2) genetic characterization of meagre broodstocks from different partners, (3) spawning induction protocols for amberjack, (4) improvement of early diets for meagre and greater amberjack, (5) optimum Essential fatty acids (EFAs) levels in weaning diets for meagre, (6) nutritional requirements for grow out diets for large meagre and greater amberjack, (7) new enrichment products to improve greater amberjack and wreckfish larval survival, (8) testing of EFAs levels to enhance reproductive performance of greater amberjack, (9) gut integrity and immune defenses in grey mullet fed soybean meals (10) skeleton morphogenesis and mineralization by staining in greater amberjack, pikeperch and grey mullet, (11) larval husbandry of greater amberjack, (10) grow out husbandry of greater amberjack, (12) protocols to prevent systemic granulomatosis in meagre through oral administration of anti-oxidants, (13) description, diagnosis and treatment of the most common infectious diseases occurring in meagre and greater amberjack, (14) prophylaxis and treatment protocols for parasitic infections based on oral administration pre-biotics or phytobiotics in greater amberjack.

**Previous Experience:** FCPCT researchers have experience in aquaculture for over 30 years and cooperated with more than 5 feed manufacturers and over 15 fish farmers in Europe. The team is very strong in broodstock, larval and juvenile nutrition, having pioneering work in nutrient requirements definition and interactions among nutrients. The team has an important expertise in the effect of nutrients on fish welfare, health and resistance to diseases, particularly in relation to fish meal and oil replacement and feed additives. The team has developed an expertise in *in vitro*, immunohistochemical and molecular markers studies to study nutritional effects on metabolism and development. The team has also expertise on selective breeding programs and genetic characterization of broodstocks. The team has been a major partner of EU FAIR, 5th, 6th and 7th FP programs, including larval studies (PUFAFEED), juveniles (RAFOA, FORM), micronutrients (COLORED), broodstock selection (FAIR-CT95-0152), molecular tools for aquaculture (AQUAFIRST), diversification (SUDEVAB), large-research facilities and disease transmission prevention (AQUAEXCEL), hatchery COST (LARVANET) or INTERREG projects, as well as National Projects (Total 165 research projects from public calls, 34 contracts with companies, 3 patents). FCPCT also includes researchers from the Ichthyopathology Department and Histopathology Service, who diagnosis and controls fish diseases for the Canary Islands fish producers since 1990. The group has more than 80 publications on fish health in peer review journals such as *Fish Pathology*, *Journal of Fish Diseases*, *J Aquat Anim Health*, *Fish and Shellfish Immunology*, *Veterinary Microbiology*, among them over 30 in relation to infectious diseases of warm water species, including diagnosis, prevention and treatments, over 20 in relation to the immune system, over 20 on fish welfare and more than 30 about nutrition and fish health. A selection of the most relevant publications in this field are provided. The FCPCT pathologists also are members of the European Association of Fish Pathologists and have presented almost 20 communications to the last year's symposiums on "Diseases of Fish and Shellfish". The group has also supervised more than 10 PhD thesis including 6 on infectious diseases in fish.

**Staff profile:** **Prof. M.S. Izquierdo** (Scientific Responsible) has more than 180 scientific publications (H index 34), as well as a dozen book chapters, participating in more than 60 national and international research



projects, including 13 EU funded, and coordinating more than a dozen. **Dr D. Montero** is the **Leader of GWP Nutrition** and works in fish nutrition and health with more than 50 articles (22 H index), and has been team leader in several EU projects (AQUAFIRST). He is a frequent referee for *Fish and Shellfish Immunology* and *Journal of Fish Disease*. He made the first descriptions of glomerulonephritis in gilthead seabream and the effect of lipids in fish welfare. He is specialized in nutrition and health and welfare in fish and the development of molecular markers of fish health in warm water species. **Dr H. Fernandez Palacios** has a more than 35 years expertise in development of rearing techniques for new species, including control of maturation and spawning, broodstock nutrition and larval rearing, he has published more than 70 peer-reviewed articles and half a dozen reviews in book chapters. In cooperation with other researchers he obtained the first F1 reproduction in meagre. **Dr L. Robaina** is an expert in alternative ingredients and feed formulation, has been team leader in several EU projects (COLORED), cooperated with several feed producers and formulated feeds for several of them. **Dr Silvia Torrecillas**, is specialized in the effect of prebiotics and probiotics in disease resistance in fish and particularly in the infection processes through the gut. **Dr M.J. Zamorano** is an expert in molecular markers. **Dr. J.M. Afonso** is specialized in design of PCR multiplex with specific microsatellites, development and standardization of fish lines and coordination of national and international programs on selective breeding. **M.J. Caballero**, is Professor of Fish Pathology and Marine Animals Health in the Veterinary Faculty. She is specialized in Fish Comparative Pathology and has been involved in a dozen of EU, national and regional projects related to fish health. She is coordinator of the Fish Health Course of the MSc and PhD in Aquaculture, awarded with Excellence mention of the Spanish Ministry of Education. She is head of the Service GIA Histopath All this researchers are members of GIA, a Canary Islands multi-institutional based aquaculture research group lead by **Prof. M.S. Izquierdo**. **Prof. Fernando Real**, Head of the Ichthyopathology Department, Full Professor of Fish Diseases and Infectious Diseases since 1990, is specialized in fish infectious diseases and develops patents for probiotics and is responsible for the definition of Sanitary Standards for the transfer of fish among European Aquaculture facilities. He has a close cooperation with several research groups working in Fish Diseases, including the Fish Pathology group of Santiago de Compostela University. **Prof. Félix Acosta** is Professor of Fish Diseases and Infectious Diseases. He is specialized in diagnosis and prevention of infectious diseases including research on IPN, VHS, vibriosis and development of molecular markers for disease diagnosis. He has cooperated since 2003 with the Immunology and Vaccine Group of the Fisheries Research Services del Marine Laboratory, Aberdeen.

### Relevant publications

- ACOSTA, F., A. PETRIE, K. LOCKHART, N. LORENZEN, A.E. ELLIS. 2005. Kinetics of Mx expression in Atlantic salmon (*Salmo salar* L.) parr and Rainbow trout (*Oncorhynchus mykiss*) in response to VHS-DNA vaccination. *Fish & Shellfish Immun.*, 18: 81-89.
- ACOSTA, F., ELLIS, A.E., VIVAS, J., PADILLA, D., ACOSTA, B., DÉNIZ, S., BRAVO, J. y REAL, F. 2006. Complement consumption by *Photobacterium damsela* subsp. *piscicida* in seabream, red porgy and seabass normal and immune serum. Effect of the capsule on the bactericidal effect. *Fish & Shellfish Immun.*, 20: 709-717.
- ACOSTA, F., J. VIVAS, D. PADILLA, J. VEGA, J. BRAVO, V. GRASSO y F. REAL. 2009. Invasion and survival of gilt-head sea bream (*Sparus aurata*) non-phagocytic cells by *Photobacterium damsela* subsp. *Piscicida*. *Journal of Fish Diseases*, 32(6): 535-541.
- BENÍTEZ-SANTANA, T., JUÁREZ-CARRILLO, E., BETANCOR, M., TORRECILLAS, S., CABALLERO, M.J., IZQUIERDO, M.S. 2012. Increased Mauthner cells activity and escaping behaviour seabream fed long chain polyunsaturated fatty acids. *British Journal of Nutrition* 107, 295-301.
- BENÍTEZ-DORTA, V., CABALLERO, M.J., IZQUIERDO, M.S., MANCHADO, M., INFANTE, C., ZAMORANO, M.J., MONTERO, D. 2013. Total substitution of fish oil by vegetable oils in Senegalese sole (*Solea senegalensis*) diets: effects on fish performance, biochemical composition and expression of some glucocorticoid receptor related genes. *Fish Physiol. Biochem.* 39, 335-349
- BETANCOR, M.B., CABALLERO, M.J., TEROVA, G., SALEH, R., ATALAH, E., BENÍTEZ-SANTANA, T., BELL, J.G. IZQUIERDO, M.S. 2012. Selenium inclusion decreases oxidative stress indicators and muscle injuries in sea bass larvae fed high DHA microdiets. *Br. J. Nutr* 108, 2115 -



2128

- BETANCOR, M.B., ESTEFANELL, J., SOCORRO, J., ROO, J., CABALLERO, M.J. 2013. First description of parasitization by *Aggregata octopiana* in common Octopus, *Octopus vulgaris*, in Canary Islands. *Bull. Eur. Ass. Fish Pathol.* 33, 13-20.
- BETANCOR, M.B., IZQUIERDO, M.S., TEROVA, G., PREZIOSA, E., SALEH, R., MONTERO, D., HERNÁNDEZ-CRUZ, C.M. CABALLERO, M.J. 2103. Physiological pathways involved in nutritional muscle dystrophy and healing in European sea bass (*Dicentrarchus labrax*) larvae. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 164, 399- 409.
- BRAVO J, REAL F, PADILLA D, OLVEIRA JG, GRASSO V, ROMÁN L, ACOSTA F. 2013. Effect of lipopolysaccharides from *Vibrio alginolyticus* on the Mx gene expression and virus recovery from gilthead sea bream (*Sparus aurata* L.) experimentally infected with Nodavirus. *Fish Shellfish Immunol.* 2013 Jan;34(1):383-6
- CABALLERO, M.J., GALLARDO, G., ROBAINA, L., MONTERO, D., FERNÁNDEZ, A. IZQUIERDO, M.S. 2006. Vegetable lipid sources affect in vitro biosynthesis of triacylglycerols and phospholipids in the intestine of sea bream (*Sparus aurata*). *British Journal of Nutrition* 95, 448- 454.
- CABALLERO, M.J., TORSTENSEN, B., ROBAINA, L., MONTERO, D. e IZQUIERDO, M.S. 2006. Vegetable oil affect composition of lipoproteins in sea bream (*Sparus aurata*). *British Journal of Nutrition* 96, 830-839.
- CABALLERO, M.J., BETANCOR, M., ESCRIG, J.C., MONTERO, D., DE LOS MONTEROS, A. ESPINOSA, CASTRO, P., GINÉS, R., IZQUIERDO, M. 2009. Post mortem changes produced in the muscle of sea bream (*Sparus aurata*) during ice storage. *Aquaculture* 291, 201-216.
- DUNCAN, N., ESTÉVEZ, A., FERNÁNDEZ-PALACIOS, H., GAIRIN, I., HERNÁNDEZ-CRUZ, C.M., ROO, J., SCHUCHARDT, D., Vallés, R. 2013. Aquaculture production of Meagre (*Argyrosomus regius*): hatcherie techniques, ongrowing and market. In: *Advances in Aquaculture Hatchery Technology* ISBN: 0 85709 119 0. Vol: 242 pp: 519-541. Woodhead Publishing Ltd, Cambridge, UK. Geoff Allan, Gavin Burnell (Eds)
- EL AAMRI, F., PADILLA, D., ARBELO, F., CABALLERO, M. J., ROO, J., BRAVO, J., VIVAS, J., REAL, F. 2010. First report of *Streptococcus iniae* in red porgy (*Pagrus pagrus*, L). *Journal of Fish Diseases* 33, 901-905.
- FERNÁNDEZ-PALACIOS, H., HERNÁNDEZ-CRUZ, C.M., SCHUCHARDT, D., IZQUIERDO, M.S., ROO, F.J. 2009. Effect of co-feeding regimes on biological performance and biochemical composition of meagre (*Argyrosomus regius* Asso, 1801) larvae. *Europ. Aquacul. Soc. Spec. Publ.* 38, 108-111. In: C.I. Hendry, G. Van Stappen, M. Mille and P. Sorgeloos. *European Aquaculture Society* (ISBN: 978-90-9024563-90).
- GANGA, R., TORT, L., ACERETE, L., MONTERO, D. e IZQUIERDO, M.S. 2006. Modulation of ACTH-induced cortisol release by polyunsaturated fatty acids in interrenal cells from gilthead seabream, *Sparus aurata*. *J. Endocrinol.* 190, 39-45.
- GANGA, R., BELL, G.J., MONTERO, D., ATALAH, E., VRASKOU, Y., TORT, L., FERNANDEZ, A., IZQUIERDO, M.S. 2011. Adrenocorticotrophic hormone-stimulated cortisol release by the head kidney interrenal tissue from sea bream (*Sparus aurata*) fed with linseed oil and soyabean oil. *British Journal of Nutrition* 105, 238-247.
- IZQUIERDO, M.S., FERNÁNDEZ- PALACIOS, H. & TACON, A.G.J. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* 197, 25-42.
- IZQUIERDO, M.S., MONTERO, D., ROBAINA, L., CABALLERO, M.J., ROSENLUND, G. & GINES, R. 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture* 250, 431-444.
- IZQUIERDO, M.S., ROBAINA, L., JUÁREZ-CARRILLO, E., OLIVA, V., HERNÁNDEZ-CRUZ, C.M and AFONSO, J.M. 2008. Regulation of growth, fatty acid composition and delta 6 desaturase expression by dietary lipids in gilthead seabream larvae (*Sparus aurata*). *Fish Physiol. Biochem.* 34, 117-127.
- IZQUIERDO, M.S., KOVEN, W., 2011. Lipids: In: (Holt J., ed) *Larval Fish Nutrition*, Wiley-Blackwell, John Wiley and Sons, pp 47-82. ISBN: 978-0-8138-1792-7.
- IZQUIERDO, M.S., SCOLAMACCHIA, M., BETANCOR, M., ROO, J., CABALLERO, M.J., TEROVA, G., WITTEN, P.E. 2013. Effects of dietary DHA and  $\alpha$ -tocopherol on bone development, early



- mineralization and oxidative stress in *Sparus aurata* (Linnaeus, 1758) larvae. *Br. J. Nutr.* 109, 1796-1805.
- LEE-MONTERO, I., NAVARRO, A., BORRELL, Y., GARCÍA-CELDRÁN, M., MARTÍN, N., NEGRÍN-BÁEZ, D., BLANCO, G., ARMERO, E., BERBEL, C., ZAMORANO, M.J., SÁNCHEZ, J.J., ESTÉVEZ, A., RAMIS, G., MANCHADO, M., AFONSO, J.M., Development of the first standardized panel of two new microsatellite multiplex PCRs for gilthead seabream (*Sparus aurata* L.). *Animal Genetics*. In press DOI: 10.1111/age.12037
- MONTERO, D., TORT, L., ROBAINA, L., VERGARA, J.M. e IZQUIERDO, M.S. 2001. Low vitamin E in diet reduces stress resistance of gilthead seabream (*Sparus aurata*) juveniles. *Fish & Shellfish Immunol.* 11 473- 490.
- MONTERO, D., KALINOWSKI, T., OBACH, A., ROBAINA, L., TORT, L., CABALLERO, M.J. E IZQUIERDO, M.S. 2003. Vegetable lipid sources for gilthead seabream (*Sparus aurata*): Effects on fish health. *Aquaculture* 225, 353-370.
- MONTERO, D., SOCORRO, J., TORT, L., CABALLERO, M.J., ROBAINA, L.E., VERGARA, J.M. AND IZQUIERDO, M.S. 2004. Glomerulonephritis and immunosuppression associated to dietary essential fatty acids deficiency in gilthead seabream (*Sparus aurata*) juveniles. *J. Fish Diseases* 27, 297-306.
- MONTERO, D., GRASSO, V., IZQUIERDO, M.S., REAL, F., TORT, L., CABALLERO, M.J. y ACOSTA, F. 2008. Total substitution of fish oil by vegetable oils in gilthead sea bream (*Sparus aurata*) diets: effects on hepatic Mx expression and some immune parameters. *Fish and Shellfish immunol.* 24 147-155
- MONTERO, D., MATHLOUTHI, F., TORT, L., AFONSO, J.M., TORRECILLAS, S., FERNÁNDEZ-VAQUERO, A., NEGRIN, D., IZQUIERDO, M.S. 2010. Replacement of dietary fish oil by vegetable oils affects humoral immunity and expression of pro-inflammatory cytokines genes in gilthead sea bream *Sparus aurata*. *Fish Shellfish Immunol* 29, 1073 – 1081.
- NAVARRO, A., ZAMORANO, M.J., HILDEBRANDT, S., GINÉS, R., AGUILERA, C., AFONSO, J.M. 2009. Estimates of heritabilities and genetic correlations for body composition traits and G × E interactions, in gilthead seabream (*Sparus auratus* L.). *Aquaculture* 295, 183-197.
- ROMÁN, L., F. REAL, D. PADILLA, F. EL AAMRI, S. DÉNIZ, V. GRASSO, F. ACOSTA. 2013. Cytokine expression in head-kidney leucocytes of European sea bass (*Dicentrarchus labrax* L.) after incubation with the probiotic *Vagococcus fluvialis* L-21. *Fish & Shellfish Immunology* (2013) 35: 1329-1332.
- ROO, F. J., HERNÁNDEZ-CRUZ, C.M; BORRERO, C; D.SHUCHARDT, D., FERNANDEZ-PALACIOS, H. 2010.Effect of larval density and feeding sequence on meagre (*Argyrosomus regius*; Asso, 1801) larval rearing. *Aquaculture* 302, 82-88.
- ROO, J., FERNÁNDEZ-PALACIOS, H., HERNÁNDEZ-CRUZ, C.M., MESA-RODRÍGUEZ, A., SCHUCHARDT, D., IZQUIERDO, M.S. First studies on spawning and larval rearing of longfin yellowtail *Seriola rivoliana* as a fast-growing candidate for European marine finfish aquaculture diversification. *Aquac. Res.* In press. DOI: 10.1111/are.12007
- SORROZA L, PADILLA D, ACOSTA F, ROMÁN L, GRASSO V, VEGA J, REAL F.2012. Characterization of the probiotic strain *Vagococcus fluvialis* in the protection of European sea bass (*Dicentrarchus labrax*) against vibriosis by *Vibrio anguillarum*. *Vet Microbiol.* 2012 Mar 23;155(2-4):369-73
- TORRECILLAS, S., MAKOL, A., CABALLERO, M.J., MONTERO, D., DHANASIRI, A.K.S., SWEETMAN, J., IZQUIERDO, M.S.2012. Effects on mortality and stress response in European sea bass, *Dicentrarchus labrax* L., fed mannan oligosaccharides (MOS) after *Vibrio anguillarum* exposure.*J. Fish Dis.* 35, 591-602
- TORRECILLAS, S., MAKOL, A., BETANCOR, M.B., MONTERO, D., CABALLERO, M.J., SWEETMAN, J., IZQUIERDO, M.S. 2013. Enhanced intestinal epithelial barrier health status on European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. *Fish Shellfish Immunol* 34, 1485-1495.
- VIVAS J., PADILLA D., REAL, F., BRAVO, J., GRASSO, V. y ACOSTA, F. 2008. Influence of environmental conditions on biofilm formation by *Hafnia alvei* strains. *Veterinary Microbiology*, 129: 150-155. 2008.

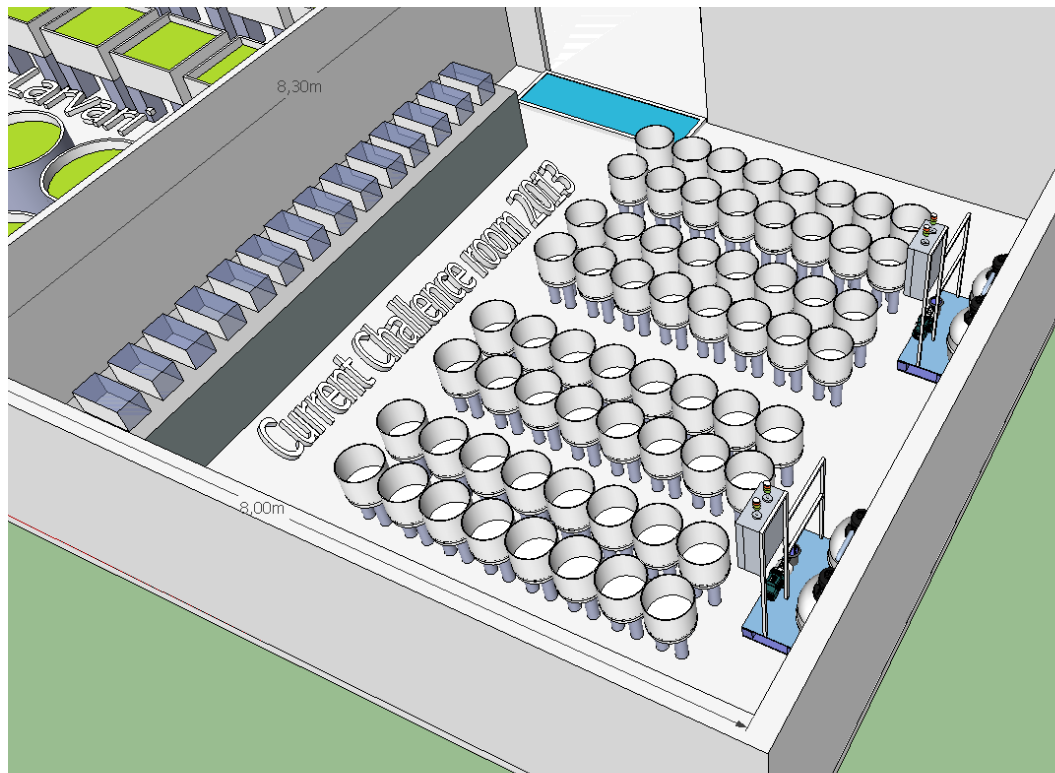




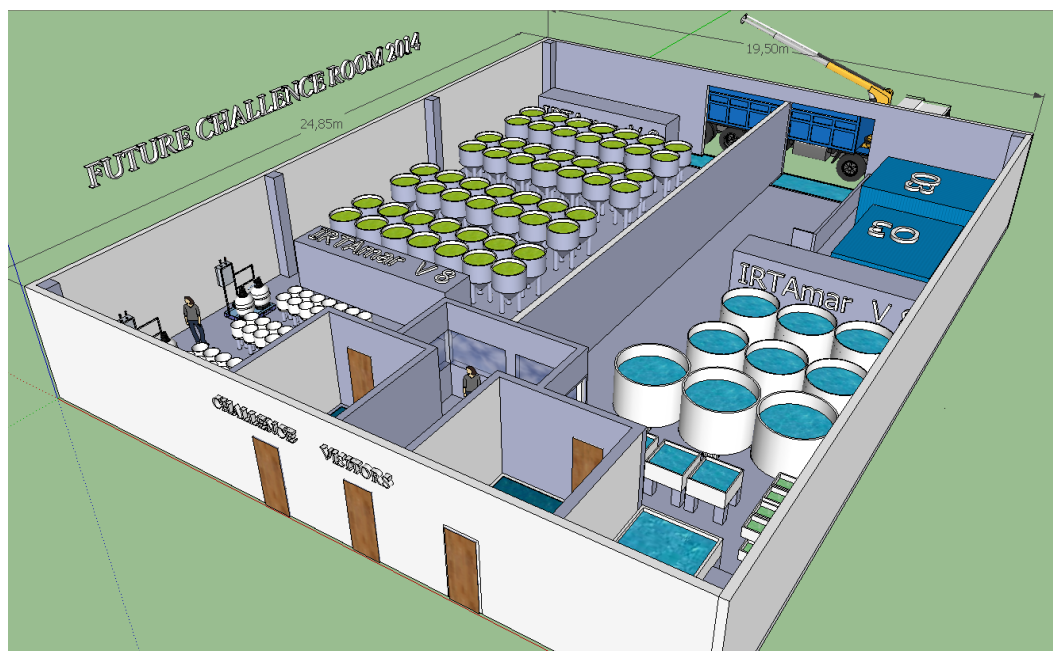
### **Partner 3. Institut de Recerca I Tecnologia Agroalimentàries (IRTA), Spain**

**Description of Organization:** The IRTA ([www.irta.es](http://www.irta.es)) is a public research institution of the Generalitat de Catalunya, which is composed of a network of 16 research centres and 8 experimental stations all over Catalonia. Its main goals are to stimulate research and technological development within the agriculture, food and environmental areas by enhancing co-ordination and collaboration with the public and private sectors. In 2011 IRTA had 571 staff, with 204 researchers, 53% women and 47% men and total available resources of 46.04 million €. **Two centres will be involved in the project.** The centre of **Sant Carles de la Ràpita** (Tarragona) placed in the Ebro River delta, one of the most important zones in terms of shellfish production in the Mediterranean, which focuses most of its research efforts on marine and brackish water aquaculture. Overall, the centre's wet facilities are state of the art, they operate mainly under recirculation, using our own RAS patented technology (P201130258). These facilities allow work under highly flexible, accurate and controlled conditions for any aquatic animal along their life cycles. Experimental challenge with infectious pathogens is preformed in ad-hoc rooms (see annex diagrams). The animal research installations are complemented by different laboratories (chemistry, molecular biology, microbiology, histology, cell culture, image analysis), which allow a high degree of self-sufficiency for multidisciplinary research. The aquaculture group has experts working on the following areas of fish aquaculture: reproduction, nutrition, health and welfare and zootechnics.

Especially for work in the Fish Health GWP, IRTA operates a challenge room equipped with 64 tanks (100 l), where key parameters can be accurately controlled (flow rate, temperature, salinity, oxygen). It can operate with flow through or under recirculation. Water filtration and UV disinfection is available in the water intake, whereas, the water effluent is treated with an overdose of ozone. Biosecurity protocols are in place both for personal and materials accessing the room. Currently a second challenge facility is under construction to increase capacity.



**The IRTA Challenge room (existing facility) of 64 m<sup>2</sup>. The whole facility runs on recirculation systems, allowing automated control of a range of parameters (Flow rate, oxygen, salinity, temperature, pH).**



The new IRTA Challenge room (under construction, 2014) of 500 m<sup>2</sup>. The whole facility runs on recirculation systems, allowing automated control of parameters (Flow rate, oxygen, salinity, temperature, pH). The new facility will include tanks able to hold a total biomass of 500 Kg each, compared to only 50 kg each for the existing facilities.

The second IRTA centre involved in DIVERSIFY is located in **Monells** and focuses on food quality and safety, and food processing and technology. This centre is equipped with industrial and pilot size equipment of the most advanced technologies for the production of food products, specially meat and fish (marination, drying, smoking, cooking, packaging, etc), as well as laboratory facilities to carry out physical (NIR, visible and microwave spectrometry, texture analyzer, colorimeter equipment), chemical (FoodScan<sup>TM</sup>, LC- and GC instruments: UPLC-DAD-MS/MS, HPLC-DAD/FLD, GC-MS/MS and GC-MS) and sensorial assessment (trained panel and consumer studies) of fresh and processed food products.

**Main Tasks:** IRTA- Sant Carles de la Ràpita is the **Species Leader for meagre** and the **Leader of GWP Reproduction and Genetics**. The tasks undertaken by IRTA include (1) broodstock studies on meagre and mullet, (2) development of larval rearing methods for meagre, mullet and pikeperch, (3) nutrition studies in meagre, (4) development of grow out methods for grey mullet and meagre, (5) feeding behaviour of meagre, (6) genetic characterization of meagre broodstock and of fast/slow growing individuals, (7) study of known pathological syndromes of meagre and the ontogeny of its immune system. IRTA-Monells is involved in (8) new product development, final product quality and consumer attitudes, values and perception of the new products.

**Previous Experience:** The expertise of IRTA includes (1) development of methods for the artificial control of reproduction using hormones, and the study of reproductive behaviour of marine fish, (2) larval rearing of marine fishes, study of the ontogeny of the digestive and feed acquisition systems, and measurement of digestive enzymes, (3) grow out of marine fishes in tanks and sea cages, (4) determination of nutrient requirements (mainly fatty acids and vitamins) of marine fish larvae and their effects on skeletogenesis, pigmentation and metabolism, (5) study of emerging pathologies in new aquaculture species, (6) use of environmental friendly products in the treatment of bacterial and parasitic aquatic diseases, (7) assessment of food quality and safety, especially fresh and processed meat and fish and (8) development of food products and processes. Particularly in the area of fish health, IRTA has three senior researchers participating in this proposal, who have each over 15 years experience working on aquatic diseases, developing and implanting molecular diagnostic techniques and environmentally friendly products for treatment of such diseases and focusing on basic epidemiology for identification of risk factors. Moreover three, more scientists work on



nutrition-health related issues such as the use of probiotics or the nutritional pathways which lead to larval deformities.

In the past 10 years, IRTA has been the recipient and/or coordinator of many research grants, mostly at a national level but also from the EU such as TRUEFOOD, ISAFRUIT, Q-PORKCHAINS (FP6), PROSAFEBEEF (FP6), LIFECYCLE, HIGHTECH EUROPE, DREAM, OPTIFEL, BIVALIFE (FP7) and/or collaborative SME projects such as REPROSEL (FP7).

#### **Relevant private contracts and collaboration with private companies in IRTA Sant Carles de la Ràpita**

2009-2010. Contract with Rubinum S.A. to evaluate the effect of Toyocerin in the diet on the growth, survival, quality and immune response of trout

2010-2013. Contract with APC Europe for the evaluation of blood hydrolisates in the diet of salmon and sea bream

2011. Contract with Rubinum S.A. to evaluate the effect of Somacyl SL 100 in diets for European eel

2011-2013.- CENIT Project with Andrés Pinaluba S.A. Development of new diets formulated with microalgae as probiotics.

2013. Contract with Biomar A/S to develop a challenge test for seabream against the parasite Sparicotyle

2013-2015. CDTI Project with SP Veterinaria S.A. to develop new formulations and carriers for the use of antibiotics and other veterinary products for aquaculture.

**Staff profile:** **Dr A. Estevez** (Scientific Responsible) is the **Species Leader for meagre** and is a marine biologist specialised in larval rearing and nutrition of marine organisms. **Dr N. Duncan** is the **leader of GWP Reproduction and Genetics** and specializes in providing solutions to reproductive dysfunctions for species new to aquaculture. Other senior and junior researchers involved in the proposal include **I. Gairin** (grow out in tanks and sea cages, health), **Dr E. Gisbert** (nutrition and larval development), and **Dr S. Morais** (lipid nutrition and metabolism and nutritional physiology) in the Sant Carles de la Ràpita Centre; and **Dr L. Guerrero** and **Anna Claret** (Consumer behaviour) and **Dr C.E. Realini** and **Dr. Pere Duran** (Food processing) in the Monells Centre.

Specifically in the area of Fish health, the senior scientists involved will be:

**Dr K.B. Andree** (parasitology, molecular biology, health). **Dr. Andree** has a PhD in Microbiology (University of California, Davis, USA, 1998), and a BaS in Biological Sciences (University of Aurora, Illinois, USA, 1985). His research has focused on the pathology of aquatic species, both molluscs and fish. He has paid particular attention to the development of new molecular diagnostic methods for detection of aquatic animal pathogens to aid in determining the health status of animal stocks in farms, and has focused his research on the study of the life cycles of these pathogens and their mode transmission. Methodologies in these areas have been implemented successfully for several parasitic, bacterial and viral pathogens. The tests developed by Dr. Andree for withering syndrome (in *Haliotis spp.*) and whirling disease in salmonid fish (USA patent No.: 5,962,227 [1996] and licensed to Biogenetic Services Inc.) have been approved for health monitoring of animals by the OIE (Office International des Epizooties, Paris, France) and AFS (American Fisheries Society, Washington DC, USA).

Since November 2004, Dr. Andree has been hired as a researcher at the IRTA center of Sant Carles de la Ràpita within the Experimental Culture unit. In addition to research responsibilities, he has been assisting in health maintenance of fish stocks kept within the facility for experimental purposes, implementation of diagnostics for identification of specific infectious pathogens, and has worked in coordination with the unit for Monitoring of the Marine Environment in the development of DNA-based systems for monitoring of blooms of harmful microalgae species.

**Dr A. Roque** (fish health and welfare). **Dr. Roque** graduated in veterinary medicine (Universidade Técnica de Lisboa, Portugal, 1989) and she did a PhD in Aquatic diseases (University of Stirling, UK, 1995). Dr. Roque did a Veterinary Epidemiology and Economics course (Royal Veterinary college, University of London UK, 2005) and one in Animal welfare (Royal Veterinary college, University of London UK, 2006). Dr. Roque worked as an independent researcher in Mexico (Centro de investigación en alimentación y desarrollo, 1996-2003), where she lead her own competitive projects national and international on aquatic health and epidemiology. She also contributed and had major input in the design and implementation of two



diagnostic laboratories for the detection of shrimp infectious diseases. Since January 2004, Dr. Roque has been hired as a researcher at the IRTA center of Sant Carles de la Rapita within the Experimental Culture unit, where she has been leading research projects on molluscs' health, fish welfare and contractual work on fish treatments. During this period she has supervised PhD, MSc and BSc students to undertake their thesis work. She has participated in three scientific opinions for the EFSA panel of Animal Health and Welfare and in the project Dataquest to develop a database on sources of information on fish diseases. Dr Roque was also one of the coordinators of WP1 in the Cost Action 867 on European Fish Welfare.

**Dr M. Dolores Furones Nozal** (fish and shellfish health). **Dr. Furones Nozal** graduated in Biology (BSc, 1981), with postgraduate research in microbiology (MSc, 1983). P hD in Fish Pathology (University of Plymouth, UK). Dr. Furones then worked for the Government of the Balearic Islands, Spain, (1991-1995) in charge of setting up and running the aquatic animal health laboratory implementing the European Union Directives for fish diseases, water quality and product safety for bivalve production areas. Later Dr. Furones moved to work for the Catalonia Government, Spain, General Directorate of Fisheries (1998-1999) where she was responsible for the National Aquaculture Centre, in charge of the research programmes and the design of facilities. And since 1999 until now, Dr. Furones works for the Catalonia Government, Spain, IRTA (1999-present) where she is the Director of IRTA Sant Carles de la Ràpita and coordinator of IRTA's Aquaculture research programme. Her own research focused on aquatic animal health (fish and shellfish) addressed to relevant diseases in Mediterranean Aquaculture, covering epidemiology, diagnostics and in vivo challenge work.

#### Relevant publications:

- Claret, A., Guerrero, L., Aguirre, E., Rincón, L., Hernández, M.D., Martínez, I., Peleteiro, J.B., Grau, A., Rodríguez-Rodríguez, C., 2012. Consumer preferences for sea fish using conjoint analysis: exploratory study of the importance of country of origin, obtaining method, storage conditions and purchasing price. *Food Quality and Preference* 26: 259-266.
- Claret, A., Guerrero, L., Gines, R., Grau, A., Hernandez, M.D., Aguirre, E., Peleterio, J.B., Fernandez-Pato, C., Rodriguez-Rodriguez, C. Consumer beliefs regarding farmed versus wild fish. *Appetite* (In Press).
- Duncan, N.J., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C.M., Roo, J., Schuchardt, D., Vallés, R., 2013. Aquaculture production of meagre (*Argyrosomus regius*): hatchery techniques, ongrowing and market. In: *Advances in aquaculture hatchery technology*. Allan, G., Burnell, G. (Eds). Woodhead Publishing Limited, Cambridge, UK.
- Duncan, N., Estévez, A., Porta, J., Carazo, I., Norambuena, F., Aguilera, C., Gairin, I., Bucci, F., Valles, R., Mylonas, C.C., 2012. Reproductive development, GnRH $\alpha$ -induced spawning and egg quality of wild meagre (*Argyrosomus regius*) acclimatised to captivity. *Fish Physiol Biochem* 38: 1273–1286.
- Fajer-Ávila E.J., Roque A., Aguilar G. and Duncan N. 2004 Patterns of occurrence of the platyhelminth parasites of the wild bullseye puffer (*Sphoeroides annulatus*) in Sinaloa, Mexico. *Journal of Parasitology*, 90: 415-418.
- Gemma Gimenez, Francesc Padros, Ana Roque, Alicia Estevez & Dolores Furones. 2006. Bacterial load reduction of live prey for fish larval feeding using Ox-Aquaculture. *Aquaculture Research*, 37: 1130-1139
- Gemma Giménez-Papiol, Francesc Padrós, Ana Roque, Alicia Estévez, Dolores Furones. 2008. Effects of a peroxide-based commercial product on bacterial load of larval rearing water and on larval survival of two species of Sparidae under intensive culture. Preliminary data. *Aquaculture Research*, 37: 1130-1139.
- Irene Salinas, Luigi Abelli, Fabrizio Bertoni, Simona Picchietti, Ana Roque, Dolores Furones, Alberto Cuesta, José Meseguer and Maria Ángeles Esteban. 2008. Monospecies and multispecies probiotic formulations produce different systemic and local immunostimulatory effects in the gilthead seabream (*Sparus aurata* L.). *Fish and Shellfish Immunology*, 25:144-123.
- Ana Roque, Hijran Yavuzcan Yildiz, Ignacio Carazo, Neil Duncan 2010. Physiological stress responses of sea bass (*Dicentrarchus labrax*) to hydrogen peroxide (H $_2$ O $_2$ ) exposure. *Aquaculture*, 304: 104-107.





- Mayra Grano-Maldonado, Ana Roque and Emma Fajer-Avila 2010 Development of *Heterobothrium ecuadori* (Monogenea: Diclidophoridae) in Bullseye Puffer Fish *Sphoeroides annulatus* under Experimental Conditions. *Fish Pathology*, 45 : 175 -178
- M. Grano-Maldonado, A. Roque, H. Aguirre and E. Fajer-Avila 2011. Egg morphology, larval development and description of the oncomiracidium of *Heterobothrium ecuadori* (Monogenea: Diclidophoridae) parasitising the bullseye pufferfish, *Sphoeroides annulatus*. *Helminthologia*, 48: 51-55,
- Mayra Grano Maldonado, Enric Gisbert, Jorge Hirt-Chabbert, Giuseppe Paladini, Ana Roque, James E Bron, Andrew P Shinn, 2011. An infection of *Gyrodactylus anguillae* Ergens, 1960 (Monogenea) associated with the mortality of glass eels (*Anguilla anguilla* L.) on the north-western Mediterranean Sea board of Spain. *Veterinary Parasitology*. 180:323-331
- Chris Noble Hernán A. Canón Jones • Børge Damsgard • Matthew J. Flood Kjell Ø. Midling Ana Roque Bjørn-Steinar Sæther Stephanie Yue Cottee. 2011. Injuries and deformities in fish: their potential impacts upon aquacultural production and welfare. *Fish Physiol Biochem*. DOI 10.1007/s10695-011-9557-1.
- Valles, R, Roque A., Caballero A., Estevez A. 2013. "Use of Ox-Aquaculture© for disinfection of live prey and meagre larvae, *Argyrosomus regius* (Asso, 1801)". Accepted in *Aquaculture Research*.
- Gomez-Gil B, Roque A, Soto-Rodríguez SA. 2011. Probiotics in the Larval Culture of Aquatic Organisms. In: Montet D, Ray RC (eds) *Aquatic Microbiology and Biotechnology*, vol II. Science Publs. Enfield, USA.
- Roque A, Soto-Rodríguez SA, Gomez-Gil B. 2009. Bacterial fish diseases and molecular tools for bacterial fish pathogens detection. In: Montet D, Ray RC (eds) *Aquatic Microbiology and Biotechnology*, vol I. Science Publs. Enfield, USA. Pp 73-99.
- Gemma Giménez Papiol and Ana Roque Uses of Hydrogen Peroxide in Aquaculture. In print.
- Gisbert, E.; Castillo, M.; Skalli, A.; Andree, K. B.; Badiola, I. (2013) *Bacillus cereus* var. *toyoi* promotes growth, affects the histological organization and microbiota of the intestinal mucosa in rainbow trout fingerlings. *Journal Of Animal Science* 91(6): 2766- 2774
- Andree, K. B.; Rodgers, C. J.; Furones, D.; Gisbert, E. (2013) Co-Infection with *Pseudomonas anguilliseptica* and *Delftia acidovorans* in the European eel, *Anguilla anguilla* (L.): a case history of an illegally trafficked protected species. *Journal Of Fish Diseases*, 36(7): 647-656.
- Darias, Maria J.; Andree, Karl B.; Boglino, Anais; Fernandez, Ignacio; Estevez, Alicia; Gisbert, Enric (2013) Coordinated Regulation of Chromatophore Differentiation and Melanogenesis during the Ontogeny of Skin Pigmentation of *Solea senegalensis* (Kaup, 1858). *PLOS ONE*, 8(5)
- Carrasco, N.; Villalba, A.; Andree, K. B.; Engelsma, M. Y.; Lacuesta, B.; Ramilo, A.; Gairin, I.; Furones, M. D. (2012) *Bonamia exitiosa* (Haplosporidia) observed infecting the European flat oyster *Ostrea edulis* cultured on the Spanish Mediterranean coast. *JOURNAL OF INVERTEBRATE PATHOLOGY*, 110(3): 307- 313.
- Boglino, A., Darias, M.J., Ortiz-Delgado, J.B., Ozcan, F., Estévez, A., Andree, K.B., Hontoria, F., Sarasquete, C., Gisbert, E. (2012) Commercial products for *Artemia* enrichment affect growth performance, digestive system maturation, ossification and incidence of skeletal deformities in Senegalese sole (*Solea senegalensis*) larvae. *Aquaculture*, 324-325: 290- 3
- Darias, M.J., Boglino, A., Manchado, M., Ortiz-Delgado, J.B., Estévez, A., Andree, K.B., Gisbert, E. (2012) Molecular regulation of both dietary vitamin A and fatty acid absorption and metabolism associated with larval morphogenesis of Senegalese sole (*Solea senegalensis*). *Comparative Biochemistry and Physiology Part A*, 161: 130-139.
- Boglino, A., Darias, M.J., Ortiz-Delgado, J.B., Gisbert, E., Estévez, A., Andree, K.B., Sarasquete, C. (2012) Isolipidic diets differing in their essential fatty acid profiles affect the deposition of unsaturated neutral lipids in the intestine, liver and vascular system of Senegalese sole larvae and early juveniles. *Comparative Biochemistry and Physiology Part A*, 162: 59-70.
- Boglino, A., Darias, M.J., Estévez, A., Andree, K.B., Gisbert, E. (2012) The effect of dietary arachidonic acid during the *Artemia* feeding period on larval growth and skeletogenesis in Senegalese sole, *Solea senegalensis*. *Journal of Applied Ichthyology*, 28: 411-418.



- Carrasco, N., Karl B. Andree, Beatriz Lacuesta, Ana Roque, Chris Rodgers, M. Dolores Furones (2012) Molecular characterization of the *Marteilia* parasite infecting the common edible cockle *Cerastoderma edule* in the Spanish Mediterranean coast. A new *Marteilia* species affecting bivalves in Europe? *Aquaculture*, 324–325: 20–26.
- Karl B. Andree, Sonia Quijano-Scheggia, Margarita Fernández, Laurence M. Elandaloussi, Esther Garcés, Jordi Camp, Jorge Diogene (2011) Quantitative PCR Coupled with Melt Curve Analysis for Detection of Selected *Pseudonitzschia* spp. (Bacillariophyceae) from the Northwestern Mediterranean Sea. *Applied and Environmental Microbiology*, 77(5) 1651-1659.
- Guerao, G., Andree, K.B. & Rotllant, G. (2011) Direct evidence of parasitism by *Copidognathus stevcici* (Acari, Halacaridae) in crabs *Maja squinado* and *M. brachydactyla* (Brachyura, Majidae) in the laboratory. *Aquaculture*, 316: 136-138.
- Caillaud, P. de la Iglesia, M. Campas, L. Elandaloussi, M. Fernandez, N. Mohammad-Noor, K. B. Andree, J. Diogene (2010) Evidence of okadaic acid production in a cultured strain of the marine dinoflagellate *Prorocentrum rhathymum* from Malaysia. *Toxicon*, 55: 633-63
- Laurence M. Elandaloussi, Noèlia Carrasco, Ana Roque, Karl B. Andree, M. Dolores Furones. (2009) First record of *Perkinsus olseni*, a protozoan parasite infecting the commercial clam *Ruditapes decussatus* in Spanish Mediterranean waters. *Journal of Invertebrate Pathology*, 100: 50- 53.
- Bendorf C. M., Kelley G. O., Yun S. C., Kurath G., Andree K. B. and Hedrick R. P. (2007) Genetic Diversity of Infectious Hematopoietic Necrosis Virus (IHNV) from Feather River and Lake Oroville, California and Virulence of Selected Isolates for Chinook Salmon (*Oncorhynchus tshawytscha*) and Rainbow Trout (*Oncorhynchus mykiss*). *Journal of Aquatic Animal Health*, 19(4): 254- 269.
- Arkush K. D., McBride A. M., Mendonca H. L., Okihiro M. S., Andree K. B., Marshall S., Henriquez V., Hedrick R. P. (2005) Genetic characterization and experimental pathogenesis of *Piscirickettsia salmonis* isolated from white seabass *Atractoscion nobilis*. *Diseases of Aquatic Organisms*, 63: 139-149.
- Gilad, O., Yun, S., Andree, K.B., Adkison, M.A., Zlotkin, A., Bercovier, H., Eldar, A., Hedrick, R.P. (2002) Characterization of the koi herpesvirus (KHV) and development of a polymerase chain reaction assay to detect the virus in koi *Cyprinus carpio koi*. *Diseases of Aquatic Organisms* 48 (2): 101- 108.
- Kent, M., Andree, K. B., Bartholomew, J., El-Matbouli, M., Desser, S., Devlin, M., Hedrick, R.P., Khattra, J., Palenzuela, O., Siddall, M., Xiao, C. (2001) Recent Advances in Our Knowledge of the Myxozoa. *Journal of Eukaryotic Microbiology*, 48 (4): 395- 413.
- Andree, K. B., Friedman, C. S., Moore, J. D., and Hedrick, R. P. (2000) A polymerase chain reaction assay for the detection of genomic DNA of a rickettsiales-like prokaryote associated with withering syndrome in black abalone (*Haliotis cracherodii*). *Journal of Shellfish Research*, 19 (1): 213- 218.
- Friedman, C. S., Andree, K. B., Beauchamp, K., Moore, J. D., Robbins, T. T., Shields, J. D., Hedrick, R. P. (2000) “*Candidatus Xenohaliotis californiensis*” a newly described pathogen of abalone, *Haliotis* spp., along the west coast of North America. *International Journal of Systematic and Evolutionary Microbiology*, 50: 847- 855.
- Antonio, D. B., Andree, K. B., Moore, J. D., Friedman, C. S., Hedrick, R. P. (2000) Detection of Rickettsiales-like prokaryotes (RLP’s) by *in situ* hybridization in Black abalone *Haliotis cracherodii* with withering syndrome. *Journal of Invertebrate Pathology*, 75: 180- 182.
- Hedrick, R. P., McDowell, T. S., Marty, G. D., Mukkatira, K., Antonio, D. B., Andree, K. B., Bukhari, Z., Clancy, T. (2000) Ultraviolet irradiation inactivates the waterborne infective stages of *Myxobolus cerebralis*: a treatment for hatchery water supplies. *Diseases of Aquatic Organisms*, 42: 53- 59.
- Antonio, D. B., Andree, K. B., McDowell, T. S., Hedrick, R. P. (1999) Detection of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss* tissues and oligochaetes using a nonradioactive *in situ* hybridization protocol. *Journal of Aquatic Animal Health*, 10(4): 338- 347.
- El-Matbouli, M., McDowell, T. S., Antonio, D. B., Andree, K. B., Hedrick, R. P. (1999) Effect of water temperature on the development, release and survival of the triactinomyxon stage of *Myxobolus cerebralis* in its oligochaete host. *International Journal of Parasitology*, 29: 627- 641.



- Andree, K. B., MacConnell, E., Hedrick, R. P. (1998) A polymerase chain reaction test for detection of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease in fish, and a comparison to existing detection techniques. *Diseases of Aquatic Organisms*, 34(2): 145- 154.
- Furones, M.D.; Alderman, D.J.; Munn, C.B.; Gilpin, M.L. (1990) Virulence of *Yersinia italica* serotype I strains is associated with a heat sensitive factor (HSF) in cell extracts. *FEMS Microbiology Letters*, 66: 339-344
- Furones, M.D.; Alderman, D.J.; Bucke, D.; Fletcher, T.C.; Knox, D.; White, A. (1992) Dietary vitamin E and the response of rainbow trout, *Oncorhynchus mykiss* (Walbaum), to infection with *Yersinia ruckeri*. *Journal of Fish Biology*, 41: 1037-1041
- Furones, M.D.; Rodgers; C.J.; Munn, C.B. (1993) *Yersinia ruckeri*, the Casual Agent of Enteric Redmouth disease (ERM) in Fish. *Annual Reviews of Fish Diseases*, 3: 105-125
- Furones, M.D.; Munn, C.B.; Gilpin, M.L. (1993) Culture Media for Differentiation of Isolates of *Yersinia ruckeri*, based on a Detection of a Virulence Factor. *Journal of Applied Bacteriology*, 74: 360-366
- Rodgers, C.J.; Furones, M. D. (1998) Disease problems in Cultured Marine Fish in the Mediterranean. *Fish Pathology*, 33 (4): 157-164
- Furones, D. (2000) Sampling for antimicrobial sensitivity testing: a practical consideration. *Aquaculture*, 196: 303-309
- Reig, L.; Furones, D.; Basurco, B. (2000) Summary report of the workshop on global quality assessment in Mediterranean aquaculture. *Cahiers Options Méditerranéennes*, 51: 135-142
- Formiga-Cruz, M.; Tofiño-Quesada, G.; Bofill-Mas, S.; Lees, D.N.; Henshilwood, K.; Allard, A.K.; Condén-Hansson, A.-C.; Hernroth, B.E.; Vantarakis, A.; Tsibouixi, A.; Papapetropoulou, M.; Furones, M.D.; Gironès, R. (2002) Distribution of Human virus contamination in Shellfish from Different Growing Areas in Greece, Spain, Sweden, and the United Kingdom. *Applied and Environmental Microbiology*, 68: 5990-5998
- Fernández-Tejedor, M.; Soubrier-Pedreño, M.A.; Furones, M.D. (2004) Acute LD<sub>50</sub> of a *Gyrodinium corsicum* natural population for *Sparus aurata* and *Dicentrarchus labrax*. *Harmful Algae*, 3: 1-9
- Fernández-Tejedor, M.; Soubrier-Pedreño, M.A.; Furones, M.D. (2007) Mitigation of lethal effects of *Karolodinium veneficum* and *K. armiger* on *Sparus aurata*: changes in haematocrit and plasma osmolality. *Diseases of Aquatic Organisms*, 77: 53-59
- Carrasco, N.; López-Flores, I.; Alcaraz, M.; Furones, M.D.; Berthe, F.C.J.; Arzul, I. (2007) Dynamics of the parasite *Marteilia refringens* (Paramyxia) in *Mytilus galloprovincialis* and zooplankton populations in Alfacs Bay (Catalonia, Spain). *Parasitology*, 134: 1541-1550
- Carrasco, N.; Lopez-Flores, I.; Alcaraz, M.; Furones, M.D.; Berthe, F.C.J.; Arzul, I. (2007) First record of a *Marteilia* parasite (Paramyxia) in zooplankton populations from a natural estuarine environment. *Aquaculture*, 269: 63-70
- Carrasco, N.; Arzul, I.; Berthe, F.C.J.; Furones, M.D. (2008) In situ hybridization detection of *Marteilia refringens* (Paramyxia) initial infective stages in its host *Mytilus galloprovincialis*. *Journal of Fish Diseases*, 31: 153-157
- Carrasco, N.; Arzul, I.; Chollet, B.; Robert, M.; Joly, J.P.; Furones, M.D.; Berthe, F.C.J. (2008) Comparative experimental infection of the copepod *Paracartia grani* with *Marteilia refringens* and *M. maurini*. *Journal of Fish Diseases*, 31: 497-504
- Carrasco, N.; Arzul, I.; Berthe, F.C.J.; Fernández-Tejedor, M.; Durfort, M.; Furones, M.D. (2008) Delta de l'Ebre is a natural Bay model for *Marteilia* spp. (Paramyxia) dynamics and life cycles studies. *Diseases of Aquatic Organisms*, 79: 65-73
- Elandaloussi, L.M.; Carrasco, N.; Roque, A.; Fernández-Tejedor, M.; Furones, D. (2008) Occurrence of *Perkinsus* sp. In two clam species (*Ruditapes philippinarum* and *R. decussatus*) from the Ebro Delta, Spain. *Bull. Eur. Ass. Fish Pathol.*, 28: 2-10
- Rodgers, C.J.; Furones, M.D. (2009) Antimicrobial agents in aquaculture: Practice, needs and issues. *CIHEAM. OPTIONS méditerranéennes. SERIES A: Mediterranean Seminars. The use of veterinary drugs and vaccines in Mediterranean aquaculture*, 86: 41-59. ISSN: 1016-121-X – ISBN: 2-85352-422-1
- Elandaloussi, L.; Carrasco, N.; Furones, D.; Roque, A. (2009) Phylogenetic relationship of *Perkinsus olseni* from the Ebro delta, Spain, to other *Perkinsus* species, based on ribosomal DNA sequences. *Diseases of Aquatic Organisms*, 86: 135-142



- Roque, A.; Lopez-Joven, C.; Lacuesta, B.; Elandaloussi, L.; Wagley, S.; Furones, M.D.; Ruiz-Zarzuela, I.; de Blas, I.; Rangdale, R.; Gomez-Gil, B. (2009) Detection and identification of *tdh* and *trh*-positive *Vibrio parahaemolyticus* from four species of cultured bivalve molluscs in the Spanish Mediterranean coast. *Applied and Environmental Microbiology*, 75: 7574-7577
- Carrasco, N.; Roque, A.; Andree, K.B.; Rodgers, C.; Lacuesta, B.; Furones, M.D. (2011) A *Marteilia* parasite and digestive epithelial virosis lesions observed during common edible cockle *Cerastoderma edule* mortality event in the Spanish Mediterranean coast. *Aquaculture*, 321: 197-202
- Lopez-Joven, C.; Ruiz-Zarzuela, I.; de Blas, I.; Furones, M.D.; Roque, A. (2011) Persistence of sucrose fermenting and nonfermenting vibrios in tissues of Manila clam species, *Ruditapes philippinarum*, depurated in seawater at two different temperatures. *Food Microbiology*, 28 (5): 951-956
- Lopez-Joven, C.; de Blas, I.; Ruiz-Zarzuela, I.; Furones, M.D.; Roque, A. (2011) Experimental Uptake and Retention of pathogenic and non-pathogenic *Vibrio parahaemolyticus* in two species of clams: *Ruditapes decussatus* and *R. philippinarum*. *Journal of Applied Microbiology*, 111 (1): 197-208
- Roque, A.; Carrasco, N.; Andree, K.B.; Lacuesta, B.; Elandaloussi, L.; Gairin, I.; Rodgers, C.; Furones, M.D. (2012) First report of OsHV-1 in Pacific oyster (*Crassostrea gigas*) cultured in Spain. *Aquaculture*, 324-325: 303-306
- Carrasco, N.; Villalba, A.; Andree, K.B.; Engelsma, M.Y.; Lacuesta, B.; Ramilo, A.; Gairin, I.; Furones, M.D. (2012) *Bonamia exitiosa* (Haplosporidia) observed infecting the European flat oyster *Ostrea edulis* cultured on the Spanish Mediterranean coast. *Journal of Invertebrate Pathology*, 110 (3): 307-313.
- López-Joven, C.; Roque, A.; Pérez-Larruscain, J.; Ruiz-Zarzuela, I.; Furones, M.D.; de Blas, I. (2013) Uptake and retention of *Vibrio parahaemolyticus* in a cohabiting population of *Ruditapes decussatus* and *Ruditapes philippinarum* under experimental conditions. *Current Microbiology*, 67: 36-40.
- Skalli, A.; Castillo, M.; Andree, K.; Tort, L.; Furones, D.; Gisbert, E. (2013) The LPS derived from the cell walls of the gram-negative bacteria *Pantoea agglomerans* stimulates growth and immune status of rainbow trout (*Oncorhynchus mykiss*) juveniles. *Aquaculture* (in press)
- Martinez-Rubio, L., Morais, S., Evensen, Ø., Wadsworth, S., Vecino, J.L.G., Ruohonen, K., Bell, J.G., Tocher, D.R. (2013) Effect of functional feeds on fatty acid and eicosanoid metabolism in liver and head kidney of Atlantic salmon (*Salmo salar* L.) with experimentally induced Heart and Skeletal Muscle Inflammation. *Fish Shellfish Immun.*, 34:1533-1545
- Martinez-Rubio, L., Morais, S., Evensen, Ø., Wadsworth, S., Ruohonen, K., Vecino, J.L.G., Bell, J.G., Tocher, D.R. (2012) Functional feeds reduce heart inflammation and pathology in Atlantic salmon (*Salmo salar* L.) following experimental challenge with Atlantic salmon reovirus (ASRV). *PLoS ONE* 7(11): e40266.
- Martins, D.A., Rocha, F., Castanheira, F., Mendes, A., Pousão-Ferreira, P., Bandarra, N., Coutinho, J., Morais, S., Yúfera, M., Conceição, L.E.C., Martínez-Rodríguez, G. (2013) Effects of dietary arachidonic acid on cortisol production and gene expression in stress response in Senegalese sole (*Solea senegalensis*) post-larvae. *Fish Physiol Biochem.* DOI 10.1007/s10695-013-9778-6.
- Martins, D.A., Rocha, F., Martínez-Rodríguez, G., Bell, J.G., Morais, S., Castanheira, F., Bandarra, N., Coutinho, J., Yúfera, M., Conceição, L.E.C. (2012) Teleost fish larvae adapt to dietary arachidonic acid supply through modulation of the expression of lipid metabolism and stress response genes. *Br J Nutr.*, 108:864-874.
- Martins, D.A., Engrola, S., Morais, S., Bandarra, N., Coutinho, J., Yúfera, M., Conceição, L.E.C. (2011) Cortisol response to air exposure in *Solea senegalensis* post-larvae is affected by dietary arachidonic acid to eicosapentaenoic acid ratio. *Fish Physiol Biochem.*, 37:733-743.





#### **Partner 4. Israel Oceanographic and Limnological Research (IOLR), Israel**

**Description of Organization:** IOLR is a non-profit government research organization that consists of 3 research centers, one of which is the National Center of Mariculture (Eilat) will participate in this proposal. The IOLR centre is internationally known as a leading research institute in the area of marine aquaculture. It is made up of nine research groups working interactively to domesticate marine species for aquaculture. The main research areas include fish reproduction, larval rearing and physiology, pathology, algal and zooplankton culture, genetic improvement of farmed marine species, development of feeds, research and development of cost effective and environmentally friendly systems for intensive fish growth as well as integrated systems for growth of fish, molluscs and algae. The professional staff at the IOLR in Eilat includes about 50 scientists, research assistants and technicians. The research infrastructure provides a continuous supply of filtered and UV treated seawater to a wide array of experimental and semi-commercial rearing tanks and ponds. In addition, the Eilat facility has a number of well-equipped analytical laboratories carrying out molecular, biochemical and biological research.

**Main Tasks:** The IOLR is the **Leader of GWP Larval Husbandry** and the **Species Leader for grey mullet**, and is primarily responsible for all studies on the grey mullet in GWP Larval husbandry, GWP Nutrition and GWP Grow out husbandry. IOLR will focus on (1) hormone-based treatments for the induction of gonadal development and maturational processes in grey mullet males and females, (2) dietary effects on grey mullet broodstock egg fecundity, quality and larval first feeding, (3) phytoplankton and nutritional effect (essential fatty acids, taurine) on grey mullet composition and performance, (4) determining the effect of co-feeding ciliates and rotifers on digestive tract maturation and enzyme production in grey mullet, (5) determining weaning time in grey mullet and type of feed according to the shift from carnivorous to omnivorous feeding, (6) determining changes in the essential fatty acid requirement as a function of developmental stage and ambient salinity in grey mullet, (7) gene expression of key enzymes in the synthesis of taurine and bile salts in grey mullet, (8) nutritional effects (essential fatty acids, taurine) on grey mullet roe (bottarga) quality and improving grow out feeds for grey mullet, (9) evaluating and maximizing the dietary incorporation of a non-GMO genetically selected soybean meal that will increase nutrient absorption and reduce DT inflammation, as well as (10) testing this diet at different stocking densities on F1 and wild caught juveniles over a range of environmental conditions.

**Previous Experience:** The Reproduction group of the IOLR has developed technologies for an efficient broodstock management of various finfish. Among the recent achievements is the establishment of hormone-based therapy for alleviating reproductive dysfunctions of grey mullet broodstock in captivity. Additionally, this group is specialized in producing bioactive recombinant proteins such as gonadotropins (including BFT-FSH and BFT-LH). The Larval Rearing and Physiology group has focused on the nutritional effects on larval growth, metamorphic success, stress response, visual acuity as well as skeletal deformity and the gene expression of key proteins in retinal function and bone formation. These two IOLR research groups have participated in EU FP grants such as FINEFISH, REPRODOTT, SELFDOTT and TRANSDOTT, as well as other international grants that include the Israel-USA BARD, Israel-Jordan and Israel-Egypt MERC and Israel-France MST.

**Staff profile:** **Dr W.M. Koven** (Scientific Responsible) is the **Leader of GWP Larval husbandry** and the **Species Leader for grey mullet**. He is well-versed on larval lipid class and fatty acid requirements and their physiological impact on growth, vision and the stress response, environmental and nutritional effects on skeletal deformity, mucosa peptide transporters, taurine metabolism as well as protocol development for the larval culture of various commercial teleosts. **Dr H. Rosenfeld** has expertise in studying fish reproductive physiology including mechanisms controlling sex differentiation, environmental and hormonal cues that initiate and regulate sexual maturity, and *in vivo/in vitro* procedures promoting gamete maturation and fertilization.

#### **Relevant publications:**

Aizen, J., Meiri, I., Tzchori, Levavi-Sivan, B., Rosenfeld, H., 2005. Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. Gen. Comp. Endocrinol. 142: 212-221.



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Izquierdo, M.S., Koven, W., 2011. Lipids: In: Holt, J. (ed.), Larval Fish Nutrition, Wiley-Blackwell, John Wiley and Sons, ISBN: 978-0-8138-1792-7, pp. 47-82.

Sandel, E., Uni, Z., Koven, W., 2010. The effect of different dietary ratios of Phosphatidylcholine and Phosphatidylinositol fed to the gilthead sea bream (*Sparus aurata*) larvae on larvae and juvenile performance. Aquaculture 304: 42-48.



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**Partner 5. The University Court of the University of Aberdeen (UNIABDN), UK**

**Description of Organization:** The UNIABDN is at the forefront of teaching and research in medicine, the humanities and science and has been involved in European projects since FP 3. It has been successful in participating in more than 600 grants funded by the European Commission, including ERC Starting and Advanced Grants. The Scottish Fish Immunology Research Centre (SFIRC), which participates in this proposal was established in 2001 and brings together fish immunology expertise across Scotland, at the Universities of Aberdeen and Stirling and at Marine Scotland. At the UNIABDN, world-leading research on fish cell-mediated immunity and the elucidation of the cytokine network has been on-going for nearly 30 years. Major facilities include state of the art molecular and fish cell culture laboratories, including FACS and clean room facilities, and marine and freshwater aquarium facilities. The latter include a pathogen containment unit and a zebrafish breeding unit. The aquarium underwent a £1M refurbishment in 2010.

**Main Tasks:** This Partner is the **Leader of GWP Fish Health** and will participate in different tasks relating to the functional characterization of the immune system of meagre (WP24) and greater amberjack (WP25). This will involve cloning a number of immune genes in both species, developing qPCR assays for expression analysis, and raising antisera to the main immunoglobulin isotypes present in these species. These assays and reagents will be used to monitor the immune responses post-vaccination or pathogen exposure, and to assess the health impact of feeding different diets.

**Previous Experience:** The UNIABDN has participated in many previous EU FP funded programmes, including recently TARGETFISH, LIFECYCLE, IMAQUANIM, SAPRO, STRESSGENES, FISHOV, AQUAFIRST. In addition, the UNIABDN has participated in many national funded programmes, supported by BBSRC, NC3Rs, DEFRA, TSB, Royal Society, etc. This Partner has unparalleled expertise in fish cytokine research, cytokine recombinant protein production and analysis of the mechanisms of disease resistance in fish. Whilst the focus is salmonids, more general interests in cytokine evolution have often required cloning of genes from other fish species, and even other vertebrate groups.

**Staff profile:** **Prof Chris Secombes** (Scientific Responsible) is the **Leader of GWP Fish Health** and has ~35 years experience of fish immunology, and in 2007 was awarded the Royal Society of Edinburgh's Alexander Ninian Bruce prize for his "outstanding contribution to our understanding of the immune system of fish, particularly salmonids". Other staff at UNIABDN include **Dr Sam Martin** and **Dr Jun Zou**, who are senior scientists within the Scottish Fish Immunology Research Centre, with a focus on fish immune-nutrigenomics and antiviral defences respectively. **Dr Martin** has pioneered functional genomics approaches to study immune system responses and most recently has employed high throughput sequencing approaches. **Dr Tiehui Wang** is a senior postdoctoral fellow within the UNIABDN, who has led work to determine the biological activity of many of the fish cytokines that have been discovered in salmonids, with a focus on recombinant protein production. The UNIABDN is supported by two laboratory and two aquarium technicians, and currently has 5 postdocs and 14 PhD students in addition to the above.

**Relevant publications:**

- Harun, N.O., Wang, T., Secombes, C.J., 2011. Gene expression profiling in naïve and vaccinated rainbow trout after *Yersinia ruckeri* infection: Insights into the mechanisms of protection seen in vaccinated fish. *Vaccine* 29: 4388-4399.
- Monte, M.M., Zou, J., Wang, T., Carrington, A., Secombes, C.J., 2011. Cloning, expression analysis and bioactivity studies of rainbow trout (*Oncorhynchus mykiss*) interleukin-22. *Cytokine* 55: 62-73.
- Wang, T., Diaz-Rosales, P., Costa, M.M., Campbell, S., Snow, M., Collet, B., Martin, S.A.M., Secombes, C.J., 2011. Functional characterisation of a nonmammalian IL-21: Rainbow trout *Oncorhynchus mykiss* IL-21 upregulates the expression of the Th cell signature cytokines IFN- $\gamma$ , IL-10 and IL-22. *J. Immunology* 186: 708-721.
- Wawra, S., Bain, J., Durward, E., De Bruijn, I., Minor, K.L., Matena, A., Löbach, L., Whisson, S.C., Bayer, P., Porter, A.J., Birch, P.R.J., Secombes C.J., van West, P., 2012. Host-targeting protein 1 (SpHtp1) from the oomycete *Saprolegnia parasitica* translocates specifically into fish cells in a tyrosine-O-sulphate-dependent manner. *PNAS* 109 (6): 2096-101.



**Partner 6. Stichting Wageningen Research (SWR) previously Dienst Landbouwkundig Onderzoek (DLO/LEI), the Netherlands**

**Description of Organization:** Stichting Wageningen Research (SWR) consists of a number of specialized institutes for applied research in healthy food and living environment. SWR collaborates with two other legal entities - Wageningen University and Stichting Van Hall Larenstein - under the external brand name **Wageningen UR (University & Research centre)** and will participate with the Agricultural Economics Research Institute or LEI. SWR has a strong track record of multidisciplinary projects and is involved in hundreds of national and international research projects. One of the strengths of Wageningen UR is that its structure facilitates and encourages close cooperation between the approximately 3000 experts from the Wageningen University and various renowned research institutes. These institutes cover a wide range of expertise including food technology, plant, animal and economic sciences.

**LEI** is the leading institute for social and economic research on agriculture, horticulture, fisheries, forestry and rural areas in the Netherlands with a work force of 295 researchers. Among LEI's clients are governments, the European Commission (DGs AGRI, SANCO, MARE and TRADE, among others), businesses and organizations. Also, LEI is performing an increasing amount of research and consultancy in developing countries. It has experience in leading large-scale international projects involving a number of different public and/or private parties. LEI's results offer a solid basis for policy and are accompanied by advice that is tailored to the client's own situation. Besides economists and econometricians, LEI is also staffed by public administration experts, marketing specialists, sociologists, ethics experts and ICT experts. LEI also has specific in-house expertise on the agricultural sectors. A group of 15 researchers works on marine governance, aquaculture and fisheries. LEI has access to unique economic data and models, which it does not only supply but also interprets on the basis of a sound knowledge of the sector and the policy environment. LEI's analyses offer strategic indications for policy.

**Main Tasks:** LEI is the **leader for GWP Socioeconomics** and will be involved in WP27, WP28, WP29, WP30. Specifically, LEI participates in the external (competitive) market analysis and the consumer research, that both are the basis for the new product development performed by other parties.

**Previous Experience:** The expertise of LEI includes consumer research, B-to-B market research, economic modelling of production systems, value-chain analysis and development, market intelligence and business model generation. This experience has been gained in a wide diversity of national, EU and international projects in the international seafood sector and also in other agri-food sectors.

**Staff profile:** **Mrs Gemma Tacken** (scientific responsible) is a Senior Researcher with an MSc in Business Economics, specialization in marketing and market research and has been working at LEI since 1997 as researcher, project manager and WP-leader in marketing, consumer and business-to-business market research projects on animal products in several EU-projects. **Mr Arie Pieter van Duijn** is a Senior Researcher with a MA in Economic Geography and an MSc in Aquaculture and Aquatic Resources Management. He started working on aquaculture in Thailand in 1997 and did his MSc in Thailand and has worked for FAO as an aquaculture and fisheries socio-economist in Vietnam. At LEI he is a senior researcher and project manager on international aquaculture projects. **Mr Willem van der Pijl** is a Researcher with an MSc in International Development Studies and started as seafood market and supply chain specialist at LEI in 2011. He focuses mainly on market access issues, market intelligence, business models and business strategies. **Mr Rik Beukers** is a Researcher with an MA in Environmental Studies and an MSc in Environmental Economics and has been working at LEI as researcher on fisheries and aquaculture in market studies, value chain analysis, international trade issues and fish processing industry. **Dr Machiel Reinders is a Researcher** and works as marketing researcher. He has expertise in marketing strategy and consumer behaviour related to food consumption. He was involved in several EU-projects (ISAFRUIT, PEGASUS and CONNECT4ACTION). **Ir. Willy Baltussen** works as senior economic researcher at LEI-Wageningen UR. He has experience in feasibility studies for national and EU purposes. He was and is involved in several EU-projects (for example "Evaluation of the feasibility of a certification scheme for high quality control posts"). He is also involved as overall project leader in EU tender and framework projects (evaluation of Regulation 1/2005; origin labelling of fresh meat of pigs, poultry, sheep and goat); as Work package leader (BoRest: evaluation of restraining device for unstunned adult cattle) and senior researcher in projects.





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**Relevant publications:**

- Baltussen, W., Gebresenbet, G., Zoest, K. de, 2011. Study on the impact of Regulation (EC) No 1/2005 on the protection of animals during transport.  
[http://ec.europa.eu/food/animal/welfare/transport/docs/study\\_report\\_en.pdf](http://ec.europa.eu/food/animal/welfare/transport/docs/study_report_en.pdf), DG SANCO/2010/D5/S12.574298
- Gebresenbet, G., Baltussen, W., Sterrenburg, P., Roest, K., de, Engstrøm, Nielsen, K., 2010. Evaluation of the feasibility of a certification scheme for high quality control posts.  
[http://ec.europa.eu/food/animal/welfare/financing/docs/call\\_10753-2010\\_feasability\\_report\\_cepost\\_en.pdf](http://ec.europa.eu/food/animal/welfare/financing/docs/call_10753-2010_feasability_report_cepost_en.pdf), SANCO/D5/2005/SI2.548887.
- Onwezen, M.C., Reinders, M.J., van der Lans, I.A., Sijtsema, S.J., Jasiulewicz, A., Guardia, M. D., Guerrero, L., 2012. A cross-national consumer segmentation based on contextual differences in food choice benefits. *Food Quality and Preference* 24 (2): 276-286.



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**Partner 7. Institute of Marine Research (IMR), Norway**

**Description of Organization:** The IMR (<http://www.imr.no>) has about 700 employees and is Norway's largest centre in marine science, as well as the second largest marine research institute in Europe. The aim of research and advice provided by IMR is to ensure that Norway's marine resources are harvested and managed in a sustainable way. Focused on aquaculture, fisheries and marine environmental studies, the present area of priority is interactions in the coastal zone, emphasizing aquaculture-fisheries interactions, implementation of the Marine Strategy Framework Directive, and general coastal zone ecology. The IMR has modern laboratories and the Austevoll Research Station has full-scale facilities for rearing of marine fish, from broodstock and eggs to harvest-size fish. The facilities and personnel hold relevant certificates for Animal Experiments according to Norwegian and European legislation. Adjacent to the rearing facilities and biological laboratories are modern laboratories for molecular biology, endocrinology, microbiology and fish pathology.

**Main Tasks:** The IMR is the **Species leader for Atlantic halibut**. Its contribution to the present project will be carried out at Austevoll Research Station and in the fish health laboratory in Bergen, by personnel in three of IMR's research groups: Reproduction and Early Life Stages, Oceanography and Fish health. The IMR tasks include studies on fecundity and spawning induction in Atlantic halibut broodstock, improvement of rearing protocols for first feeding of Atlantic halibut larvae, and methods for protection against nodavirus infections (VNN).

**Previous Experience:** The aquaculture-related expertise of IMR includes reproductive biology, early life history, growth regulation, welfare, animal health, environmental impact, genetics and molecular biology. In the past 10 years, the IMR has been the recipient and/or coordinator of many research grants, and is participating in national and EU projects involved with the study of fish physiology and development of aquaculture methods, including WEALTH (FP5), PUBERTIMING, AQUAMAX (FP6), LIFECYCLE, COPEWELL, COEXIST, PROEEL and AQUAEXCEL (FP7).

**Staff profile:** **Dr Birgitta Norberg** (Scientific Responsible) is the **Species Leader for Atlantic halibut** and was the Regional Research Director at IMR, Austevoll Research Station 2007-2010 and Adjunct professor at University of Nordland, Bodø 2003-2010. Research experience includes broodstock management, puberty regulation and techniques for sex reversal in marine fish, as well as immunoassay development. She has >70 peer-reviewed publications, mainly within reproductive physiology, puberty and growth regulation (H-index 25). **Dr Øivind Bergh** (Principal Scientist) was a Programme manager 1998-2003 and Head of IMR's Fish Health group 2004-2008, and is an Adjunct Professor at the University of Bergen since 2005. He has >90 peer-reviewed publications, mainly on bacterial diseases and microbial ecology of marine fish and shellfish (H-index 23). **PhD candidate Torstein Harboe** (Scientist) has focused on development of an intensive, year round rearing method for Atlantic halibut fry, including use of algae during first feeding, flow-trough systems for yolk-sac larvae incubation and automatic cleaning systems for rearing tanks. He has >20 peer-reviewed publications, mainly within marine larval rearing technology and nutrition (H-index 12). **Dr Nina Sandlund** (Scientist) is involved in Fish Health, especially bacterial diseases of early life stages of fish and shellfish, and ecological roles of pathogens in wild populations. **Dr. Sonal Patel** (Scientist) holds a PhD in Fish Health (2009): Immune related genes in Atlantic halibut, Expression of B- and T cell markers during ontogenesis. He has publications on teleost immune systems, especially Atlantic halibut, including work on vaccine development against nodavirus (VNN). Currently he holds a personal grant for Excellent Younger Scientists from the Research Council of Norway. **Professor Audun H. Nerland** (Scientist) has long experience in vaccine development, documentation and production (including the first commercial recombinant vaccine for use in aquaculture) from research institutions and the private industry. He has been project leader for several projects dealing with different aspects of vaccination against bacterial and viral infections.

**Relevant publications**

Babiak, J., Babiak, I., Harboe, T., Haugen, T., van Nes, S., Norberg, B., 2012. Induced sex reversal using an aromatase inhibitor, Fadrozole, in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* 324-325: 276-280.



- Harboe, T., Mangor-Jensen, A., Moren, M., Hamre, K., Rønnestad, I., 2009. Control of light condition affects the feeding regime and enables successful eye migration in Atlantic halibut juveniles. *Aquaculture* 290 (3-4): 250-255.
- Nerland, A.H., Øvergård, A.C., Patel, S., 2011. Viruses of fish. In: *Studies in Viral Ecology*, Christon, J. and Hurst (eds.), Wiley-Blackwell, ISBN: 978-1-1180-2458-4.
- Øvergård, A.C., Patel, S., Nøstbakken, O.J., Nerland, A.H., 2013. Atlantic halibut (*Hippoglossus hippoglossus* L.) T-cell and cytokine response after vaccination and challenge with nodavirus. *Vaccine* 31(19): 2395-2402.
- Overgård, A.C., Nerland, A.H., Fiksdal, I.U., Patel, S., 2012. Atlantic halibut experimentally infected with nodavirus shows increased levels of T-cell marker and IFN $\gamma$  transcripts. *Dev. Comp. Immunol.* 37(1): 139-150.
- Patel, S., Øvergård, A.C., Nerland, A.H., 2008. CD8 $\alpha$  and CD8 $\beta$  in Atlantic halibut, *Hippoglossus hippoglossus*: cloning, characterization and gene expression during viral and bacterial infection. *Fish Shellfish Immunol.* 25(5): 570-580.
- Rekecki, A., Ringø, E., Olsen, R., Myklebust, R., Dierckens, K., Bergh, Ø., Laureau, S., Cornelissen, M., Ducatelle, R., Decostere, A., Bossier, P., Van den Broek, W., 2013. Luminal uptake of *Vibrio (Listonella) anguillarum* by shed enterocytes – a novel early defense strategy in larval fish. *Journal of Fish Diseases* 36: 419-426.
- Sandlund, N., Rødseth, O.M., Knappskog, D.H., Fiksdal, I.U., Bergh, Ø., 2010. Comparative susceptibility of turbot, halibut and cod yolk-sac larvae to challenge with *Vibrio* spp. *Diseases of Aquatic Organisms* 89: 29-37.
- Vadstein, O., Bergh, Ø., Gatesoupe, F.-J., Galindo-Villegas, J., Mulero, V., Picchetti, S., Scapigliatti, G., Makridis, P., Olsen, Y., Dierckens, K., Defoirdt, T., Boon, N., De Schryver, P., Bossier, P., 2013. Microbiology and immunology of fish larvae. *Reviews in Aquaculture*, in press.
- Weltzien, F.-A., Andersson, E., Andersen, Ø., Shalchian-Tabrizi, K., Norberg, B., 2004. The brain-pituitary-gonad axis in male teleosts, with emphasis on the flatfish (Pleuronectiformes). *Comp.Biochem. Physiol. A* 137:447-477.



## **Partner 8. Instituto Español de Oceanografía (IEO), Spain**

**Description of Organization:** The IEO is a public research organization, involved with research in marine fisheries resources assessment, aquaculture and marine environment. The main objective of the Aquaculture Department is to investigate the commercial culture technologies of different species of fish, molluscs and algae, and has several Experimental Culture Facilities and laboratories fully equipped allowing the development of studies on a wide range of fields related to aquaculture. In this proposal, the Culture Units from **IEO-Vigo (wreckfish)** and **IEO-Canarias (greater amberjack)** will participate and the expertise of the Culture Unit of **IEO-Murcia** in larval rearing of large pelagic fishes (Atlantic bluefin tuna and various Sparids) will be also available for the success of the project.

**Main Tasks:** This Partner is the **Species Leader for wreckfish** and will be involved in tasks related to the wreckfish and greater amberjack including (1) reproductive behaviour, natural spawning conditions and hormonal induction treatments, (2) *in vitro* fertilization and sperm cryopreservation, (3) development of larval rearing protocols, (4) nutrition studies for the improvement of spawning quality and larval and weaning performance and (5) immune response, hematological and biochemical indicators of fish health and welfare.

**Previous Experience:** The IEO has been involved in development of aquaculture production methods for many years. Main research areas include (1) rearing protocols for new species for aquaculture such as greater amberjack, wreckfish, sparids, flatfish and cephalopods, (2) reproductive performance and physiology, (3) hatchery rearing techniques, (4) weaning and grow out in tanks and cages, (5) nutritional requirements, especially lipids and carotenoids of cultured marine species, (6) health and welfare, immune system and vaccine development, (7) genetic analysis for selective breeding of aquaculture stocks. During the last 10 years IEO has been involved in EU and national projects related with diversification in aquaculture: 4 European projects (IFD97-1695, SARDYN, REPRODOTT (FP5), SELFDOTT (FP7)) and 19 National projects, 9 Regional projects and 2 Private foundations projects.

**Staff profile:** **Dr B. Álvarez-Blázquez** (animal husbandry) is replacing **Dr J.B. Peleteiro** (Scientific Responsible) as the **Species leader for wreckfish**, due to his recent retirement. **Dr S. Jerez**, is responsible of the studies on greater amberjack at the IEO and specializes on reproduction and broodstock nutrition, larval rearing and feeding strategies. The team includes also senior researchers, **Dr M. Pérez** (genetics applied to aquaculture), **Drs J.R. Cejas, E. Abellán** and **M. Arizcun** (new species culture development), **Drs E. Almansa** and **P. Domingues** (marine fish and mollusk nutrition), **Dr R.M. Cal** and (animal husbandry), **Drs M.V. Martín, V.C. Rubio** and **E. Chaves-Pozo** (fish physiology, fish health and welfare).

### **Relevant publications:**

- Figueiredo-Silva, C., Corraze, G., Kaushik, S., Peleteiro, J.B., Valente, M.L.P., 2010. Modulation of blackspot seabream (*Pagellus bogaraveo*) intermediary metabolic pathways by dispensable amino acids. *Amino Acids* 39: 1401-1416.
- Jerez, S., Samper, M., Santamaría, F.J., Villamandos, J.E., Cejas, J.R., Felipe, B.C., 2006. Natural spawning of greater amberjack (*Seriola dumerili*) kept in captivity in the Canary Islands. *Aquaculture* 252: 199-207.
- Rodríguez-Barreto, D., Jerez, S., Cejas, J.R., Martín, M.V., Acosta, N.G., Bolaños, A., Lorenzo, A., 2012. Comparative study of lipid and fatty acid composition in different tissues of wild and cultured female broodstock of greater amberjack (*Seriola dumerili*). *Aquaculture* 360-361: 1-9.



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**Partner 9. Université de Lorraine – INRA (UL), France**

**Description of Organization:** Composed by 32 professors and associated professors, 18 engineers and technicians and 20 PhD students, the Research Unit on Animal and Functionality of Animal Products (UR AFPA) belongs to the axis "Agronomy, Agro-industry and Forestry" of the University of Lorraine (UL) and is organized into three research teams: It will participate with the Domestication in Inland Aquaculture (DAC) unit, with a team of 2 professors, 6 associated professors or researchers, 1 engineer, 2 technicians and 5 PhD students, who are involved in research on the domestication of new species --mainly percids- and inland aquaculture diversification. In relation with the development of its research, UL will have new and modern facilities built over the year 2013 (project of 3.5 million Euros with 2.5 million Euros of investment, facilities available in December 2013). These experimental facilities will be adapted for freshwater fish reproduction, larval rearing and grow out, and more especially multifactorial experimental designs (with 16 independent experimental rooms). The main skills of the team are fish husbandry, physiology, behaviour and development.

**Main Tasks:** This Partner is the **Species Leader for pikeperch** and its research tasks will be in pikeperch. The UL will be directly concerned with WP16 (larval husbandry) and WP22 (growth and husbandry). For these two tasks, several experiments will be carried out in our new experimental aquaculture platform. To understand the multifactorial determinism of the performance of pikeperch larval rearing and growth, UL will carry out complex factorial designs, be responsible for the sampling strategies in collaboration with other Partners (IRTA, FUNDP, DTU), carry out analyses of the results and the modelling of the performances. Experiments will also involve a study of the ontogeny of cannibalism emergence in pikeperch larval rearing.

**Previous Experience:** The UL has a long expertise on the domestication of new species, mainly percids, for inland aquaculture diversification and has been working on percid fish culture for 15 years. Its main skills concern fish domestication (development of a generic approach), reproduction (control of reproductive cycle), and larval rearing (optimization of rearing protocols and regulation of size heterogeneity). The UL has a specific know-how in the use of particular methodology such as complex multifactorial designs and modelling. The recent recruitment of 3 associated professors (B. Schaerlinger, F. Teletchea, S. Milla) and the integration of two new colleagues (A. Pasquet, D. Chardard) have brought new skills in UL. The UL has participated in 6 EU projects such as Fair-CT96-1572, Fair-CT98-9241, ACRAPEP, PERCATECH, LUCIOPERCIMPROVE (FP5), REPROFISH (FP7).

**Staff profile:** **Prof P. Fontaine** (Scientific Responsible) is the **Species Leader for pikeperch** and is a specialist of percid fish reproduction and more especially of the environmental control of reproductive cycle (out-of-season spawning). **Dr F. Teletchea** is mainly competent on fish biology (reproductive fish strategy, larval development). **Dr S. Milla** is a fish physiologist (stress) and immunologist. **Dr A. Pasquet** is an expert in animal behaviour.

**Relevant publications:**

- Abdulfatah, A., Fontaine, P., Kestemont, P., Gardeur, J.-N., Marie M., 2011. Effects of photothermal kinetic and amplitude of photoperiod decrease on the induction of the reproduction cycle in female Eurasian perch *Perca fluviatilis*. *Aquaculture* 322-323: 169-176.
- Abdulfatah, A., Fontaine, P., Kestemont, P., Milla, S., Marie, M., 2013. Effects of the thermal threshold and the timing of temperature reduction on the initiation of reproduction cycle in female of Eurasian perch *Perca fluviatilis*. *Aquaculture* 376-379: 90-96.
- Kestemont P., Jourdan S., Houbart M., Mélard C., Paspatis M., Fontaine P., Cuvier A., Kentouri M., Baras E., 2003. Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. *Aquaculture* 227: 333-356.
- Teletchea, F., Fontaine, P., 2013. Levels of domestication in fish: implications for the sustainable future of aquaculture. *Fish and Fisheries* (DOI:10.1111/faf.12006).



### **Partner 10. Technische Universiteit Eindhoven (TU/e), the Netherlands**

**Description of Organization:** Eindhoven University of Technology (TU/e) is a research university specializing in engineering science & technology. With advanced quality research, the university contributes to the progress of technical sciences and thus the development of technological innovations. The university wants to give significant impetus to the knowledge-intensive industries and other social sectors with a high or rapidly evolving..... The TU/e emphasizes and focuses on “knowledge valorization”, that is research results are translated into successful innovations and serve as a basis for creating new products, processes and enterprises. TU/e will participate with its group “Innovation, Technology entrepreneurship and Marketing” (ITEM), which is specialized in this area and complements the university’s start-ups organized in and via its Incubator.

**Main Tasks:** This Partner’s tasks focus on GWP Socioeconomics, in particular the business model development and development of the marketing launch strategy for the new fish fillets and value products. The Partner will lead the effort for developing a business model together with the SME Partners along the value chain and apply simulation models to forecast marketing strategies. The Partner will contribute to (1) the opportunities and barriers for growth, and (2) idea generation and selection for new product development. Specifically contributions pertain to analyses of success cases and using market and business criteria, next to technical criteria.

**Previous Experience:** The expertise of the ITEM group of TU/e includes (1) business models for new technology and technology start-ups, (2) technology “valorization”, i.e. transferring knowledge from universities to the market, (3) knowledge development regarding eco systems for technology development, and (4) developing marketing and sales programs for selling new products. In the past 10 years, the ITEM group of TU/e has been the recipient and/or coordinator of many research local and EU grants, including: FP7 Socio economic Sciences and Humanities, Creativity for Innovation & Growth in Europe (2013-2016), KIC InnoEnergy S.E., Energy Technology Commercialization (2012-2016), STW, study regarding Technology Commercialization – Selection, Support and Development (2012-2013), Interreg IVC, EURIS programme European Collaborative and Regional Open Innovation Strategies project: Business Models for Open Innovation (lead partner, 2011-2012), DG ENTR/CIP/09: E- (8/2009-2/2011), KIC InnoEnergy SE, SEE CC (2011-2012), Erasmus Mundus Joint Doctorate Program Select+ (2012-2017), RAAK: Integration of Social Media in the Product Development Process of SME’s (2012-2014).

**Staff profile:** **Prof. Dr E.J. Nijssen** (Scientific Responsible) is a professor of technology marketing and has unique expertise in the area of marketing and selling radical new technologies. Other senior and junior researchers involved in the proposal include **Dr M. van der Borgh** (selling new products and services, and expert in the area of innovation eco systems) and **Dr B. Walrave** (System dynamics expert).

#### **Relevant publications**

- Berends, J.J., Jelinek, M., Reymen, I.M.M.J., Stultiens, R.G.L., 2012. Product Innovation processes in small firms: combining entrepreneurial effectuation and managerial causation. *Journal of Product Innovation Management* (in press).
- Borgh, W. van der, Cloudt, M.M.A.H., Romme, A.G.L., 2012. Value creation by knowledge-based ecosystems: evidence from a field study. *R&D Management* 42 (2): 150-169.
- Borgh, W. van der, 2012. Selling new products. Eindhoven: Technische Universiteit Eindhoven. ((Co-) promoters: Nijssen, E.J. and Jong, A. de). Doctoral dissertation.
- Nijssen, E.J., van Trijp, H., 1998. Branding fresh food products: Exploratory empirical evidence from the Netherlands. *Eur. Rev. Agric. Econ.* 25 (2): 228-242.
- Nijssen, E.J., Hillebrand, B., de Jong, J.P.J., Kemp, R.G.M., 2012. Strategic Value Assessment and Explorative Learning Opportunities with Customers. *Journal of Product Innovation Management* 29 (S1): 91-102.





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**Partner 11. Aarhus Universitet (AU), Denmark**

**Description of Organization:** The Centre of Research on Customer Relationships in the Food Sector (MAPP) (<http://badm.au.dk/AU/>) belongs to the Department of Business Administration, School of Business and Social Sciences, AU, and is regarded as a world-class centre of excellence on consumer science in the food area.

**Main Tasks:** The AU contributes in all WPs with focus on socio-economic aspects. More specifically, AU's main task is to lead WP29 on 'Consumer value perceptions and behavioral change'. In WP29 AU will be the main responsible for the analysis and understanding of value perceptions of consumers with regard to cultured fish in general, and the developed fish species in particular, for the evaluation of consumer sensory perceptions towards the new products developed, for the optimization of the new products developed in terms of ideal extrinsic product attribute combinations, and for the determination of the effectiveness of market communication in consumer behaviour change in relation to the fish species considered and the new added value products (raw products included) developed. Moreover, AU will be responsible for Activity 28.1 on 'Product concept development (technical and consumer-driven)' in WP28, according to which AU will run a qualitative research with consumers and experts to generate input for new product development and will screen new product development ideas on their ability to attract customer demand. Finally, AU will contribute to all other Activities of WPs 27, 28 and 30.

**Previous Experience:** Since 1991, AU has conducted research on marketing-related problems in the food sector. Major areas of research have been consumer behaviour with regard to the purchase of food, decision-making of retailers, cooperation among suppliers and between suppliers and retailers in food value chain and the management of market-oriented product development in food companies. The AU has a long track record of participating in EU FP programmes (e.g., RECAPT, SEAFOODPLUS, QPORKCHAINS, PROSAFEFEF, EURECCA, CROSSENZ, SENIORFOODQOL, SUSPORQUAL, CONDOR), as well as numerous collaborative projects with industry.

**Staff profile:** **Dr. Klaus G. Grunert** is Professor of Food Marketing at the Department of Business Administration, AU, and the founder and director of the MAPP Centre. As director of MAPP, he has carried out more than 75 collaboration projects with the food industry, including many EU FP projects.

**Relevant publications**

- Grunert, K.G., Lähteenmäki, L., Boztug, Y., Martinsdottir, E., Ueland, O., Åström, A., Lampila, P., 2009. Perception of health claims among Nordic consumers. *J Cons Pol.* 32: 269-287.
- Grunert, K.G., Wills, J.M., 2007. A review of European research on consumer response to nutrition information on food labels. *J Pub Health* 15: 385-399.
- Krystallis, A., de Barcellos, M.D., Grunert, K.G., Perrea, T., Verbeke, W., 2012. Consumer attitudes towards sustainability aspects of food production: insights from three continents. *J. Mark. Manag.* 28: 334-372.
- Krystallis, A., Vassallo, M., Chrysochoidis, G., 2012. The usefulness of Schwartz's 'Values Theory' in understanding consumer behaviour towards differentiated products. *J. Mark. Manag.* 28: 1438-1463.
- Krystallis, A., Chrysochou, P., 2012. Do prior knowledge about and type of functionality influence consumer perceptions about unhealthy foods? The Case of Functional Children Snacks. *Agribus* 28: 86-102.
- Krystallis, A., Chrysochou, P., 2011. Health claims as communication tools that enhance brand loyalty: The case of low-fat claims within the dairy food category. *J. Mark. Comm.* 17: 213-228.



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**Partner 12. Asociación Empresarial de Productores de Cultivos Marinos (APROMAR), Spain**

**Description of Organization:** APROMAR is Spain's Marine Aquaculture Producers Association. Its members include almost all Spanish marine fish farmers, plus shellfish hatcheries, algae producers and fish feed manufacturers. APROMAR is recognized by the EU as a Producer's Organization (OP-30).

**Main Tasks:** This Partner's tasks include mainly work in GWP Socioeconomics and in WP31 Dissemination. Specifically, APROMAR will participate in WP27 in the tasks External competitive analysis, Internal competitive analysis and Opportunities for growth; in WP30 in the tasks Business model innovation, New product marketing strategy development and Recommendations regarding industry development. In WP31 Dissemination it will participate in the organization of workshops, in obtaining the involvement of the European aquaculture industry, in approaching and working with the food industry and consumers federations, and in the production and revision of documents and actions.

**Previous Experience:** APROMAR has participated and coordinated many National projects, mainly in the fields of aquaculture's sustainability, worker's safety and life-long training courses for aquaculture farms personnel. Scientific projects have been developed in the design of epidemiological maps, on the absence of *Anisakis* spp. in aquaculture fish and on the fabrication of biomass counters for offshore cages.

**Staff profile:** **Mr Javier Ojeda** (Scientific Responsible) has an MSc in Marine Biology and is the General manager of APROMAR. He has wide experience in marine fish farming, including both hatchery and on-growing production of gilthead sea bream, European sea bass, Senegalese sole and Atlantic salmon. He is also active in supporting sustainable aquaculture, product certification, environmental impacts, product quality and works to improve the legislative framework of the aquaculture industry in Spain and the EU. **Mr Juan M. Fernández** (Biologist; technical advisor) has a long professional background in fish farming and in fish feed production. He has managed important aquaculture firms and has worked in developing production techniques for mass production of new species such as meagre. **Mr Rafael Gómez** (Shipping Engineer; Administrative officer) is responsible for administration and office activities in APROMAR.

**Relevant publications**

APROMAR publishes every year an Annual Report that explains the situation of marine aquaculture in the world, in Europe and particularly in Spain. It includes detailed statistics and analysis of the situation of the industry. Other publications include guidelines for sustainable aquaculture, consumer information and technical manuals.





### **Partner 13. Università degli Studi di Bari "Aldo Moro" (UNIBA), Italy**

**Description of Organization:** The University of Bari Aldo Moro, founded in 1924, includes 24 Departments and is one of the most important Italian Universities. The Section of Veterinary Clinics and Animal Production of the Department of Emergency and Organs Transplant (D.E.T.O.), as well as the Department of Bioscience, Biotechnology and Biopharmaceutical will be involved in the present proposal. UNIBA has well-equipped laboratories to carry out histological, biochemical and molecular biology analyses. In particular, the laboratory of histology is equipped with stoves for paraffin wax embedding, cryostats, microtomes, light microscopes, scanning electron microscope (SEM), image analyzer for morphometric analyses and ELISA Microplate Reader. Moreover, this laboratory is equipped with a IsoMet® Low Speed Saw for spines and vertebrae cutting and stereo microscopes for fish age determination. The molecular biology laboratory has equipment for molecular biology studies, including thermocycler, real-time PCR, NanoDrop spectrophotometer.

**Main Tasks:** The UNIBA will work on the reproduction of greater amberjack and grey mullet. As regards the greater amberjack, UNIBA will be involved in the following tasks: (1) assist in the capture of live wild fish and transportation to Ittica Caldoli farm, (2) sampling of wild and captive-reared specimens, (3) shipment of biological samples to all the Partners involved in GWP Reproduction and Genetics, (4) description of the reproductive cycle of wild and captive-reared individuals, (5) determination of fish size and age at first sexual maturity, (6) study of male germ cell proliferation and apoptosis in both wild and captive-reared individuals and in different periods of the reproductive cycle, (7) analysis of liver vitellogenin gene expression in both wild and captive-reared specimens and in different periods of the reproductive cycle, (8) analysis of oocyte vitellogenin receptor gene expression in both wild and captive-reared fish and in different periods of the reproductive cycle. In grey mullet, UNIBA will be involved in the effect of dietary components in roe (bottarga) quality, looking at oocyte yolk accumulation and quantity (ovary mass).

**Previous Experience:** The research activity of UNIBA is mainly focused on: (1) fish reproductive cycle, (2) fish gametogenesis in wild and captive condition, (3) fish size and age at first maturity, (4) effect of stress on oocyte atresia, (5) male germ cell proliferation and apoptosis in wild and captive-reared fish, (6) expression of genes involved in fish reproductive process, such as vitellogenin and vitellogenin receptors, (7) study of Atlantic bluefin tuna trophic and reproductive migration by conventional and electronic tagging. In the last years UNIBA participated or led many national and EU projects, including: EU project n. 97/029 "Major improvements in our knowledge of eastern Atlantic bluefin tuna in the Mediterranean", SIDS (FP5), REPRODOTT (FP5), SELFDOTT (FP7), ALLOTUNA (Apulia Region, Italy).

**Staff profile:** **Dr Aldo Corriero** (Scientific Responsible) has a long experience on fish gonad histology, gametogenesis, germ cell proliferation and apoptosis. **Prof. C. De Giorgi** is a molecular biologist and leads the molecular biology work on Atlantic bluefin tuna vitellogenin and vitellogenin receptors in the SELFDOTT project. The scientific team will include also **Prof. L. Passantino** (histology and biochemical analyses), **Dr N. Santamaria** (sampling, immunohistochemistry and age determination), **Dr C. Pousis** (sampling, biomolecular analysis), **Dr R. Zupa** (sampling, histology, immunohistochemistry and morphometry) and **Dr M. Deflorio** (sampling, statistical data analysis). **Dr G. Centoducati**, expert of animal science, will be involved in grey mullet bottarga studies.

#### **Relevant publications**

- Corriero, A., Zupa, R., Bello, G., Mylonas, C.C., Santamaria, C.A., Deflorio, M., Genovese, S., Basilone, G., Buscaino, G., Buffa, G., Pousis, C., De Metrio, G., 2011. Evidence that severe acute stress and starvation induce rapid atresia of ovarian vitellogenic follicles in Atlantic bluefin tuna, *Thunnus thynnus* (Osteichthyes: Scombridae). *J. Fish Dis.* 34: 853-860.
- Pousis, C., Santamaria, N., Zupa, R., De Giorgi, C., Mylonas, C.C., de la Gandara, F., Vassallo-Agius, R., Bello, G., Corriero, A., 2012. Expression of vitellogenin receptor gene in the ovary of wild and captive Atlantic bluefin tuna (*Thunnus thynnus* L.). *Anim. Reprod. Sci.* 132: 101– 110.
- Zupa, R., Fauvel, C., Mylonas, C.C., Santamaria, N., Valentini, L., Pousis, C., Papadaki, M., Suquet, M., de la Gandara, F., Bello, G., De Metrio, G., Corriero, A., 2013. Comparative analysis of male germ cell proliferation and apoptosis in wild and captive Atlantic bluefin tuna *Thunnus thynnus*. *J. Appl. Ichthyol.* 29: 71-81 (doi 10.1111/j.1439-0426.2012.02045.x).



## **Partner 14. Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), France**

**Description of Organization:** The IFREMER is an industrial and commercial public body entirely devoted to marine research and the exploitation of marine resources, operating under the auspices of ministries of Education, Research and Agriculture. It will participate in the project with its department of Biology of Exploited Marine Organisms (BOME) operating at the Experimental Aquaculture Station of Palavas (Montpellier). This station is a leading R&D laboratory in the field of Aquaculture, focusing its research actions to Sustainability and Systems, Reproduction and Genetics. It has been recognized as a large research European Infrastructure (ASEFAF, FP5, AQUAEXCEL, FP7) and operates accurately controlled and well-monitored facilities for experimental rearing of larvae, juveniles and broodstocks. The station is also equipped with analytical laboratories for water analyses, proteomics and image analysis, and it is linked to Montpellier University genopole and research platforms through combined research units (UMR).

**Main Tasks:** The IFREMER will contribute to the field of sperm characterization for different species following a standardized method. This will involve (1) setting the procedure on samples in the different broodstock rearing sites, (2) transferring the procedure to field researchers/Partners and finally stakeholders, and (3) compiling/storing/analyzing the whole data set on sperm characteristics in the species examined in DIVERSIFY. The final aim is different among the species: in meagre (WP2, task 2.3) and wreckfish (WP6 task 6.4) the objective is to prepare and to define protocols for artificial fertilization and cryopreservation; in greater amberjack (WP3 task 3.1 and 3.3) and mullet (WP7 task 7.1) the objective is to accurately define the best period for reproduction and to assess the effect of heterologous hormonal stimulation.

**Previous Experience:** The IFREMER has developed a strong expertise in controlled reproduction in shellfish as well as in finfish aquaculture for more than 20 years. In order to facilitate quantitative genetic programs, the team focused on the setup of artificial fertilization and, as a prerequisite, on the determinism of gamete quality. The reproduction team fruitfully collaborated with French as well as European expert teams in fish physiology and fish aquaculture such as HCMR (Greece), IEO (Spain) and INRA (France) in bilateral, national and European projects including REPRODOTT, HERITABOLUM (FP5), COMPETUS and GRRAS (FP6), SELFDOTT, AQUAEXCEL and REPROSEED (FP7).

**Staff profile:** **Dr C. Fauvel** (Scientific Responsible) is a reproductive physiologist and currently studies egg intrinsic quality (genome and proteome inheritance in eggs) and works simultaneously on sperm quality in relation to its physiology and on the development of conservation protocols (chilled and cryopreservation). He is the leader in the Working Group 1 (gamete quality assessment) of the COST action AQUAGAMETE (2012-2017), aimed at improving and standardizing the methods used for the evaluation of aquatic gametes. **Dr M. Suquet** (reproductive physiology) has a strong expertise in sperm motility and energetics by previous collaborations with Prof. R. Billard and Dr J. Cosson, both world-class specialists of fish spermatology. **Mr G. Dutto** has 20 years of experience as a husbandry technician, specialized in broodstock management and reproduction process control both in temperate and tropical fish species.

### **Relevant publications**

- Crespel, A., Rime, H., Fraboulet, E., Bobe, J., Fauvel, C., 2008. Egg quality in domesticated and wild seabass (*Dicentrarchus labrax*): A proteomic analysis. *Cybium, Suppl. 8th ISRPF* 32, 2: 205.
- Fauvel, C., Suquet, M., Cosson, J., 2010. Evaluation of fish sperm quality. *J. App. Ichthyol.* 26: 636-643.
- Fauvel, C., Boryshpolets, S., Cosson, J., Wilson Leedy, J.G., Labbé, C., Haffray, P., Suquet, M., 2012. Improvement of seabass sperm chilled conservation by the use of a cell culture medium. *J. App. Ichthyol.* 28: 961-968.
- Zupa, R., Fauvel, C., Mylonas, C.C., Santamaria, N., Valentini, L., Pousis, C., Papadaki, M., Suquet, M., de la Gándara, F., Bello, G., De Metrio, G., Corriero, A. 2012. Comparative analysis of male germ cell proliferation and apoptosis in wild and captive Atlantic bluefin tuna *Thunnus thynnus*. *J. Appl. Ichthyol.* 29: 71-81 (doi 10.1111/j.1439-0426.2012.02045.x).



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**Partner 15. Universidad de La Laguna (ULL), Spain**

**Description of Organization:** La Laguna University (ULL) (<http://www.ull.es/>), particularly the Faculty of Biology, has a long-term teaching and research experience in Marine Research including Aquaculture. A new Master Degree in “Marine Biology: Biodiversity and Conservation” has been developed and will be offered from 2013/14. The **Nutrition Group**, a small group with a wide experience in Aquaculture and Marine Physiology which has been collaborating for more than 20 years with the Spanish Institute of Oceanography, Oceanographic Centre of the Canary Islands. This cooperation has led to the creation of AQUAFISMAR research group ([http://aquafis.webs.ull.es/index\\_en.htm](http://aquafis.webs.ull.es/index_en.htm)). **Aquaculture Nutrition Group** research activities can be summarized in more than 12 competitive national and regional funded projects, 40 peer-reviewed publications and more than 10 research PhD grants and resultant associated PhD theses in the aquaculture field in the last 10 years. The group facilities include well equipped laboratories with HPLC, GC, GC-MS for detailed analysis of lipids, fatty acids, vitamins and carotenoids, scanning densitometers for lipid class analysis, NIR spectroscopy for food quality control, Kjeldahl equipment, fluorimeters, and all the necessary tools for the analyses of digestive enzymes, ATPase and other enzymatic activities, TBARS, lipid peroxides, hormones and eicosanoids. A cell culture lab with incubators, autoclave, laminar flow hoods and microscopes, a Molecular biology lab equipped with PCR, RT-PCR and electrophoresis equipment for genotyping, and a Metabolism lab with isotope facilities for nutrient metabolic studies are also available. Staff from the Tropical Disease and Public Health Research Institute of La Laguna University <http://www.ull.es/view/institutos/tropicales/Miembros/es> which also participate in the proposal, will be an essential contributor for the actions included in GWP Fish health (fish health), in particular studies focusing on monogenean parasitism control in greater amberjack.

**Main Tasks:** ULL will participate in nutritional and physiology studies of the six selected species drawn up in several actions of GWP Reproduction and genetics, Nutrition and Socioeconomics. The team will be mainly involved in tasks related to (1) evaluation of underlying nutritional status of captive and wild broodstock of greater amberjack, wreckfish and grey mullet, (2) effects of dietary composition on digestive physiology and ATPase activities in greater amberjack, meagre and Atlantic halibut larvae, (3) determination of oxidative stress enzymes regarding stocking density at different sizes of greater amberjack juveniles, (4) assessment on the nutritional value of lipids and carotenoids in enrichment products for greater amberjack larvae and broodstock diets, (5) characterization of pikeperch essential fatty acid metabolism in relation to dietary composition and stress tolerance to salinity rearing conditions, (6) evaluation of the osmoregulatory epithelia integrity of greater amberjack juveniles affected by monogenean parasites infection and (7) evaluation of final product nutritional quality of meagre and grey mullet.

**Previous Experience:** The nutrition group has extensive experience on the determination of the nutritional requirements (mainly lipid and fatty acids) of finfish with particular emphasis to physiological and metabolic aspects related to nutrition (reproductive success, larval survival and development, growth potential of juveniles, nutrients and electrolytes gut uptake, digestive enzymes and ATPases activities), and also on lipid metabolism studies by using C<sup>14</sup>-radiolabelled fatty acids in isolated cells and larvae. The expertise of ULL includes studies in a wide variety of marine fish species (*Sparus aurata*, *Dicentrarchus labrax*, *Diplodus sargus*, *Pagrus pagrus*, *Seriola dumerili*) and cephalopods (*Octopus vulgaris*, *Sepia officinalis*). Other recent investigations have assessed the effect of global change (temperature and salinity) in fish lipid metabolism, the use of alternative lipid sources to fish oil in aquafeeds and the role of carotenoids and retinoids in fish physiology and pigmentation. The Aquaculture Nutrition Group of ULL has also been involved in several granted projects funded by the Spanish Government jointly with the regional aquaculture industry, providing scientific advisory to fish producers chiefly in terms of nutritional evaluation of feeds and product quality. Our current research mainly deals with the effect of dietary fatty acid and carotenoid profiles on greater amberjack reproductive performance. The Department of Parasitology, ecology and Genetics of La Laguna University, has focused on parasitology of vertebrates primarily helminth fauna of wild and domestic vertebrates (faunistic, taxonomy, systematic, epidemiology, ecology), phylogeny and genotyping of parasites: helminthes and protozoa.



**Staff profile:** **Dr C. Rodríguez** (Scientific Responsible), is a senior lecturer with 20 years research and teaching experience in aquaculture with main emphasis on fish and cephalopod lipid nutrition and fatty acid metabolism. More novel activities within the group under her responsibility are the studies of uptake, esterification and modification of radiolabelled fatty acids by hepatocytes, enterocytes and gill cells isolated from marine fish species of aquaculture industry; application of biotechnology to the search of useful biomarkers for the evaluation of global change impact in fish and partial substitution of fish oils with vegetable oils in farmed fish feeds. **Prof. A. Lorenzo**, head of the Aquaculture Nutrition Group, and **Dr A. Bolaños**, have more than 30 years of teaching and research experience on animal physiology, specialized for the last 20 years in the physiology aspects of fish nutrition at different life stages (larvae, juveniles, broodstock). **Mrs. N.G. Acosta**, senior technician and PhD student, with over 15 years experience in biochemical analyses methodology (including HPLC, GC, GC/MS), is responsible for the laboratory facilities. **Mrs. D. Rodríguez-Barreto**, a PhD fellow, whose thesis research has focused on assessing the effect of dietary fatty acid profiles on greater amberjack broodstocks, and **Dr. P. Foronda**, senior lecture and member of the ULL Tropical Disease and Public Health Research institute, with expertise in parasitology and helminthic fauna in vertebrates

### Relevant related publications:

- Díaz-López, M., Pérez, M.J., Acosta, N.G., Jerez, S., Dorta-Guerra, R., Tocher, D.R., Lorenzo, A., Rodríguez, C., 2010. Effects of dietary fish oil substitution by *Echium* oil on enterocyte and hepatocyte lipid metabolism of gilthead seabream (*Sparus aurata* L.). *Comparative Biochemistry and Physiology B* 155: 371-379.
- Feliu, C., López, M., Gómez, M.S., Torres, J., Sánchez, S., Miquel, J., Abreu-Acosta, N., Segovia, J.M., Martín-Alonso, A., Montoliu, I., Villa, M., Fernández-Álvarez, A., Bakhoun, A., Valladares, B., Orós, J. and Foronda, P., 2012. Parasite fauna of rodents (Murinae) from El Hierro (Canary Islands, Spain): a multidisciplinary approach. *Acta Parasitologica* 57 (2): 171-178.
- Fernandez, A., Exposito, Y., Valladares B., Castillo, A., Foronda, P. Microbiological and parasitological analyses of fish from Tenerife, Canary Islands. *Journal of Food* (To be submitted).
- Fonseca-Madriral, J., Pineda-Delgado, D., Martínez-Palacios, C., Rodríguez, C., Tocher, D.R., 2012. Effect of salinity on the biosynthesis of n-3 long-chain polyunsaturated fatty acids in silverside *Chirostoma estor*. *Fish Physiology and Biochemistry* 38(4): 1047-1057.
- Jerez, S., Rodríguez, C., Cejas, J.R., Martín, V., Bolaños, A., Lorenzo, A., 2012. Influence of age of female gilthead seabream (*Sparus aurata* L.) broodstock on spawning quality throughout the reproductive season. *Aquaculture* 350-353: 54-62.
- Martín, M.V., Almansa, E., Cejas, J.R., Bolaños, A., Jerez, S., Lorenzo, A., 2011. Effects of a diet lacking HUFA on lipid and fatty acid content of intestine and gills of male gilthead seabream (*Sparus aurata* L.) broodstock at different stages of the reproductive cycle. *Fish Physiology and Biochemistry* 37(4): 935-949.
- Rodríguez, C., Acosta, C., Badía, P., Cejas, J.R., Santamaría, F.J., Lorenzo, A., 2004. Assessment of lipid and essential fatty acids requirements of black seabream (*Spondilyosoma cantharus*) by comparison of lipid composition in muscle and liver of wild and captive adult fish. *Comparative Biochemistry and Physiology B* 139: 619-629.
- Rodríguez-Barreto, D., Consuegra, S., Jerez, S., Cejas, J.R., Martín, V., Lorenzo, A., 2013. Using molecular markers for pedigree reconstruction of the greater amberjack (*Seriola dumerili*) in the absence of parental information. *Animal Genetics* DOI: 10.1111/age.12039.
- Rodríguez-Barreto, D., Jerez, S., Cejas, J.R., Martín, M.V., Acosta, N.G., Bolaños, A., Lorenzo, A., 2012. Comparative study of lipid and fatty acid composition in different tissues of wild and cultured female broodstock of greater amberjack (*Seriola dumerili*). *Aquaculture* 360-361: 1-9.
- Tejera, N., Cejas, J.R., Rodríguez, C., Jerez, S., Pérez, J.A., Felipe, B., Lorenzo, A., 2010. Pigmentation, carotenoids, lipid peroxides and lipid composition of red porgy (*Pagrus pagrus*) skin reared under open-cage conditions. *Aquaculture Research* 41 (7): 1043-1053.





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**Partner 16. Facultes Universitaire Notre-Dame de la Paix de Namur (FUNDP), Belgium**

**Description of Organization:** The FUNDP is a non-profit organization funded by the Wallonia-Brussels Federation of Belgium. It will participate to this project with the Research Unit in Environmental and Evolutionary Biology (URBE), including a staff of 35 persons (4 academics, 6 senior researchers, 20 junior scientists, mainly PhD students and 5 technicians). URBE is dealing with different aspects of aquatic biology including aquaculture of freshwater species. Aside classical RAS facilities for fish reproduction, nutrition and immunology (class II rooms for pathogens) experiments, URBE has at its disposal several biotechnological platforms for endocrinology, biochemistry and physiology analyses, *in vitro* experimentation, transcriptomics and proteomics equipment, flow cytometry, immunohistochemistry autostaining, ABI sequencer 16 capillaries and pyro-sequencing.

**Main Tasks:** The FUNDP will be involved in WP10 Nutrition-pikeperch and in WP22 Grow out husbandry-pikeperch. In GWP Nutrition, research activities will be focused mainly on Task 10.1 (improvement of larval nutrition of pikeperch), including enzymatic analyses, histology of target tissues and *in situ* hybridization, transcriptomic analyses of target genes expression, proteomics on specific organs (principally liver) in selected experiments and resistance of larvae/juveniles to bacterial and stress challenge tests. In WP22 Grow out husbandry, research activities will investigate 1) the effects of husbandry practices and environmental factors on pikeperch growth, immune and physiological status under experimental and farm conditions and 2) the effects of fish domestication level and geographical origin on growth and stress sensitivity.

**Previous Experience:** In direct relation to this project, FUNDP has got a long expertise in the physiology and aquaculture of percid fish species, including (1) the larval development (digestive enzyme activities, digestive tract histology, gene and protein expression profiles, biochemical composition) according to dietary variables, (2) the identification of environmental and nutritional variables affecting the growth performances and immune status of juveniles, with a special emphasis on stress response. In the past 10 years, FUNDP has been the recipient and/or coordinator of many research grants, the EC-funded projects FISH COMPETITION (FP 4), LUCIOPERCA (FP5), PERCATECH, LUCIOPERCIMPROVE (FP6). FUNDP is also involved in a large 5-year collaborative project including 5 Belgian Universities and dealing with multiple stress in aquaculture (Aquastress).

**Staff profile:** **Prof. Dr P. Kestemont** (Scientific Responsible) is a fish physiologist involved in the reproduction, nutrition and immunology of freshwater fish species (mainly percid fishes). **Dr S.N.M. Mandiki** (WP22) is a fish endocrinologist and immunologist. **Dr J. Douxfils** (WP22) is a fish physiologist involved in stress-immune system interactions. **Dr F. Geay** (WP10) is a fish nutritionist (lipid metabolism).

**Relevant publications**

- Bich Hang, B.T., Milla, S., Gillardin, V., Phuong, N.T., Kestemont, P., 2013. In vivo effects of *Escherichia coli* lipopolysaccharide on regulation of immune response and protein expression in striped catfish (*Pangasianodon hypophthalmus*). *Fish and Shellfish Immunology* 34: 339-347.
- Douxfils, J., Deprez, M., Mandiki, S.N.M., Milla, S., Henrotte, E., Mathieu, C., Silvestre, F., Vandecan M., Rougeot, C., Mélard, C., Dieu, M., Raes, M., Kestemont, P., 2013. Physiological and proteomic responses to single and repeated hypoxia in juvenile Eurasian perch under domestication - Clues to physiological acclimation and humoral immune modulations. *Fish and Shellfish Immunology* 33: 1112-1122.
- Douxfils, J, Mandiki, S.N.M., Marotte, G., Silvestre, F., Henrotte, E., Milla, S., Vandecan, M., Rougeot, C., Mélard, C., Kestemont P., 2011. Does domestication process affect stress response in juvenile Eurasian perch *Perca fluviatilis*? *Comparative Biochemistry and Physiology A. Molecular & Integrative Physiology* 159: 92-99.
- Douxfils, J., Mathieu, C., Mandiki, S.N.M., Milla, S., Henrotte, E., Wang, N., Vandecan, M., Dieu, M., Dauchot, N., Pigneur, L.-M., Li, X., Rougeot, C., Mélard, C., Silvestre, F., van Doninck, K., Raes, M., Kestemont, P., 2011. Physiological and proteomic evidences that domestication process differentially modulates the immune status of juvenile Eurasian perch (*Perca fluviatilis*) under chronic confinement stress. *Fish and Shellfish Immunology* 31: 1113-1121.



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- Hamza, N., Kestemont, P., Khemis, I., Mhetli, M., Cahu, C., 2012. Effect of different sources and levels of dietary phospholipids on performances and fatty acid composition of pikeperch (*Sander lucioperca*) larvae. *Aquaculture Nutrition* 18: 249-257.
- Hamza, N., Methli, M., Ben Khemis, I., Cahu, C., Kestemont, P., 2008. Effects of dietary phospholipids levels on performance, enzyme activities and fatty acid composition of pikeperch *Sander lucioperca* larvae. *Aquaculture* 275: 274-282.
- Hamza, N., Silvestre, F., Mhetli, M., Ben Kemis, I., Dieu, M., Raes, M., Cahu, C., Kestemont, P., 2010. Differential protein expression profile in the liver of pikeperch (*Sander lucioperca*) larvae fed with increasing levels of phospholipid. *Comparative Biochemistry and Physiology, Part D, Genomics and Proteomics* 5: 130-137.
- Milla, S., Mandiki, S.N.M., Hubermont, P., Rougeot, C. Mélard, C., Kestemont P., 2009. Ovarian steroidogenesis inhibition by constant photothermal conditions is caused by a lack of gonadotropin stimulation in Eurasian perch. *General and Comparative Endocrinology* 163: 242-250.
- Wang, N., Xu, X., Kestemont, P., 2009. Effect of temperature and feeding frequency on growth performances, feed efficiency and body composition of pikeperch juveniles (*Sander lucioperca*) *Aquaculture* 289: 70-73.



### **Partner 17. National Institute of Nutrition and Seafood Research (NIFES), Norway**

***This Partner has been taken over universally by P7. IMR, who is from now on responsible for the implementation of the work originally assigned to NIFES, and has also received its remaining budget.***

**Description of Organization:** NIFES is a governmental research institution that has responsibility to develop knowledge and give advice on fish nutrition and risk/benefits of seafood in human nutrition. The institute has a well-developed laboratory infrastructure, with analyses of all nutrients and many contaminants and is a National Reference Laboratory within nutrient analysis. We also have labs for biochemistry, cell biology, microbiology, histology and molecular biology, including genomics and proteomics. The institute will participate in the project with the Department of Embryo and Larva within the Research Program for Fish Nutrition.

**Main Tasks:** NIFES will participate in the project mainly within WP11 Nutrition Atlantic halibut (1) comparing nutrient retention in Atlantic halibut larvae reared in recycling aquaculture system (RAS) vs flow-through system (FTS) and (2) examining the effects of *Artemia* type (nauplii or on-grown *Artemia*) on the nutritional status and juvenile quality of Atlantic halibut larvae and post larvae. NIFES will also participate in meagre mineral nutrition (WP8 Nutrition meagre), with minor contributions.

**Previous Experience:** The expertise of NIFES includes Nutrition in broodstock, larvae and juveniles of marine fish and specifically effects of nutrition on juvenile quality in Atlantic halibut. NIFES has been an active partner in EU funded projects since the 5th EU frame program including RAFOA, FORM and AQUAMAX, having coordinated the two latter.

**Staff profile:** **Dr K. Hamre** (Scientific Responsible) is a senior scientist at NIFES and has achieved qualifications equivalent to professor in Fish Nutrition in 2001 and in Developmental Biology in 2006. Her research includes nutrition in broodstock, larvae and juveniles of marine fish and red-ox balance in fish in general. **Dr Øystein Sæle** holds a PhD in biology, with focus on eye migration in Atlantic halibut. His main research since 2008 has been on lipid nutrition in Atlantic cod and Ballan wrasse, larvae and juveniles. **Dr Mari Moren** is head of research in the department of Embryo and Larvae. She holds a PhD on vitamin A requirement and metabolism in Atlantic halibut and received a grant from The Research Council of Norway for excellent young scientists in 2008-2011 on her research in Retinoic acid and arachidonic acid interactions in Atlantic cod larvae.

#### **Relevant publications**

- Hamre, K., Yufera, M., Ronnestad, I., Boglione, C., Conceição, L., Izquierdo, M. (In press). Fish larval nutrition and feed formulation - knowledge gaps and bottlenecks for advances in larval rearing. Reviews in Aquaculture.
- Hamre, K., Moren, M., Solbakken, J., Opstad, I., Pittmann, K., 2005. The impact of nutrition on metamorphosis in Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture 250: 555-565.
- Lie, K.K., Moren, M., 2012. Retinoic acid induces two osteocalcin isoforms and inhibits markers of osteoclast activity in Atlantic cod (*Gadus morhua*) ex vivo cultured craniofacial tissues. Comp. Biochem. Physiol. A 161: 174-184.
- Sæle, Ø., Nordgreen, A., Olsvik, P.A., Hamre, K., 2011. Characterization and expression of secretory phospholipase A2 group IB, during ontogeny of Atlantic cod (*Gadus morhua*). Brit. J. Nutr. 105: 228-237.



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**Partner 18. Fundacion Centro Tecnologico de Andalusia (CTAQUA), Spain**

**Description of Organization:** CTAQUA is a newly established non-profit, private foundation, which aims at developing applied research, technology, innovation, sustainability and training within the aquaculture sector, thus improving the competitiveness of Andalusian aquaculture companies. CTAQUA gives support to aquaculture companies and creates a platform for collaboration between the research community and the private sector. This general objective is achieved by (1) providing services to the aquaculture community focused on the development of R&D projects, (2) applying new technologies in aquaculture companies, (3) transfer of knowledge from the academic sector to the industry and (4) promoting the application of the new markets standards in the actual aquaculture practices in the farms. Several farms, feed companies and equipment suppliers are members of CTAQUA. The organization has large experience in publicizing its activities and projects results within the aquaculture sector via the periodic meetings with its members, weekly updates in its website ([www.ctaqua.es](http://www.ctaqua.es)), organizing seminars and workshops and frequent participation in the media.

The Foundation also operates an Experimental Centre equipped with several recirculation systems to carry out lab and pilot trials in applied nutrition. Among its activities CTAQUA can provide technical assistance, design protocol development and on provide spot follow up of farm trials.

**Main Tasks:** CTAQUA is the **WP31 Dissemination** leader and will be involved in the following tasks: (1) optimization of grow out trials and evaluation of finishing diets in grey mullet, (2) screening of ideas and development of new concept for new fish product development (NPD), (3) elaboration and production of new fish products from meagre, greater amberjack, grey mullet and pikeperch, (4) monitoring and evaluation of the technical quality of the new products, (5) perform a sensory descriptive analysis, (6) evaluation of consumer value perceptions and behavioural changes, (7) consumer sensory perceptions, (8) development of tools to increase communication effectiveness in the fish product markets, (9) development of business models and marketing strategies for the aquaculture production companies, (10) elaboration of recommendations concerning industry development and market expansion and (11) coordination of the dissemination activities of the project.

**Previous Experience:** The expertise of CTAQUA is based on different projects developed in the area of aquaculture product marketing and market studies, as well as in its R&D experience within applied aquaculture studies. Among others, CTAQUA has developed an innovative project concerning the marketing of aquaculture products: “Alternatives for processing, packaging and presentation of the aquaculture products: Market study and evaluation”. This project has involved producers, retailers and supermarkets. CTAQUA has carried out also the project “Consumer perception of aquaculture products”, focused on the national market. Together with the Spanish producers association (APROMAR) CTAQUA developed the project “Market study to promote the consumption of meagre (*Argyrosomus regius*) in Spain”. The Experimental Center of CTAQUA has conducted rearing trials under strictly defined and controlled environmental conditions both in fresh and seawater species. One of the main lines of research is the development of diets with very low inclusion of fishmeal as protein source for fish feeds, focused mainly on the two species mostly produced in the Mediterranean (European seabass, *Dicentrarchus labrax* and Gilthead seabream, *Sparus aurata*). The members of CTAQUA provide an industrial field to complete the pilot trials developed in the Experimental Center and evaluate the impact of the research in real production conditions.

**Staff profile:** **Mrs Rocio Robles** (Scientific Responsible) is the Technical Director of CTAQUA. She is the **Leader of WP31 Dissemination** and has great experience in applied research in aquaculture, mainly in the field of applied nutrition, early growth and grow-out of aquatic species and applied microbiology in aquaculture. She has participated in EU funded projects such as AQUALITY and AQUAMAX. **Mr Juan Manuel García de Lomas** (Manager), has founded the Andalusian Association of Aquaculture Producers and is a well-known professional in the aquaculture sector, with broad expertise in marketing and aquaculture business promotion. **Mrs Maria Avivar Valderas** is an economist and market analyst, specialized in marketing techniques and marketing research and has participated in national projects focused on fish market studies and sales analyses.





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### **Relevant publications**

Márquez, L. Robles, R., Morales, G.A., Moyano, F.J., 2012. Gut pH as a limiting factor for digestive proteolysis in cultured juveniles of the gilthead sea bream (*Sparus aurata*). *Fish Physiology and Biochemistry* 38 (3): 859-869.

Robles, R., Lozano, A.B., Sevilla, A., Márquez, L., Nuez-Ortín, W., Moyano, F.J., 2013. Effect of partially covered butyrate on growth and intestinal metabolism in sea bream (*Sparus aurata*). *Fish Physiology and Biochemistry*. DOI 10.1007/s10695-013-9809-3



### **Partner 19. Consellería do Medo Rural e do Mar - Xunta de Galicia (CMRM), Spain**

**Description of Organization:** The CMRM has, amongst its competences, the promotion of applied marine research and the technological dissemination regarding its sectors and intervention fields. It will participate with its Marine Research Centre (**CIMA**) and Galician Institute of Aquaculture Training (**IGafa**). The basic function of CIMA is to develop research activities directed to a rational management of marine resources in the areas of competence of Galician Fisheries Administration. Its scientific activity is developed in Aquaculture, Marine Resources, Pathology and Coastal Oceanographic Process areas. The IGafa is an Institute for Aquaculture Training placed on the Island of Arousa (Pontevedra), the area where there is the highest concentration of marine culture in Galicia. Its specific goal is to respond to the demand for basic and intermediate staff for companies in the aquaculture sector. CIMA and IGafa work very closely, with the main objectives of developing and improving the culture process and systems of species with a bigger commercial interest. IGafa has land-based facilities to undertake research in reproduction, larval rearing, nutrition, feeding behaviour and CIMA has well-equipped laboratories to do nutrition studies and also has some sea cages to culture fish.

**Main Tasks:** This Partner's tasks include (1) broodstock maintenance, study reproductive function and develop spawning induction methods of wreckfish, (2) development of larval culture conditions, (3) larval quality evaluation and feeding protocols in two culture systems (RAS and flow-through) in wreckfish, (4) definition of nutritional requirements and design of adequate feeding regimes for broodstock to optimize reproduction success, (5) study of the effect of enrichment nutritional quality on larval quality in wreckfish.

**Previous Experience:** The expertise of CIMA and IGafa includes the larval rearing of many marine fishes like turbot, sea bream, blackspot sea bream, common sole, senegalese sole, sea bass and pollack using different systems (RAS and flow-through), grow out of marine fishes in land-based and sea cages, studies of larval and on-growing nutrition of different marine species. In the past 10 years, the CMRM has been the recipient and/or coordinator of many Regional (5 Research projects) and National projects funded by National Advisory Board for Mariculture, JACUMAR (10 Research projects) and has also participated in some EU projects (IFD97-1695, FEDER, POCTEC, Iberomare 2007-2013) related with the domestication of marine fishes and species diversification. In the last years, CIMA and IGafa in collaboration with other centres (IEO, CIIMAR of Porto, University of Santiago de Compostela) have been involved in the development of sustainable diets for *Solea senegalensis* replacing fishmeal by plant-protein sources with the aim to contribute to achieve a sustainable aquaculture economically and environmentally. Furthermore, in the last 3 years CIMA and IGafa have been working on wreckfish on-growing and the first results were exposed at different international congresses.

**Staff profile:** **Mrs Fátima Linares** (Scientific Responsible) is a researcher attached to CIMA and she works in the field of aquaculture mainly in the area of farming new species of marine fish. For several years now, her work has focused primarily in the field of nutrition at different stages of development of fish in intensive farming as well as an octopus and she is the responsible of these programmes at the CIMA. During the period 2005-2009, she was General Director of Innovation and Development at the Regional Department and Fisheries and Maritime Affairs. **Mr Jose Luis Rodriguez** is teacher in the IGafa and he collaborates in research projects with other institutions and he is a specialist in fish culture in all phases of development of different species of fish, marine and freshwater. In recent years he has been working in the development of recirculation systems for marine and freshwater aquaculture.

#### **Relevant publications**

- Andrade, C.A.P., Nascimento, F., Conceição, L.E.C., Linares, F., Lacuisse, M., Dinis, M.T., 2012. Red Porgy, *Pagrus pagrus*, Larvae Performance and Nutritional Condition in Response to Different Weaning Regimes. *Journal of the World Aquaculture Society* 43 (3): 321-334.
- García de La Banda, I., Lobo, C., León-Rubio, J.M., Tapia-Paniagua, S., Balebona, M.C., Moriñigo, M.A., Moreno-Ventas, X., Lucas, L.M., Linares, F., Arce, F., Arijo, S., 2010. Influence of two closely related probiotics on juvenile Senegalese sole (*Solea senegalensis*, Kaup 1858) performance and protection against *Photobacterium damsela* subsp. Piscicida. *Aquaculture* 306: 281-288.



- Linares, F., Rodríguez, J.L., Peleteiro, J.B., Alvarez-Blázquez, B., Pazos, G., Otero, M., Valente, L.M.P., 2012. Ongrowing and lipid composition of Senegalese sole *Solea senegalensis* fed with commercial and plant-based diets. European Aquaculture Society 2012.
- Ribeiro, L., Couto, A., Olmedo, M., Álvarez-Blázquez, B., Linares, F., Valente, L., 2008. Digestive Enzyme Activity at Different Developmental Stages of Blackspot Seabream, *Pagellus bogaraveo* (Brunnich 1768). Aquaculture Research 39 (4): 339-346.
- Rodríguez, J.L., Laporta Miguels, M., Vega González, E., Linares, F., 2011. Preongrowing of flatfish in a recirculation aquaculture system in Galicia, Spain. Actas World aquaculture. Aquaculture for a changing world. Natal, Brasil.
- Rodríguez, J.L., Vega, E., Linares, F., 2011. Design and application of a recirculation aquaculture system for marine fish larvae rearing. Actas World aquaculture. Aquaculture for a changing world. Natal, Brasil.
- Rodríguez, J.L., Linares, F., Pazos, G., Soto, N., 2010. Larval culture of senegal sole, *Solea senegalensis*, in closed and open circuit. Aquaculture Europe 2010: Seafarming Tomorrow. European Aquaculture Society Publication, 170.
- Rodríguez Villanueva, J.L., Peleteiro, J.B., Pérez Rial, E., Soares, E.C., Álvarez-Blázquez, B., Mariño, C., Linares, F., Mañanós, E., 2011. Growth of wreckfish (*Polyprion americanus*) in Galicia, Spain. Actas European Aquaculture 2011.
- Valente, L.M.P., Linares, F., Villanueva, J.L.R., Silva, J.M.G., Espe, M., Escórcio, C., Pires, M.A., Saavedra, M.J., Borges, P., Medale, F., Álvarez-Blázquez, B., Peleteiro, J.B., 2011. Dietary protein source or energy levels have no major impact on growth performance, nutrient utilization or flesh fatty acids composition of market-sized Senegalese sole. Aquaculture 318: 128–137.



## **Partner 20. SKRETTING Aquaculture Research Centre (SARC), Norway**

**Description of Organization:** Skretting Aquaculture Research Centre (SARC) is the central R&D unit for global fish feed company Skretting (Nutreco Head Office in Amersfoort, The Netherlands). SARC's main objective is to provide research and technical support regarding fish and shrimp feeds. Thus SARC core competencies are within fish nutrition, feed raw materials including quality control, feed manufacturing processes and feed management. Its facilities in Europe include a research station with land-based tanks, a pilot plant for feed production and a well-equipped modern chemical laboratory. The research station is designed for running trials with salmonids through all life stages with different water qualities (temperature and salinity). The laboratory has methods established for taking most relevant quality analyses of feeds and fish. The pilot plant has 2 extruders and flexibility to run all types of common fish feed. The production capacity is up to 200 kg/hour. All facilities are located in or in the vicinity (station) of Stavanger. For its work, it also uses processing plants, farms and health challenge unit owned by and located in its sister Nutreco companies in many areas of the world. SARC has about 70 employees of which about 50% have degrees corresponding to MSc or PhD levels.

**Main Tasks:** The main task of this Partner is in GWP Nutrition. SARC will formulate and produce the experimental diets for the growing experiments of meagre and amberjack. SARC will take care of the raw material, as well as the feed analysis and quality. This work also includes discussions of trial design and results related to diets.

**Previous Experience:** Skretting ARC has extended experienced as a participant in previous EU-funded projects, where the contribution has been to produce the experimental feeds for the other Partners, running experimental trials in its own facilities and Researcher staff contribution. Among others, SARC has participated in the following projects:

- Researching Alternatives to Fish Oil (RAFOA). EU Framework V.
- New strategies to improve grain legumes in food and feed (Grain Legumes). EU Framework VI.
- Sustainable Aquafeeds to maximize health benefits of farmed fish to consumers (AQUAMAX). EU Framework VI.
- Maximizing marine Omega-3 retention in farmed fish: sustainable production of healthy food (Omega3max). EU Framework VII.

**Staff profile:** **Dr. Ramon Fontanillas** (Scientific Responsible) Senior researcher at SARC, has been involved in animal nutrition for 15 years, working in the fish feed industry for 8 years, involved in the evaluation of raw materials as well as nutrient requirements for marine species. **Dr Grethe Rosenlund**, principal researcher at SARC, has been involved in fish nutrition research for more than 30 years and has worked in the feed industry for 22 years. She has experience from nutrition studies on both salmonids and marine finfish including broodstock and larval nutrition as well as use of different lipid sources in aquafeeds.

### **Relevant publications**

- Bogevik, A.S., Natario, S., Karlsen, Ø., Thorsen, A., Hamre, K., Rosenlund, G., Norberg, B., 2012. The effect of dietary lipid content on stress and egg quality in farmed Atlantic cod (*Gadus morhua* L.). J.Fish Biol. 81: 1391-1405.
- Bonaldo, A., Parma, L., Mandrioli, L., Sirri, R., Fontanillas, R., Badiani, A., Gatta, P.P., 2011. Increasing dietary plant proteins affects growth performance and ammonia excretion but not digestibility and gut histology in turbot (*Psetta maxima*) juveniles. Aquaculture 318: 101-108.
- Kullgren, A., Jutfelt, F., Fontanillas, R., Sundell, K., Samuelsson, L., Wiklander, K., Kling, P., Koppe, W., Larsson, D.G.J., Björnsson, B.T., Jönsson, E., 2013. The impact of temperature on the metabolome and endocrine metabolic signals in Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. Part A. 164: 44-53.
- Liland, N.S., Rosenlund, G., Berntssen, M.H.G., Brattelid, T., Madsen, L., Torstensen, B.E., 2012. Net production of Atlantic salmon protein (FIFO, Fish in Fish out < 1) with dietary plant proteins and vegetable oils. Aquacult. Nutr. 19: 289-300. doi: 10.1111/j.1365-2095.2012.00958.x



## **Partner 21. Danmarks Tekniske Universitet (DTU), Denmark**

**Description of Organization:** DTU is a private foundation conducting research, education and research-based consulting services to the public authorities in Denmark. DTU comprises 18 institutes. One is the National Institute of Aquatic Resources (DTU Aqua, <http://www.aqua.dtu.dk>). DTU Aqua, Section for Aquaculture in Hirtshals, conducts applied aquaculture research and counselling, including research in fish feed and nutrition, fish physiology, recirculation technology, and environmental impacts of fish farming, organic aquaculture and certification, fish welfare. DTU Aqua has several state of the art recirculation full-scale rearing facilities and advanced laboratory facilities.

**Main Tasks:** This Partner is involved mainly with pikeperch. In GWP Nutrition, studies will include the effects of lipid origin and fatty acids in pikeperch larvae, neural development using visual and mechanosensory acuity during avoidance responses as proxies and verified from ultrastructural examination by light and electron microscopy. Also, influence of early lipid nutrition in pikeperch and effects on long term metabolism, physiology, fish behaviour and stress (i.e. tolerance of sub-lethal physiological stresses of larvae /post larvae (responses to temperature change or hypoxia). Finally, studies will examine the influence of dietary PUFA levels and rearing salinity levels on LC-PUFA synthesis (DHA), growth performance and stress resistance. In GWP Larval husbandry the tasks include development of larval rearing methods to increase survival and minimize cannibalism. In GWP Grow out husbandry, DTU will examine the influence of stressful husbandry practices and environmental conditions (i.e. lighting, rearing density, handling, sorting, water temperature, salinity, pH, TAN, NO<sub>2</sub>-N, NO<sub>3</sub>-N, oxygen level, tank design/rearing system) on physiological stress responses in pikeperch. DTU will also be involved in studies on the effects of lipid origin and fatty acids in meagre larvae.

**Previous Experience:** DTU Aqua provides scientific based advisory for the Danish Ministry of Food, Agriculture and Fisheries and other public authorities, the commercial fisheries, the aquaculture industry and international commissions. The expertise of DTU AQUA includes: (1) larval rearing of marine and freshwater species in intensive RAS systems, (2) influence of lipid classes and fatty acids involving pigmentation and stress parameters, inflammatory responses and production of eicosanoids, (3) Lipids and feeding strategies to optimize FA utilization and product quality, (4) Behavioural and neuroendocrine reaction to stress of fish in aquaculture and (5) Bioenergetics of fish in aquaculture and influence of dissolved gasses. DTU AQUA has been coordinator or partner in various national, Nordic and EU projects within aquaculture, such as AQUABEST, ORAQUA, COPEWELL (FP6), BIFINE, PROEEL (FP7).

**Staff profile:** **Dr Ivar Lund** (Scientific Responsible) is a Senior Researcher working on fish physiology in relation to lipid nutrition and fatty acids with specific reference to the importance of LC –PUFAs (long chain polyunsaturated fatty acids) on nutrient quality, ontogenic development and short and long term physiological responses in fish larvae or juvenile fish and also on the influence of dietary lipid content and its composition for conversion and deposition in various tissues. Senior researcher **Dr Erik Höglund** (Senior scientist) is the head of the fish welfare research group, studying physiological and behavioural changes related to stress (e.g., stocking density), and how these changes could be applied to assess welfare in fish. **Dr Peter Vilhelm Skov** (Associate Professor) works on bioenergetics and the utilization of energy intake and how aquaculture conditions influence energy partitioning between basal metabolism, activity and growth. A post doc employment may be established partly financed by the DTU budget and internal DTU fundings.

### **Relevant publications**

- Hoglund, E., Gjoen, H.M., Pottinger, T.G., Overli, O., 2008. Parental stress-coping styles affect the behaviour of rainbow trout *Oncorhynchus mykiss* at early developmental stages. *J. Fish Biol.* 73: 1764-1769.
- Lund, I., Steinfeldt, S.J., 2011. The effects of dietary long chain essential fatty acids on growth and stress tolerance in pikeperch larvae (*Sander lucioperca* L.). *Aquaculture Nutrition* 17: 191-199.
- Lund, I., Skov, P.V., Hansen, B.W., 2012. Dietary supplementation of essential fatty acids in larval pikeperch (*Sander lucioperca*); short and long term effects on stress tolerance and metabolic physiology. *Comp. Biochem Physiol.* 162 (4): 340-348 (<http://dx.doi.org/10.1016/j.cbpa.2012.04.004>).



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**Partner 22. Stirling White Halibut (SWH), Norway**

**Description of Organization:** Sterling White Halibut (SWH) is the largest Atlantic halibut farm in the world. The broodstock and hatchery is located at Reipholmen in Rørvik in Nord-Trøndelag county. The juvenile fish are moved to further ongrowth at Imsland and Helland in Vindafjorden in Rogaland County in southwestern Norway. The grow out cages are located in Vassvik and Kjeurda in Hjelmeland in Ryfylke near Stavanger in Rogaland county. SWH is 100% owned by the largest salmon producer in the world (Marine Harvest) and was founded in 2001. SWH produces 1000 mt of high quality halibut a year. The goal is a stable production of 350,000 juveniles for grow out per year and slowly increase the amount of juveniles produced and building the market for halibut.

**Main Tasks:** SWH will participate in GWP Reproduction and Genetics, and GWP Larval husbandry. In reproduction, SWH will contribute with Atlantic halibut broodstock for hormone injection. The personnel at the hatchery will take part in the whole process of husbandry of the broodstock, as well as assist with injection of hormones, together with the involved scientists. In larval rearing, SWH will be a part of the planning and designing of the RAS at Austevoll together with IMR. We will also participate in the making of a reliable on-grown *Artemia* protocol. SWH will be involved also in GWP Socioeconomics.

**Previous Experience:** Sterling White Halibut are mainly fish producers but we do participate in R&D projects that will benefit our production in the future. Currently we have a project together with IMR on developing All Female production. We have also participated in national funded projects such as ACCELERATED PRODUCTION OF ATLANTIC HALIBUT - NFR 180088/120.

**Staff profile: PhD candidate B. Erstad** (Scientific Responsible) has more than 10 years of experience with farming cold water species. He was the larval and live feed manager at Marine Harvest Cod (formerly known as Cod Culture Norway) hatchery from 2002-2008. He has been a biologist at Sterling White Halibut's value chain from 2008. He has been working with production problems (larval rearing, eye health on juvenile, early maturation in sea cage fish, photoperiod issues, etc).

**Relevant publications**

- Hamre, K., Mollan, T. A., Sæle, Ø., Erstad, B., 2008. Rotifers enriched with iodine and selenium increase survival in Atlantic cod (*Gadus morhua*) larvae. *Aquaculture* 285: 190-195.
- Remø, S.C., Erstad, B., Imsland, A.K., Waagbø, R., 2011. Eye health in juvenile Atlantic halibut, *Hippoglossus hippoglossus* L., at two commercial production densities. *Aquaculture* 321:21–25.





### **Partner 23. ARGOSARONIKOS Aquacultures (ARGO), Greece**

**Description of Organization:** ARGOSARONIKOS fish farms ([www.argofish.gr](http://www.argofish.gr)) have been operating in the sector of Mediterranean fish since 1987, producing such fish as gilthead sea bream, European sea bass and meagre. In fact, ARGO was the first company in Greece that introduced meagre in the local market. Currently, the firm's entire production is exported to European countries. Production capacity comes to 800 t of fish a year and the firm's modern installations are located in privately owned land of Pyrgiakoni Bay, on the south side of the island of Salamis and consist of:

- A modern hatchery for the production of fish from broodstock.
- Modern and automated pre-growing units where the fish are reared for two months, until they have reached the ideal size to continue their lives in the sea.
- An 800-t capacity sea cage facility, where the fish grow in their natural environment, in much lower-than-average population densities.
- A modern packing unit, located a mere 100 meters from the production units, so that the fish are packed immediately after being harvested to be delivered fresh to consumers.

ARGO has developed into a modern, vertically integrated unit; its services comply with the ISO 9001 international model (certificate No 629335 guardian), and HACCP. Furthermore, strict control systems are followed at every stage of fish production and growth, while the ARGO has implemented a tracking system that ensures full traceability, as each fish is unique with its own identity. An important aspect contributing to fish quality is the fish food used, which is selected on the basis of strict criteria to ensure consumers' safety and health. The fish feed used is certified in regard to European Legislation specifications and demands.

ARGO, showing respect for the environment and believing in sustainable development, keep the waters in Pyrkogiani Bay as clean as they were before the farm's cages were installed. This is verified with periodic sampling and measurements and by using materials that are friendly to the environment. The company has recently installed a 100 KW photovoltaic park to meet the Company's power needs, thus producing energy from sources that are friendly to the environment.

**Main Tasks:** ARGO will participate in WP3 Reproduction and Genetics - greater amberjack, in Task 3.2 Development of an optimized spawning induction protocol for captive greater amberjack in the Mediterranean and Task 3.5 Spawning induction of greater amberjack and egg collection in cages. The company is in charge of obtaining mature breeders from the wild and maintaining them in cages in the facility for the duration of the project. The broodstock will be used for spawning induction experiments, using hormone treatment if necessary and for the development of egg collection methods from sea cages. ARGO also participates in WP20 Grow out husbandry – meagre in Task 20.2 Effect of rearing environment, where the effect of light intensity will be examined on fish distribution and growth performance. ARGO will also contribute to WP30-Socioeconomics: business model and marketing strategy development, and will assist the RTD partners involved in this WP in developing a business model for the new species.

Due to a total mortality of the ITTICAL broodstock obtained for the activities of Task 3.1 (WP3), this Task was moved to ARGO, who succeeded in acquiring an adequate broodstock and offered to give this to the project. Therefore, a significant part of the original budget from ITTICAL (75,000 EU contribution) was transferred mainly to ARGO. Smaller budget transfers were also made to partners HCMR, IOLR, UNIBA, IFREMER and UL, to cover the additional cost incurred by the samplings that were now going to be done in Greece, in partner ARGO.

**Previous Experience:** The expertise of the owner and Scientific Responsible Mr. Anastasios Raftopoulos in the field of fish farming starts in 1987, when ARGO was founded. Since then, ARGO has grown into a modern, vertically integrated unit with a hatchery, a modern unit for fry pre-fattening, feeding units in the open sea, as well as a modern packaging and standardization unit. In 2007, ARGO participated in the European Programme ALFA (FP6), for the continuous production of live feed in aquaculture hatcheries.

**Staff profile:** **Mr Anastasios Raftopoulos** (Scientific Responsible) is the owner and managing director of ARGO. He has 25 years of experience in the production of marine fish. **Mr Manolis Daniil**, Mechanical Engineer, is the technical manager of ARGO and the responsible person for all technicians and technical activities.



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**Partner 24. Azienda Agricola Ittica Caldoli Srl (ITICAL), Italy**

**Description of the Organization:** Ittica Caldoli Srl is a private enterprise located in the Puglia Region, south of Italy, specialized in the reproduction and grow-out of marine and freshwater fish species. It is active since 1986, at the beginning the main activity was eels weaning but after some years it evolved in sea bass reproduction and grow-out. Recently, a new modern hatchery has been realized based on closed recirculation systems. Actually 12 persons are involved in the production activity. The company goal is to increase the actual annual 4 million seabass and seabream fry production to 10 million fries per year with the breeding of new and high valued fry species.

**Main Tasks:** ITICAL will be involved in GWP Reproduction and Genetics - greater amberjack setting an adequate tank to maintain a broodstock in the optimal conditions for reproduction and investigate reliable larval rearing protocols. The tasks will include (a) setting a broodstock tank, (b) recruitment of wild-caught animals and their shipment in the farm, (c) adaptation to captivity, (d) maintenance of broodstock to photo-thermal conditions, (e) support to fish angling during hormonal induction operations performed by UNIBA staff. Due to a total mortality of the broodstock obtained for the activities of Task 3.1 (WP3) and the difficulties faced by the company to acquire another stock, this Task was moved to another Partner (P23. ARGO), who succeeded in acquiring an adequate broodstock and offered to give this to the project. Therefore, a significant part of the original budget from ITICAL (75,000 EU contribution) was transferred mainly to ARGO. Smaller budget transfers were also made to partners HCMR, IOLR, UNIBA, IFREMER and UL, to cover the additional cost incurred by the samplings that were now going to be done in Greece, in partner ARGO.

Also, ITICAL will participate in GWP Larval husbandry of greater amberjack (a) monitoring the spawning events and collection of eggs, (b) shipping fertilized eggs or larvae to the other Partners involved in the same WP, (c) incubating eggs and rearing of larvae for the optimization of a husbandry protocol. Finally, ITICAL will be also involved in the GWP Nutrition - grey mullet, to optimize a rearing broodstock protocol aimed to obtaining high quality gonads to be used for bottarga production, by (a) capture of wild animals (b) adaptation and maintenance in captivity (c) optimization of nutrition and feeding management of broodstock (d) support UNIBA staff during the operations of gonad sampling. The Partner will also have a small contribution in WP30-Socioeconomics: business model and marketing strategy development, and will assist the RTD partners involved in this WP in developing a business model for the new species.

**Previous Experience:** Ittica Caldoli, over the main activity of breeding and rearing marine fish species, is participating in national and EU funded projects for the diversification of species and products: (a) “Pilot project for the reproduction and restocking of sea urchin (*Paracentrotus lividus*) along the sea shore of south of Italy” (European Fund for Fishery (FEP) 2007-2013, Project n° 59/OPI/010), (b) “Organic hatchery productions: technical and economical evaluations” (European Fund for Fishery (FEP) 2007-2013, Project n° 55/OPI/010), (c) “Technologies for the enhancement and extension of shelf life in processed fish products of high nutritional value” (PON R&C 2007-2013, Project PON 01-01962).

**Staff Profile:** **Dr Fulvio Cepollaro** (Scientific Responsible) is the technical manager of hatchery and he is directly involved in the plant operation, broodstock management, evaluation of reproduction phases, breeding and raising of larvae, grow out of fingerlings. **Mr A. Novelli, MSc** (General Manager) has expertise in commercial reproduction of marine and freshwater species, he has long experience in design and management of fish farms, leading in the past some of the main marine hatcheries of Italy.

**Relevant Publications:**

Bertotto, D., Cepollaro, F., Libertini, A., Barbaro, A., Francescon, A., Belvedere, P., Colombo, L., 2005. Production of clonal founders in the European sea bass, *Dicentrarchus labrax* L., by mitotic gynogenesis. *Aquaculture* 246: 115-124.

Francescon, A., Rancescon, A., Barbaro, A., Bertotto, D., Libertini, A., Cepollaro, F., Richard, J., Belvedere, P., Colombo, L.,. Assessment of homozygosity and fertility in meiotic gynogens of the European sea bass (*Dicentrarchus labrax* L.). *Aquaculture* 243: 93-102.





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**Partner 25. DOR DGEY YAM Ltd (DOR), Israel**

**Description of the Organization:** DOR is a well-known and respected fish-breeding center that covers an area of about 70 hectares and is ideally situated on the coast of Dor, Near Haifa, Israel. With the benefit of water supply combining fresh sea water with a high-quality saline well, a new and state-of-the art hatchery has been established, with large focus on development of new and high-value species.

**Main Tasks:** DOR will participate in WP7 Reproduction and Genetics – grey mullet and in WP19 Larval husbandry – grey mullet to evaluate an improved grey mullet larval rearing protocol, based on work carried out in WP13 (Nutrition) and WP23 (Grow out husbandry), in one of three commercial systems (depending on the number of eggs) that include twenty 7 m<sup>3</sup> tanks overall. In addition, DOR will participate in WP5 (23.2) where it will grow over 1 year F1 grey mullet fingerlings in two 6000-m<sup>2</sup>, which will differ in their stocking density (0.5 and 1 juvenile m<sup>2</sup>). The fish will be fed an improved grey mullet grow-out diet and DOR staff will evaluate fish performance in terms of FCR, PER, SGR, overall weight gain and survival. An economic evaluation of the trial will also be calculated. These results will be compared to (1) growing F1 juveniles in cement bottom tanks in Eilat, Israel (IOLR), as well as (2) growing wild juveniles in earthen and cement bottom tanks in Greece (GEI) and Spain (CTAQUA). The Partner will also have a small contribution in WP30-Socioeconomics: business model and marketing strategy development, and will assist the RTD partners involved in this WP in developing a bussines model for the new species.

**Previous Experience:** DOR Aquaculture and its founding partner, Mr Hagay Sarusi, have been a catalyst to the progress made in the development of white grouper (*Epinephelus aeneus*) reproduction in Israel. Deploying knowhow and skills nourished over 20 years of research and business relationships around the world, DOR has been able to situate itself at the forefront of the Israeli Aquaculture Industry.

**Staff Profile:** **Mr Hagay Sarusi** (Scientific Responsible) is the founding partner of DOR. **Mr. Asaf Elkayam, Bsc.** is the hatchery manager and a senior member of DOR's biology team, he has over 5 years of hands-on experience in both research and practical hatchery management, including broodstock management, food chain management and larval rearing. Mr. Elkayam has played a major role in developing larval rearing processes for grey mullet, white grouper and blufin tuna while working at the IOLR.



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**Partner 26. VAS. GEITONAS & Co Ltd EE (GEI), Greece**

**Description of the Organization:** V. GEITONAS & Co Ltd is a private enterprise established in 1985, and is operating in the northwest part of Greece (region of Arta) with a staff of 16. GEI specializes in the production of freshwater fish species (e.g., freshwater eel, tilapia and grey mullet), with the major product of the company being the freshwater eel. After 10 years of personal work and the investment of a lot of funds, the company established efficient methods of farming, beginning from the smallest commercial size of 150 g and reaching to the biggest 1300 g. The company achieved to produce and offer the European and Greek markets a variety of eel products (Live, Fresh and Smoked). Each year GEI exports 170 - 220 tons of its products. **The company applies the Food Safety Management System (ISO 22000:2005) - HACCP.**

**Main Tasks:** V. GEITONAS & Co Ltd will be involved in WP Nutrition-grey mullet 13. The tasks will include (a) recruitment of wild-caught animals and their shipment in the farm, (b) adaptation to captivity, (c) maintenance and feeding of fish during the experimental period, (d) monitoring and recording parameters as the daily oxygen levels and temperature and (e) support to the HCMR staff during the experimental period.

Wild caught mullet juveniles will be stocked at two different densities (4 and 6 juvenile m<sup>2</sup>) in 6 cement ponds (20 m<sup>2</sup>) where each density will be tested in triplicate tanks over 1 year. The fish will be fed an improved mullet extruded feed as described in Task 23.1. Fish performance will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival and the trial will be supervised by the HCMR staff.

**Previous Experience:** The company has more than 10 years experience in grey mullet culture. The mullets are reared under semi-intensive conditions in small earth ponds (280 m<sup>2</sup>), which allow fish to grow in their natural environment. The mullets are caught and immediately packaged. GEI produces more than 6 tons of mullet per year targeting to increase its production to more than 20 t of grey mullet annually. One of the company's visions is to produce and offer high quality mullet for the customer-consumer in the European and Greek market.

**Staff Profile:** **Mr V. Geitonas** (Scientific Responsible) is the General Manager and is an agronomist with over 30 years experience in commercial production of different fish species, and in design and management of fish farms. **Mrs. Evaggelia Zania** (Technical Manager) is an ichthyologist, and she directly oversees fish farm activities and operation.



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**Partner 27. Aquaculture Forkys A.E. (FORKYS), Greece**

**Description of Organization:** Forkys Aquacultures SA is a private enterprise located in the Chios Island, specialized in the reproduction and grow-out of marine fish species. It is active since 1994. The main activity is the production of European sea bass and gilthead sea bream in sea cages. Also new species such as common sea bream, common dentex, sharpsnout sea bream and meagre are being cultivated at Forkys net cages. Recently, FORKYS acquired a hatchery and a fish farm in Crete, Greece and started with hatchery production of both species. Forkys employs more than 120 persons both at reproduction, grow-out and packing. FORKYS has also begun recently the production of meagre. The company's goal is to increase the annual production of hatchery to 15 million fry production and the production of grow-out farms to 10,000 metric tonnes. In addition, FORKYS is interested in diversifying its production with new/emerging species such as meagre, the greater amberjack and wreckfish.

**Main Tasks:** FORKYS will be involved in GWP Larval husbandry and GWP Grow out husbandry. In WP15 Larval husbandry-greater amberjack incubation of eggs and rearing of larvae for the optimization of a husbandry protocol will be done in 2 periods of 4 months each at the facilities of Forkys hatchery in Crete. In WP21 Grow out husbandry-greater amberjack, in a period of 12 months, the optimum grow-out depth will be determined at net cages in the grow-out farm of Forkys in Crete.

**Previous Experience:** FORKYS is participating in a national project for the diversification of species titled "Development of reproduction and culture methods for meagre (*Argyrosomus regius*) as a means to improve the competitiveness of the aquaculture industry through species diversification." The programme is financed by the General Secretariat for Research & Technology Operational Program: "COOPERATION 2011. Partnerships of Production and Research Institutes in Focused Research and Technology Sectors", Project n° 09SYN-24-424.

**Staff profile:** **Mr Ioannis Diakogeorgakis, MSc** (Scientific Responsible) is the Quality Assurance Manager of Forkys Aquacultures and has a lot of experience participating in National and EU funding programs. **Mr Babis Sirigos** (Hatchery manager) has long-term experience in marine aquaculture, as a technical staff of a number of commercial operations. **Mr Dimitrios Pagonis** (Hatchery technical manager) has great experience in broodstock management of marine fish. **Mr Nikolaos Vasilakis** (Grow-out farm technical manager) has a lot of experience in grow-out of marine fish.



### **Partner 28. Canarias Explotaciones Marinas S.L. (CANEXMAR), Spain**

**Description of the Organization:** Canarias Explotaciones Marinas S.L. is a private enterprise established in 1998, operating in the west coast of Gran Canaria, with a staff of 12 employees. The facility has 10 cages of 20 m diameter, 4 cages of 25 m diameter, 4 cages of 12 m diameter, 1 boat and 2 zodiacs. CANEXMAR is specialized in the production of gilthead seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*) with a capacity 1200 tons per year, maintains an average production during last years of 650 tons (60% seabream and 40% seabass). In 2006 the company was renewed and started to promote programs of research and development (R&D) towards diversification, being the pioneers in the production of meagre (*Argyrosomus regius*) in the Canarian Archipelago. Also CANEXMAR actively collaborates with different public R&D institutions in projects to diversify marine aquaculture, such as:

- “Mejora de las técnicas de cría de larvas de (*Seriola rivoliana*): determinación de requerimientos de ácidos grasos esenciales en su etapa larvaria y optimización de la secuencia alimentaria (METSER)” where its main activity is related with the on-growing of amberjack and striped jack juveniles in cages (on-going studies) (2011-2013).
- “Proyecto piloto para la implantación de técnicas de reproducción y cultivo larvario en cautividad del medregal (*seriola sp.*) y del jurel dentón (*Pseudocaranx dentex*) - RAPCREC” which successfully caught and adapted amberjack and striped jack broodstock to captive conditions (2010-2011).
- “Nutrición y alimentación de paralarvas y subadultos de pulpo de roca (*Octopus vulgaris*), where the company participated in the on-growing of octopus juveniles in benthic cages (2009-2012).
- “Programa piloto de repoblación de especies de interés pesquero: bocinegro (*Pagrus pagrus*) y almeja canaria (*Haliotis tuberculata coccinea*)” where the company adapted red porgy juveniles to cages culture (2009-2010).
- “Desarrollo de un programa piloto de mejora genética en dorada (*Sparus aurata*)”, where the company reared genetically selected seabream juveniles in sea cages (2009-2011).
- “Acuicultura integrada: experiencia piloto para el desarrollo de sistemas de cultivo multitrofico”, where the company reared *Haliotis tuberculata* in specific culture systems (ortex) (2007-2010).
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**Main Tasks:** CANEXMAR S.L will be involved in WP9 Nutrition-greater amberjack) and WP21 Grow out (greater amberjack).

The tasks in WP 9 will include: a) maintenance and feeding of fish during the experimental period, (b) monitoring and recording parameters as the daily oxygen levels and temperature and (c) support to the FCPCT staff during the experimental period. Amberjack juveniles will be stocked in regular cages (20 m diameter, 10 m depth) to test the developed novel diets at a commercial farm level over one year. Two growing cycles will be performed over 2 years. Fish performance will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival and the trial will be supervised by the FCPCT staff.

The tasks in WP 21 will include: a) maintenance and feeding of fish during the experimental period, (b) monitoring and recording parameters as the daily oxygen levels and temperature and (c) support to the FCPCT staff during the experimental period. Amberjack juveniles will be stocked in regular cages and submerged cages (20 m diameter, 10 m depth) for 2 successive rearing periods of 12 months each. The fish will be fed an optimized diet. Fish performance will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival and the trial will be supervised by the FCPCT staff.

The Partner will also have a small contribution in WP30-Socioeconomics: business model and marketing strategy development, and will assist the RTD partners involved in this WP in developing a bussines model for the new species.

**Staff Profile:** Mr A. Mormeneo (Scientific Responsible).



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**Partner 29. ASIALOR SARL (ASIALOR), France**

**Description of Organization:** ASIALOR is a new French private company located in the Lorraine Region, North-East of France, specialized in perch and pikeperch production in recirculating aquaculture system (RAS). Founded in 2009, ASIALOR began fish production in 2011. Recently new modern out-of-season rooms, hatcheries, juvenile and grow out systems have been realized based on recirculation system technology. During two years, ASIALOR grew step by step in two sites in Moselle. The first one is located in Pierrevillers for broodstock management and hatchery/nursery activities. The second, located in Dieuze, concerns fish grow out, transformation and commercialization (whole fish, fillet). Seven persons are currently involved in the production activity. The company's goal is to increase the annual production up to 60-80 tonnes in 2015-2016.

**Main Tasks:** ASIALOR will be involved in WP10 Nutrition – pike perch, WP16 Larval husbandry-pike perch and WP22 Grow out husbandry-pike perch. Specifically, ASIALOR will be involved in the (a) identification of the main influencing environmental, population and nutritional factors to improve larval rearing protocols (Task 16.1) and (b) identification and characterization of husbandry practices and environmental factors on pikeperch growth, immune and physiological status (WP22). ASIALOR will also test in farm conditions (a) new protocols to improve juvenile production by decreasing cannibalism, such as initial larvae quality, grading or feeding strategies (Task 16.2), and (b) a new diet more adapted to pikeperch requirements to improve larval nutrition (WP10) and to reduce stress sensitivity with optimal early fatty acid enrichment (WP10). The Partner will also have a small contribution in WP30-Socioeconomics: business model and marketing strategy development, and will assist the RTD partners involved in this WP in developing a business model for the new species.

**Previous Experience:** Since 2011, ASIALOR has participated in many national and European projects (European Fisheries Fund EFF). In 2012, ASIALOR has obtained the Label “Young Innovative Company” (JEI in French) and took advantage of the research tax credit. The research tax credit is a government-funded aid to businesses investing in research and development.

**Staff profile:** **Mrs Ly Tu-Lihn** (Scientific Responsible) is the Technical Manager of the grow-out site and is directly involved in the plant operation, transformation and commercialization of end product. **Mr J. Saint-Sevin** is the Technical manager of the hatchery and is involved in the plant operation, broodstock management, evaluation of reproduction phases, breeding and larval rearing. **Mr M. Buffet** is a technician of the grow-out site and **Mr S. Freyheit** is the technician of the hatchery. Two other persons are involved in fish processing (production of fillets). **Mr M. K. Ly** is the Managing Director and will be involved in financial management of the project.



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**Partner 30. CULMAREX S.A. (CULMAREX), Spain (the organization exited the consortium in 2015, Amendment 2)**

**Description of the Organization:**

In 1986 a group of Spanish investors created Culmárex with the intention of rearing greater amberjack and oysters in the Bahía del Hornillo, Águilas, Spain. In 1990 Marine Farms acquired part of the company and introduced gilthead sea bream and European sea bass farming. During more than twenty years, Culmárex has been dedicated to the rearing and marketing of these two species. Nowadays, Cooke Aquaculture is the mother company of CULMAREX, and CULMAREX is the mother company of Culmárex Group, the largest in Spain with a production of 14,000 metric tons in 2013. CULMAREX Group is a total of eleven fish farms located in Andalucía, Murcia, Alicante and Valencia and a hatchery situated in Mallorca. In this moment the Group is composed of 330 workers and 85 million of live fish.

CULMAREX Group has pioneered improvements in systems of quality control and traceability, as well as systems of sustainable production. At the same time, Culmárex is totally committed to the protection of the environment, and incorporates policies based on the prevention, and minimization of environmental impact. In 2008 the entire Group was certified in an Integrated Management System based on the Standards ISO 9001:2000, ISO 14001:2004 and OSHAS 18001:2007.

CULMAREX Group promoted various projects to optimize and innovate the processes that allow the development of sustainable activity. This investment in R&D has led the Group to play a leading role in research programmes within the sector, collaborating with different public and private entities.

**Main Tasks:** CULMAREX will participate in WP20 Grow out husbandry - meagre. The tasks include testing different feeding systems on a commercial scale in commercial cages. Feeding strategies using an automated feeding system with demand type characteristics will be compared with existing hand feeding strategies. It is expected that the experiments will (a) improve the acclimation period in the weeks after juvenile transfer to cages and (b) give consistent predictable reduced size variation and FCR during grow-out. This work will reduce production costs and facilitate the business plan with reliable estimates of harvest size and time.

**Previous Experience:** CULMAREX participated in COST/215/96 (17/01/1996-16/01/2001): “Regulation of voluntary feed intake in fish”, the adequation of feeding strategy to the spontaneous feeding rhythms in cultured fish, experimental activities at pre-Industrial scale are performed at CULMAREX fish farm placed in the coast of Águilas (Murcia)

CULMAREX started with grow-out of meagre in 2013 and aims to continue production of meagre as a third production species. BERSOLAZ (incorporated to Culmárex Group in November 2013) has experience in meagre grow-out, producing 3000 t of meagre during the period 2002-2009.

**Staff Profile:** **Dra. María Dolores López Belluga** (Scientific Responsible) M.Sc., PhD in Biology from the University of Murcia, specialist in Marine Ecology. Since 1997 she works in Culmárex Group, in this time her work has evolved from technical Responsible in the production area, to quality manager and, since 2008, to R&D and New Products Responsible. Since 2008, she manages the R&D Department of CULMAREX Group. **Dr. Mezian Azzaydi**, PhD in Biology from the University of Murcia, specialist in Physiology of fish, is the Production Manager of CULMAREX Group. His research studies are relative to feed intake in seabass, specifically “Effects of Feeding Time on Feed Intake and Growth”. He worked in CULMAREX since 1999 and has an extensive experience in rearing sea bass and sea bream on a commercial scale.





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**Partner 31. IRIDA S.A. (IRIDA), Greece**

**Description of Organization:** IRIDA S.A. is a dynamic and independent company that produces and supplies aquafeed to the Mediterranean aquaculture industry. The products include high quality fish feeds manufactured with a diameter range from 1.0-12.0 mm for species such as gilthead sea bream, European sea bass, meagre, sharpsnout seabream and red porgy. IRIDA is the first fish feed company in Greece that has been certified for Organic Fish Feed production.

IRIDA invests in research and innovation in order to support in the best way the Mediterranean aquaculture industry. Main research areas for the R&D efforts include improvement of taste and quality for farmed fish, development of sustainable fish feeds and species diversification.

**Main Tasks:** IRIDA will formulate and produce the experimental diets for the growing experiments of grey mullet for WP23 Grow out husbandry-grey mullet. IRIDA will take care of the raw material, as well as the feed analysis and quality.

**Previous Experience:** IRIDA has extended experience as a participant in previous projects, where the contribution has been to produce the experimental feeds for the other Partners. Among others, IRIDA has participated in the following projects:

- “Development of methods for reproduction and rearing of meagre (*Argyrosomus regius*) as measure to enhance the competitiveness of Aquaculture with the introduction of new species” (Funded from General Secretariat of Research and Technology).
- “Reproduction of Bluefin Tuna (*Thunnus, thynnus*) in captivity and fingerling production for grow out” (Funded from General Secretariat of Research and Technology).

**Staff profile:** **Mr Nikos Papaioannou** (Scientific Responsible) is an Animal Scientist and holds an MSc degree from Stirling University, Institute of Aquaculture in Scotland and a BSc degree from the Agricultural University of Athens, Animal Science. Today, as Technical Director of IRIDA S.A. he coordinates the fish feed production and the consulting services that the company offers to its customers. He is also a main shareholder of the company. **Dr. Sotiris Papasolomontos** is an Animal Scientist and holds degrees from The University of London, the University of Newcastle (MSc and PhD), and the London Business School. For a period of thirty years he has been involved in animal nutrition and feed industry in the UK and Greece, responsible for all technical and advisory services.

**Relevant publications:**

Chatzifotis, S., Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, I., Mylonas, C.C., 2010. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. *Aquaculture* 307: 65–70




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**Partner 32. Ayuntamiento de A Coruna – Aquarium Finisterre (MC2), Spain**

**Description of Organization:** Museos Científicos Coruñeses “mc<sup>2</sup>” belongs to La Corunna City Council. It is a three science network devoted to the popularization of science: Domus (human kind), Casa de las Ciencias (physical sciences and astronomy) and “Aquarium Finisterrae” (marine ecosystems). Their mission includes the development of exhibitions, educational activities, films, publications, science fairs, lectures, research projects and any kind of initiatives to promote a better understanding of science among public of all kinds. According to the European and Spanish regulations, Aquarium Finisterrae as a Zoological institution must have among their aims, not only the environmental education but also the research programmes concerning Oceans preservation and of course aquaculture sustainability.

**Main Tasks:** The MC2 will be involved in the WPs related to wreckfish (1) reproductive behaviour, natural spawning conditions and hormonal induction treatments, (2) development of larval rearing protocols, (3) nutrition studies for the improvement of spawning quality and larval and weaning performance.

**Previous Experience:** Collaborating with surrounding Universities, fish farms, and public research organization as IEO and CSIC, the Aquarium Finisterrae, thanks to its facilities and staff background, has been and is still involved with some research programmes not only affecting Ornamental Aquarium species but also Aquaculture local species.

**Staff profile:** All staff involved is experienced in aquarium species husbandry and reproduction of a wide variety of aquarium and aquaculture species: Mr. **A. Vilar Peron** (Biologist) Animal husbandry “Curator of exhibits”, leader (mc<sup>2</sup>) has been taking care of the wreckfish stock and reproductive efforts at the Aquarium since 1999. He is responsible for the natural reproduction and larval development and in the DIVERSIFY programme he will be involved in (1) reproductive behaviour, natural spawning conditions and hormonal induction treatments. Mr. **C. García Soler** (Biologist) Animal husbandry “Curator of exhibits” is experienced in “*Labrus bergylta*” and “*Scyllarus arctus*” captive breeding and in the DIVERSIFY programme he will be involved in (2) development of larval rearing protocols. **Dr A. Veiga Villar** “Head Laboratory and live food management” will be involved in (3) nutrition studies for the improvement of spawning quality and larval and weaning performance.

**Relevant publications**

- A. Perez Cribeiro, A. Vilar Peron, M Ferrant Rojo, C. García Soler, 2003. Survival and adaptation to captivity of Anglerfish *Lophius piscatorius*. Influence of Capture method transport acclimatization and feeding. EUAC, Oct 3, 2003.
- Planas, M., , Chamorro, A., Quintas, P., Vilar, A., 2008. Establishment and maintenance of threatened long-snouted seahorse, *Hippocampus guttulatus*, broodstocks in captivity. Aquaculture 238 (1-4): 19-28 (10.1016/j.aquaculture.2008.06.023).





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**Partner 33. Federation of Greek Maricultures (FGM), Greece**

**Description of Organization:** Established in 1991, the Federation of Greek Maricultures (F.G.M.) represents the Greek aquaculture companies that produce Mediterranean euryhaline fish species and mainly Gilthead sea bream and European sea bass. FGM is now associated with Greek companies providing technology and services to the industry, including fish feed. FGM currently represents 80% of the Greek aquaculture production and 2/3 of the total employees working in the sector. FGM is the official representative of the fish farmers to the State, the Public Authorities, European Institutions and International Organizations and since 1993 member of the Federation of European Aquaculture Producers (FEAP). Its main objective is to promote the sustainable development of the sector and maintain the leading position of its members in the production of Mediterranean species. Apart from the Board and the secretariat, FGM comprises of three experts - Committees (Marketing, Scientific Advice and Institutional Affairs) in order to tackle effectively the complex issues affecting the sector.

**Main Tasks:** The FGM will be involved in WP31 Dissemination, by creating a link on its website to the DIVERSIFY website and distributing the project brochure to its members (Task 31.1). In addition, it will cooperate with the WP31 leader and the Species leaders in developing and then translating the Technical leaflets (one per species) and distribute them to their members (Task 31.7).

**Previous Experience:** FGM has been working with HEPO to establish a quality trademark for sea bream and sea bass farmed in Greece. A further Quality Assurance Scheme has been funded by PESCA Project. Lately, FGM coordinated INTRANEAMMA, a project funded by the EU Lifelong Learning Programme through the Greek National Agency. The project aimed to identify priority vocational skill needs in the Mediterranean mariculture industry and in response, to develop pilot innovative sector-led programmes to improve vocational skills and therefore to ensure sustainability in sea bass and sea bream aquaculture.

**Staff profile:** **Mr Ioannis Pelekanakis** is the General Manager of the FGM, he has worked for the Fisheries Committee of the European Parliament, participated in the assessment of the FP6 and has considerable experience with the promotion of the sustainable development of the aquaculture sector. **Mrs Madgalini Armanidou**, FGM secretariat staff, has significant experience with previous EU funded programs run by FGM.




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**Partner 34. German Federation for Fish Processors and Wholesalers (BVFi), Germany**

**Description of Organization:** The BVFi represents the interests of the German fish processing and fish wholesale industry.

**Main Tasks:** The main objectives and activities from the BVFi are:

- Representation of interests
- Advise and dissemination of information to members
- Enabling fair competition on the German fish market

The BVFi represents the German processing and wholesaling sector at national and EU-level. On a national level the federation has contacts with several Governmental organizations, the fishing industry, NGOs and the media, in order to support its members and to promote the image of the German fish-processing sector. On the EU-level BVFi is a member of AIPCE/CEP.

The federation informs its members about relevant issues for fish processing and wholesaling, such as import tariffs and tariff contingents, EU-policies, quality standards and certification initiatives. The federation is one of the major founders of the science-based internet platform [www.fischbestaende-online.de](http://www.fischbestaende-online.de) and will release a new website of aquaculture information at the end of the year 2013.

In DIVERSIFY, the BVFi will be involved in WP31 Dissemination, by creating a link on its website to the DIVERSIFY website and distributing the project brochure to its members (Task 31.1). In addition, it will cooperate with the WP31 leader and the Species leaders in developing and then translating the Technical leaflets (one per species) and distribute them to their members (Task 31.7).

**Previous Experience:** The association was partner in the following EU-funded projects: (1) “Freshlabel”, Integrated approach to enable traceability of the cold chain of fresh, chilled meat and fish products by means of taylor-made time/temperature indicators, (2) “Smartcatch”, the development of a novel remote stress sensing system to increase safety, efficiency and reduce environmental effects in fishing and mooring applications.

**Staff profile:** **Dr. Matthias Keller**, managing director of the following professional organizations: Bundesmarktverband der Fischwirtschaft e.V. (Federal market association of fisheries), Bundesverband der deutschen Fischindustrie und des Fischgroßhandels e.V. (German fish processing and wholesale association), Fisch-Informationszentrum e.V. (Fish-Informationcenter) and „Stiftung seeklar“ – Verein zum Schutz der Meere e.V. („Foundation seeklar“ – Foundation for the protection of the sea).

**Relevant publications:** Editor and author of the book „Behr’s Handbuch für Fisch, Krebs- und Weichtiere“, updated 3 times a year, ISBN: 978-3-86022-185-3; co-author of the AIPCE-„Fin Fish Study“ 2011 and earlier years and author of the annual report of the association of fish processors and fish wholesalers.




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**Partner 35. Hungarian Aquaculture Association (MASZ), Hungary**

*This Partner has been taken over universally by the HUNGARIAN AQUACULTURE AND FISHERIES INTER-BRANCH ORGANISATION (MA-HAL) MAHAL, who is from now on responsible for the implementation of the work originally assigned to MASZ, and has also received its remaining budget.*

**Description of Organization:** The Hungarian Aquaculture Association (MASZ) is a non-profit organization registered in Hungary in 2010. MASZ is a major producers' organization in Hungary that is also member of the Federation of European Aquaculture Producers (FEAP). The Association has 44 members mainly fish producers, using various systems (fish ponds, integrated systems, intensive production facilities) for the production of major freshwater species including pikeperch. MASZ has been established to give momentum to aquaculture development in Hungary mainly through the active representation of the members in stakeholders' consultation and the promotion of innovation. The Association regularly organizes workshops and disseminates information to members through the MASZ website and written materials. MASZ is collaborating with producers association in the region and with the Network of Aquaculture Centers in Central and Eastern Europe (NACEE).

**Main Tasks:** The MASZ will be involved in WP31 Dissemination, by creating a link on its website to the DIVERSIFY website and distributing the project brochure to its members (Task 31.1). In addition, it will cooperate with the WP31 leader and the Species leaders in developing and then translating the Technical leaflets (one per species) and distribute them to their members (Task 31.7).

**Previous Experience:** The MASZ, through its members has been involved in various national and international aquaculture development projects. Some of these projects were aimed at the development of systems and technologies of new or emerging species (e.g. FP7 projects: Lucioperca; Sustinaqua; Aquaredspot). MASZ has been actively involved in the preparation and implementation of the Fisheries Operational Program in Hungary.

**Staff profile:** **Dr. Laszlo Varadi** president of MASZ was the director of the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI) for 20 years and has been involved in the preparation and implementation of numerous national and international aquaculture development projects. **Ms. Emese Bekefi** secretary of the Association (part time) is the head of the Training and Extension Department of HAKI.

**Relevant publications**

Békefi, E., Lengyel, P., Váradi, L., 2006. Review of producers associations and their role in aquaculture development in Eastern Europe. Proceedings of the EIFAC Symposium on Aquaculture Development – Partnership between Science and Producers Associations. Wierzba, Poland, 26-29 May, EIFAC Occasional Paper No. 37, Rome, FAO 2006, 51-55 p.

Varadi, L. Species diversification - Upcoming species: Eastern Baltic Aquaculture Conference“European Fisheries Fund and opportunities for the Development of Eastern Baltic Aquaculture” 6-7 May 2008, Riga, Latvia.



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**Partner 36. Asociación Nacional de Fabricantes de Conservas de Pescados y Pariscos (ANFACO), Spain**

**Description of Organization:** ANFACO is the National Association of Fish and Seafood Canning Manufactures and Technical Centre for the Preservation of Fish, Seafood and Aquaculture Products.

The Centre is a non-profit organization that embodies a main institution for the whole fish processing industry. ANFACO also has opened its R&D activity to the whole of the food industry. The organization maintains a close communication, interaction and collaboration with different entities spread all over the world, it includes Research Institutes, Universities, Technological centres, and many more.

ANFACO has more than 200 associate members from a multisectorial cluster linked to seafood producers and industrial processing sector, which includes fish and seafood canning, semi preserved and salted fish, frozen, chilled and processed products, fish meals, oils and raw materials. It also involves among others, machinery, casting and packing of sea products, marketer organizations, associations and auxiliary services.

ANFACO has a strong history of participation in research projects, both funded by public sources at European, national and regional levels and by private companies. This experience encompasses research activities, coordination of partnerships and implementation and economic management and extensive collaboration with national and international research institutions and private companies.

The main areas in ANFACO are: Health and food, Genomics and proteomics applied to the marine industry and food, Food Safety, Aquaculture, Environment and Valuation of sea products, engineering and energy efficiency and New preservation technologies.

**Main Tasks:** The ANFACO will be involved in WP31 Dissemination, by creating a link on its website to the DIVERSIFY website and distributing the project brochure to its members (Task 31.1). In addition, it will cooperate with the WP31 leader and the Species leaders in developing and then translating the Technical leaflets (one per species) and distribute them to their members (Task 31.7).

**Previous Experience:** A long experience in the coordination and participation in research activities characterizes the equipment of ANFACO that has participated in more than 12 V, VI and VII Framework projects. Nowadays, the centre carries out around 60 projects including contract with industries. Also, ANFACO has broad experience in research results dissemination among the involved productive sectors (producers, transformer industry, traders and consumers).

**Staff profile:** **Dr. Sandra Rellán**, R&D Coordinator at the organization. She coordinates the different activities in research and dissemination towards the business sector, coordinating the participation of ANFACO-CECOPESCA in the different calls and adapting the research to all interested in the Seafood sector. **Mr Xose Ramon Vázquez**, Coordinator of the Technological Transfer Office at ANFACO-CECOPESCA. He has strong relationships with several industry organizations and with several regional and international technological platforms. He is also involved in the PTGAL (Galician Agri-Food Technology Platform). He has participated in several projects at national and European level. **Mrs Victoria Fernández** Degree in oceanography. Nearly two years in the Technological Transfer Office of ANFACO - CECOPESCA performing coordination tasks, execution and management of business innovation projects with companies. Currently she is the International Project Manager at ANFACO-CECOPESCA.



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**Partner 37. European Food Information Council (EUFIC), Belgium**

**Description of Organization:** EUFIC (<http://www.eufic.org>) is a non-profit organization, which provides science-based information on food safety and quality and health and nutrition to the media, health and nutrition professionals, educators and opinion leaders, in a way that promotes consumer understanding. EUFIC's mission is to enhance the public's understanding of such issues and to raise consumers' awareness of the active role they play in safe food handling and choosing a well-balanced and healthy diet. With its offices located in Brussels (Belgium), EUFIC counts on and liaises with a European network to enhance the impact and outreach of its communication instruments and programs in other countries. EUFIC actively participates in European initiatives together with the European Commission Directorate Generals for Research (DG Research) and for Health and Consumers (DG SANCO), where it contributes to a number of projects as a research and dissemination partner.

**Main Tasks:** EUFIC will participate in WP31 Dissemination, and will be involved with disseminating relevant, current information from DIVERSIFY to consumers, through a specific link (“quadrant”) in the organization website, leaflets to create awareness for the project and articles in its magazine FOOD TODAY.

**Previous Experience:** EUFIC issues several publications, such as FOOD TODAY, a newsletter that highlights subjects of current interest and is distributed to over 45,000 scientists, educators, media and other communication multipliers in 11 languages, EUFIC Reviews on topical issues, or leaflets on healthy eating for kids and adults. EUFIC also uses new technologies such as podcasts and webinars to disseminate key messages quickly and efficiently. EUFIC also regularly conducts research to gauge consumer attitudes towards specific nutrition and food safety issues. EUFIC has participated in a variety of EU projects, in the area of consumer issues and dissemination.

**Staff profile:** **Dr. Josephine Wills** was appointed to Director General of the European Food Information Council in 2006 and has been involved many FP projects since then. Previously Jo worked for the private sector in science, communication, and regulatory roles for 18 years, latterly as European Head of Scientific and Regulatory Affairs for all product categories. Jo has published over 80 scientific papers, edited four books and lectured extensively worldwide. **Dr. Laura Fernández Celemín** is Director of Nutrition & Health, and Deputy Director General at EUFIC. Laura holds a degree in human nutrition and a PhD in Biomedical Sciences from the Catholic University of Louvain, Brussels, Belgium. Laura's roles include deciding on the Nutrition and Health strategy for EUFIC and the supervision of EUFIC's role in EU-funded research projects. She was in charge of coordinating the FLABEL project on behalf of EUFIC. **Mr. Adrian Giordani** is Communications Manager EU Projects at EUFIC since January 2013. Adrian has a MSc in Science Communication from Imperial College, London and a B.A. in Software Systems. Adrian previously worked as an Editor at CERN for a European Commission FP7 project, and he has wide experienced in editing, sub-editing, interviewing, writing and publishing. In his current position he deals with communications for EU Projects and other science-related content.



**Partner 38. Kentro Meleton Agoras kai Koinis Gnomis Anonimi Etairia - Hellenic Research House (HRH), Greece**

**Description of Organization:** The Hellenic Research House S.A. (HRH) was founded in 1992 in Athens, Greece. Since its foundation, HRH has undertaken hundreds of projects, like B2C and B2B surveys, tracking and ad-hoc studies. It is a full market research institute that provides all kinds of consumer research, opinion poll, research at the point of sales, industrial and medical research, convenience goods research, as well as sociological, statistical, economical and political research. The company's overall objective is to optimise marketing decisions through quality of research design, execution and interpretation.

For the last 20 years, HRH plays an active role in survey design, where it internally creates structured questionnaires, builds and maintains databases, or conducts web-based applications. The company has in its disposal a complete array of technological equipment to meet the needs of a project. HRH employs the necessary statistical packages (SAS, SPSS) to deal with conventional research topics and also to perform online research solutions (GMI's Net-MR). The company is licensed to operate one of the most comprehensive web-based data collection systems, as well as to disseminate research information and results by assigning exclusive access with outmost confidentiality to its collaborators and clients (with a unique user ID and password).

HRH's Qualitative Research techniques include: focus groups and in-depth interviews, conducted by experienced psychologists using advanced and up-to-date psychological methods. Focus groups are carried out in our offices, which are fully equipped, including viewing facilities (closed TV or PC circuit). Quantitative research includes surveys that are carried out using all major data collection methodologies: Door-to-door (national interviewing force), Telephone (in-house CATI on-line network), Hall tests (central Athens or other city location), Business-to-business (specialized national interviewing force), Mystery visits and Observational Research, and Internet Research, where HRH recruits and maintains a panel of respondents, administers questionnaires via e-mail or web site, collects data and performs analysis in real time through a web browser. One of the distinctive features of this method is that the agency creates a client account with a unique id and password for overlooking research development and results in real time.

**Main Tasks:** HRH will be involved in testing of new product strategies performed in the 5 countries selected (i.e. UK, D, ES, F, I) for GWP Socioeconomics. Specifically, HRH will participate in the external (competitive) market analysis and the consumer research, that both are the basis for the new product development performed by other parties. HRH will identify and recruit the external market research agencies or third party knowledgeable individuals/research groups in United Kingdom, Germany, Spain, France, and Italy. HRH will, also, undertake/supervise data collection project management in all these countries.

**Previous Experience:** During its 20 years of operation HRH has implemented more than 2,500 different market research or public opinion studies and has served more than 400 different clients. Furthermore, the company is known in the market research industry as one of the few that knowledgeably and consistently performs the techniques and methods of Applied Statistics. It is worth mentioning that HRH collaborates with several research companies in Greece and abroad. This feature offers an add-on value to the agency's experience. Through the years HRH has managed, conducted or consulted on research projects that involved such categories as food snacks, ready food, food assortments, candies, agricultural products, as well as tracing the origin of food (TRACE – EU Funded Project through the Sixth Framework Programme under the Food Quality and Safety Priority). The agency has participated in a number of EU proposals, in the area of consumer issues and dissemination. HRH has succeeded and continues to do so in applying cost-effective research solutions to the food industry.

**Staff profile:** **Mrs. Hellas-Maria Saltavarea**, the Founder and Managing Director of the HRH will be the Project Leader. In her thirty-six years in market research she has been involved in retailing auditing, statistical analysis, consulting services and other fields of applied statistics. Mrs. Saltavarea holds a Bachelor degree in Mathematics from University of Athens and Master of Science degrees in Business Administration from University of Economics and Business in Athens and in Applied Statistics from Loughborough University of Technology, UK. **Dr Athanasios Krystallis** (Scientific Responsible) is Senior





Marketing Consultant at HRH and ex-Professor of Food Consumer Choice at the Department of Business Administration, AU. His expertise lies in statistical modelling of food consumer behaviour with emphasis on food quality and safety management and new product development and consumer acceptance. He also has expertise in strategic management and brand management. Dr A. Krystallis has participated in more than 5 EU-funded research projects under FPs 5, 6 and 7. **Mrs. Teta Rekka**, the Total Quality and Client Relations Director, will be appointed as the Project Coordinator. In her 30 years of professional career, she has conducted consumer, business, media and social research on over 2000 projects. Mrs. Rekka holds a Bachelor degree in Economics from University of Piraeus and a Post-graduate Certificate in Demography from London School of Economics and Political Science, UK. **Mr. Constantinos Larentzakis** is the Director of Statistical Applications and Methods. His role in the project will be the Lead Statistician and Senior Expert on Sampling Procedures, Data Management and Statistical Analysis. Mr. Larentzakis holds a Bachelor of Science in Mathematics from University of Ioannina. **Ms. Angeliki Manousi** is senior Research Executive and will be the Project Supervisor. She has prepared numerous survey designs and training materials for field researchers. Ms. Manousi holds a Degree in Crop Science from Technological Educational Institute of Epirus and a Master of Science in Marketing and Communication with New Technologies from University of Economics and Business in Athens. **Mrs. Ioanna Zara** serves as the Field Manager and will have the role of Field Coordinator in the project. She has extensive experience in preparing fieldwork materials, sampling frame construction, mapping, data collection, data entry, data editing, tabulation, and applying quality control measures. Mrs. Zara holds a degree in Business Accounting from Technological Educational Institute of Larissa.



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**Partner 39. Fish2Be NV (F2B), Belgium**

**Description of Organization:** Fish 2 Be (F2B) is a fish hatchery in Belgium specialised in the out-of-season juvenile production of pikeperch. The company was established in 2013 by founder Ir. Jiri Bossuyt, a seasoned aquaculturist with 10 years of experiences in cultivating Mediterranean species, such as Sea bream (*S. aurata*) and sea bass (*D. labrax*), and freshwater species of the sturgeon (*Acipenseridae*) and percids family (*Percidae*). F2B has done research in the past on breeding stock nutrition. This was partly subsidised by the Flemish government (IWT 140895). Recently we applied for a continuation of this project and to improve also on bio-security and fish health.

**Main Tasks:** F2B will participate in WP 10 “Nutrition – Pikeperch”, both in Task 10.1 “Increase knowledge of nutrients essential for first feeding of pikeperch” and Task 10.2 “Develop specific enrichment products and formulated diets to improve pikeperch larval performance”. F2B will perform validation tests for both tasks at a commercial scale level to evaluate the best performing protocols, enrichment products and formulated diets that resulted from the work done in lab conditions.

F2B will also participate in WP 16 “Larval husbandry – pikeperch”, in Task 16.1 “Optimal combinations of factors to improve larval rearing” and Task 16.2 “Development of an industrial protocol”. F2B will establish a working protocol from the results of Task 16.1 and perform an evaluation test in year 5 to improve larval growth and to reduce significantly cannibalism and larval mortality.

F2B will also participate in WP 22 “Growth and husbandry – pikeperch” in Task 22.2 “Characterisation of pikeperch growth, immune and physiological status in farm conditions”. Based on the results from Task 22.1 an evaluation test is going to be performed by F2B. Growth and physio-immunological status of fish from 10 g to 800 g will be compared, in farm conditions, between standard husbandry conditions usually applied by F2B and its customers and the best rearing conditions identified in Task 22.1.

F2B will also participate in in WP 30 “Socioeconomics – Business model and marketing strategy development” in Task 30.1 “Business models” and Task 30.2 “Recommendations for industry development and international market expansion”. F2B will aid with both Tasks by providing data and reviewing evaluations done by the workpackage leaders.

**Previous Experience:** F2B has done research in the past on breeding stock nutrition. This was partly subsidised by the Flemish government (IWT 140895). Recently we applied for a continuation of this project and to improve also on bio-security and fish health.

**Staff profile:** **Mr Ir. Jiri Bossuyt** (Scientific Responsible) is the owner and managing director of F2B. He has 10 years of experience in the production of marine fish. There is currently a staff of three people at F2B. Fish 2 be employs also an aquaculture professional, recently graduated from the Wageningen University, The Netherlands, who will be most involved in the practical implementation of the work. Because of seasonal variations, our staff can be quickly expanded to follow the need of the company with part-time contracts as we have done in the past. Also F2B will be hiring two additional technicians for 2018 following a scheduled expansion.





#### **Partner 40. Galaxidi Marine Farms S.A. (GMF), Greece**

**Description of Organization:** The Galaxidi Marine Farms S.A. (GMF), was founded in, GALAXIDI MARINE FARM S.A. (GMF) is a vertically integrated marine aquaculture company, which was established in 1987, and it is situated near the town of Galaxidi on the northern coast of Corinthian Gulf in the central Greece. The species produced include Gilthead Sea Bream (*Sparus aurata*), European Sea Bass (*Dicentrarchus labrax*), Red Porgy (*Pagrus pagrus*), Pandora (*Pagellus erythrinus*), Meagre (*Argyrosomus regius*) and Sharp – Snout Sea Bream (*Puntazzo puntazzo*). The past 29 years, 97% of its production has been exported, most of the European Community countries, mainly to Spain, Italy, Germany, France and Austria.

GMF operations indicate that it is a technological leader in the fish industry. The company has five growing cage units (one of them for organic production), two hatchery sites (one of them certified to produce organic juveniles of sea bass and sea bream) and a modern packing and processing station designed to latest EU specification with automatic grading and weighting machines. The company produces annually 25 million juveniles and 7,000 tons of commercial size fish.

GMF was the first Greek marine farm to be certified, since July 2000, for its entire vertical production, from egg stage to whole fresh fish, according to EN ISO 9002:1994 standard. GMF, today, applies a food safety management system (HACCP) and a quality management system for the production of fry, fish farming, packaging, and the distribution of fresh fish according to EN ISO 22000:2005 and EN ISO 9001:2008 standards respectively, certified by TUV Austria Hellas.

In 2008 the firm acquired Certificate of Compliance Management System Biological Products, according to Standard NATURLAND STANDARDS FOR ORGANIC AQUACULTURE: 08, for the Breeding, Packaging and Sale –inspected from "IMO GmbH" lots- bream and sea bass. Today, the entire organic production is certified by BIO HELLAS and Galaxidi Marine Farm S.A. is the only company in Europe certified to produce organic juveniles of sea bass and sea bream.

Also in 2015, the company acquired the Certificate of Compliance Management System of Integrated Farm under GLOBAL G.A.P. Protocol, AQUACULTURE, IFA 4.0-2: 13, for Fry Production, Breeding, Packaging and distribution of fresh seafood. In July 2016, our packing station has been inspected in order to be certified by TUV Austria Hellas, to acquire the IFS Food Standard Version 2014.

Galaxidi Marine Farm S.A. is one of the oldest and most successful aquaculture companies in Greece and the Mediterranean region. Its success comes not only from the solid management and focusing on high quality products, but also on forward thinking and the employment of the latest technologies on production methods. The company has a long tradition in testing and using innovative technologies primarily created for other species but which had been adapted to the needs of Mediterranean fish farming. In addition to the above, the company has also participated in National and European projects and also has private contracts with research organizations and universities, in order to support its strategic growth in the sector.

GMF S.A. is a company that always wants to try new challenges, technologies and actively contribute to the development of the aquaculture sector. Specifically for greater amberjack the company has maintained a large broodstock (n=28) of wild-caught fish for the past 3 years, and has successfully induced spawning and obtained eggs. For the past 2 years, a small number of juveniles have also been produced through the operation of the companies' hatchery facilities. Therefore, Galaxidi is an important commercial partner for this proposal, as it will provide both a reproductive stock and eggs, but also juveniles of greater amberjack. Galaxidi is currently the only aquaculture company in Greece that has any experience with the larval rearing of this species, and only one of two companies in Greece with experience in the broodstock management and egg production of this species. A new independent broodstock department for the installation and reproduction of our new species Greater amberjack (*Seriola dumerilli*) and Dentex (*Dentex dentex*) is under construction.

**Main Tasks:** GMF will participate in WP3 Reproduction and Genetics - greater amberjack, in Task 3.2 Development of an optimized spawning induction protocol for captive greater amberjack in the



Mediterranean and Task 3.5 Spawning induction of greater amberjack and egg collection in cages. The company has obtained mature breeders from the wild and maintains them in cages in the facility for the duration of the project. The broodstock will be used for spawning induction experiments, using hormone treatment and for the development of egg collection methods from sea cages. Eggs obtained from these experiments will be used for the rearing of the larvae and the production of juveniles for the other WPs of the project.

### Previous Experience

1. **REPROSEL (FP7-SME-2010-1):** REPROduction protocols and molecular tools for mass spawning and communal rearing based on SElective breeding schemes applied to multiple-spawning marine fish.
2. **National Program on Development of the Industrial Research and Technology (2005):** Exploitation of solar energy for the operation of a capillary pump heat transfer system and its implementation in aquaculture heating systems.
3. GMF currently runs a **breeding selection program** for gilthead sea bream and European sea bass, supervised by the University of **Wageningen**, Animal Breeding & Genomics Center, Aquaculture Department.
4. GMF has private contracts with **HCMR** on the broodstock management, reproduction, larval rearing etc. of Greater amberjack (*Seriola dumerilli*).

**Staff Profile:** **Ms Kalliopi D. Tsakoniti** (Principle Investigator), R&D Department. Ichthyologist, M.Sc. in Aquaculture Nutrition. Responsible for the company's breeding program. Six years of experience in data analysis and production planning (e-mail: [k.tsakoniti@gmf-sa.gr](mailto:k.tsakoniti@gmf-sa.gr)). **Mr George Iakovopoulos**, On-growing and Hatchery Production Manager. 28 years of experience in aquaculture, hatchery and on-growing management, trained by "Marine Farm Technology Ltd" in Greece and abroad. **Mrs Harikleia Tsouni**, Hatchery Manager. 20 years of experience in broodstock, rotifer, artemia, larvae and juveniles production.



## B 2.3 Consortium as a whole

### *Describe:*

*How the beneficiaries collectively constitute a consortium capable of achieving the project objectives, and how they are suited and are committed to the tasks assigned to them. Show the complementarity between participants.*

*Explain how the composition of the consortium is well-balanced in relation to the objectives of the project.*

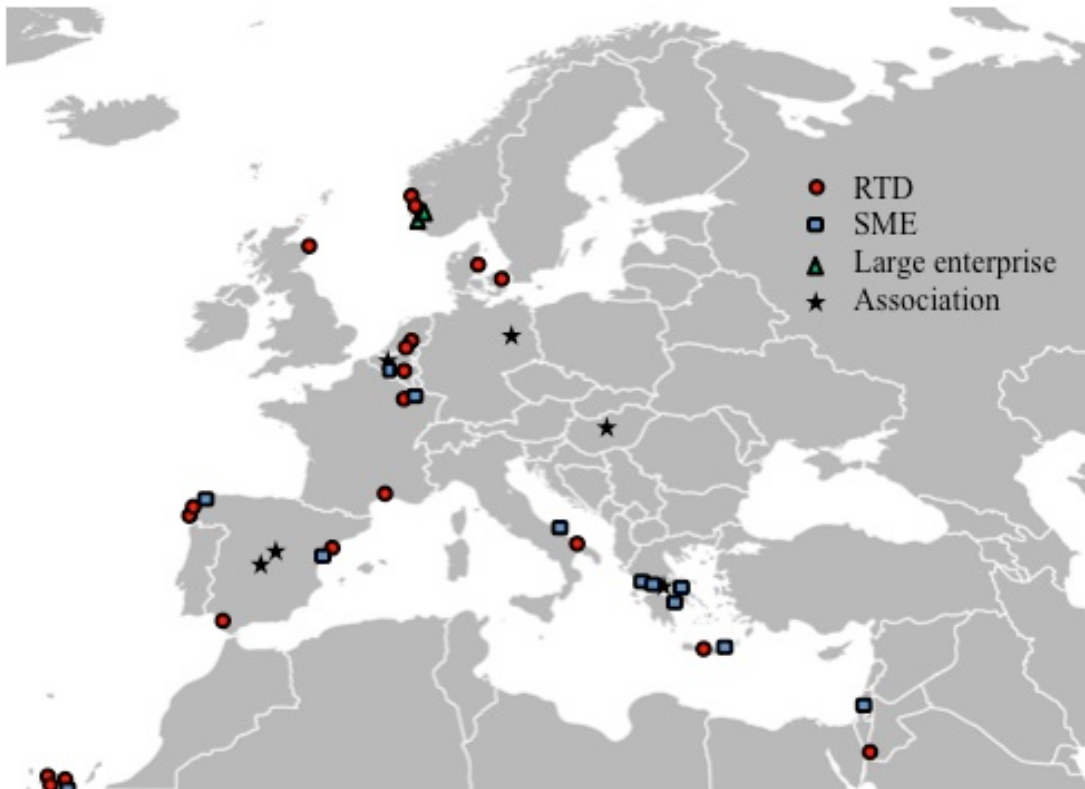
*If appropriate describe the industrial/commercial involvement to ensure exploitation of the results, and*

*If appropriate, show how the opportunity of involving SMEs has been addressed.*

The DIVERSIFY Consortium consists of 38 Partners selected on the basis of their excellence and the requirements of the proposal to meet the aims of the Call. All Partners have verifiable excellence and expertise in the subjects and priorities of the Call text, namely the (a) **Diversification of fish species and products in European aquaculture**, (b) the species selected: meagre, greater amberjack, pikeperch, Atlantic halibut, wreckfish and grey mullet and (c) the required research areas: reproduction, genetics, nutrition, fish health larval rearing and grow out husbandry, as well as in aspects related to market dynamics, new product development, adding value to raw products, processors', consumers' and retailers' preferences, etc.

In the summer of 2018, an Amendment 4 was launched to account for the Universal Take over of two partners. Partner P35. MASZ was taken over by a new Partner (MAHAL), and Partner P17. NIFES was taken over by P7. IMR. Partner MAHAL and P7. IMR, take full responsibility of the activities of P35. MASZ and P17. NIFES, respectively, as regards the project and all activities described in the DOW.

### Distribution of Partners around Europe




**Participant vital-statistics, Country of origin**

- 10 European Union countries (Belgium, Denmark, France, Greece, Italy, Netherlands, Spain United Kingdom, Hungary and Germany)
- 2 associated countries (Norway and Israel).

**Type of organization**

- 20 universities/research institutions (7 from Spain, 2 each from Denmark, France, Norway and Netherlands and 1 each from Greece, Belgium, Italy, United Kingdom and Israel)
- 9 SMEs (6 from Greece, and 1 each from Spain, Belgium and Israel). Two SMEs from France and Italy have exited during 2016 and have been replaced)
- 6 Associations, aquaculture, fisheries, food and fish processing (2 from Spain and 1 each from Greece, Belgium, Hungary and Germany)
- 2 Large enterprises (Norway)

Before describing the consortium as a whole, it is necessary to discuss the selection process and criteria for the species involved in DIVERSIFY, the identification of research areas and the allocation of resources. These factors guided strongly the selection of Partners for the consortium.

**1. Objectives of the Proposal, species selection and allocation of funds**

The aim of DIVERSIFY is to explore the biological and socio-economic potential of new/emerging candidate fish species for aquaculture and subsequently support the diversification of the activity in terms of species, products and markets. The first consideration to ensure this aim for DIVERSIFY, was the **selection of species and allocation of RTD funds**. This was made with three basic considerations:

- The candidates should be large and/or fast growing species that can be processed into new value added products,
- A focus on Mediterranean species was chosen, as the European Aquaculture Technology and Innovation Platform (EATIP) clearly identifies that the Mediterranean both requires and has greater potential for species diversification (EATIP, 2012),
- An inclusive approach should be followed that would provide new species/products for all types of European aquaculture --such as cold marine water in northern Europe, cool marine water on the Atlantic coast, warm marine water in the Mediterranean and fresh water, as well as extensive and semi-intensive pond aquaculture.

**Species selection criteria and allocation of resources**

Species	Inclusion in industry	Fast growth, large size	Processing to new products	Potential for high output production	Allocation of resources*
Meagre	+++	+++	+++	+++	22.9%
Greater amberjack	+	+++	+++	+++	31.3%
Pikeperch	+++	+++	+++	++	14.2%
Atlantic halibut	+++	++	+++	++	13.2%
Wreckfish	0	+++	+++	+++	7.1%
Grey mullet	++	++	+++	++	11.3%

\*Percent of RTD budget



Meagre and greater amberjack offer excellent potential for Mediterranean cage culture, both near and offshore and are, therefore, priorities for DIVERSIFY. Meagre was indicated by EATIP as a species to diversify Mediterranean aquaculture and this high potential combined with a complex bottleneck of variable growth in grow out indicates a budget of 25.3% is justified to achieve the aims. Greater amberjack exhibits superior growth and species of the same genus and family have been demonstrated to be aquaculture successes around the world with a global aquaculture production of 185,000 t in 2010 (FAO, 2012). The high market price, 14 € kg<sup>-1</sup> (FAO, 2012) and global market has attracted industry interest and 4 SMEs and 1 enterprise have entered DIVERSIFY for this species. This potential for mass production, identification of many bottlenecks and allocation of budget to incorporate industry Partners dictates a budget of 33.3% for greater amberjack.

Wreckfish and grey mullet are two more species with a potential for the Mediterranean. The grey mullet is a herbivorous fish and can promote sustainable marine fin-fish culture with diets free from fish products. Bottlenecks are fewer, as an industry exists using wild juveniles and although this will provide a highly sustainable product the potential appears lower than the other two species (partly due to its limited application to cage culture), and consequently the budget is 9.2%. Wreckfish offers both potential for the Mediterranean and cool water (Atlantic coasts of Portugal, Spain, France, etc.) cage culture, which to date has been poorly studied (and not yet exploited by the aquaculture industry) and do not feature in the EATIP Vision Document (EATIP, 2012). Providing the biological knowledge to culture wreckfish strengthens the possibility for offshore aquaculture in the Atlantic and adds a fourth species for the Mediterranean. However, despite of the excellent potential of wreckfish, few studies have been made on the biology of the specie in captivity and this factor of the “unknown” makes wreckfish a higher risk and consequently the budget is the smallest of all selected species (7.5%).

Pikeperch is a fresh water species, growing fast in RAS to a large size for processing. The EATIP identifies that species diversification and RAS are important for fresh water aquaculture. Pikeperch is in production and has been studied, bottlenecks are fewer and fresh water offers a more confined potential for expansion compared to marine species and, therefore, a budget of 15.3% was allocated. Lastly, cold-water aquaculture is dominated by Atlantic salmon and EATIP does not expect this to change, the ideal production areas are taken by salmon and future expansion is expected by increased efficiency of salmon production and possible offshore culture. However, there exist good possibilities for moderate expansion into other species and flatfish are expected to gain in importance. Atlantic halibut is a large species that can be reared in cages and processed into added-value products, and is an obvious candidate for aquaculture expansion in cold waters. The Atlantic halibut has received extensive funding in the past and recently an important bottleneck of poor male growth has been overcome. DIVERSIFY will address the remaining bottlenecks to promote expansion of this species with a budget of 9.5%.

## *2. Partner selection and consortium as a whole*

So, given the above species selection, the selection of the **RTD Partners** for DIVERSIFY was based on their **knowledge and involvement in ongoing initiatives with the selected fish species**, a demonstrated research focus in line with the research priorities of the call text and the expected impact of the project. Therefore, in accordance with the call text, DIVERSIFY Partners will build (without overlapping) on recent and ongoing initiatives (see Table 2.3a at the end of this section), and will focus on the targeted issues. All RTD Partners are **leaders within their fields** with internationally demonstrated state-of-the-art knowledge, expertise and methodologies, and a strong vision on how to advance. As mentioned at the beginning, all Partners have verifiable excellence and expertise in the subjects and priorities of the Call both in the species selected and the required research areas. Partners included are some of the best known in research areas of the chosen species --such as the IMR in Atlantic halibut, IOLR in Grey mullet, UL/DTU/FUNDP in pikeperch, IEO/FCPCT/HCMR in greater amberjack, IRTA/HCMR/FCPCT in meagre and the IEO/IFREMÉR/HCMR in wreckfish-- as well as in the scientific disciplines required. A producer association (APROMAR) is also involved and will contribute with the knowledge of the production issues and industry limitations.

Some of the RTD Partners in DIVERSIFY have been working together in various previous and current projects, both nationally and EU funded ones, and have developed already very close interactions, and





efficient communication and data/information exchanges. Their involvement in DIVERSIFY to bring efficiency and effectiveness in implementing the various activities planned in the project. The inclusion of new Partners, or groups of Partners that have not worked with others in the consortium so far, is also considered invaluable, as it will contribute to expanding and strengthening the European research area, by developing more interactions among RTD centres from throughout Europe. Also, to ensure a smooth and effective project operation regarding management, integration between Partners and effective dissemination, Partners with long and successful track records of prior EU funded collaborations, including several coordinating responsibilities, have been chosen.

The Partners responsible for GWP Socioeconomics are uniquely qualified to achieve the objectives of the project. The three key Partners (SWR, AU and TU/e) have world-class expertise in the food market and/or business research and are perfectly complementary with each other, with different competencies in terms of disciplinary and methodological approach, bringing to the project their extensive expertise and experience. The GWPL (SWR) is the leading institute for social and economic research on fisheries and other food sectors in the Netherlands and among the best in Europe. SWR has long-term experience in food-related consumer marketing research and marketing related to new food product development, and considerable experience with EU FP projects. Also, SWR has considerable experience in EU FP projects. The major experience of AU is in analyzing the social-psychological determinants of food choice and eating habits, with special emphasis on the marketing and communication parameters affecting food choice. TU/e specializes in engineering science & technology and the translation of research results into successful innovations and business plans that serve as a basis for creating new products, processes and enterprises, which is particularly relevant for the industrial/commercial involvement of the participating SMEs and large enterprises, in order to ensure successful exploitation of the results. The above three key Partners are coupled with a number of Partners with the necessary expertise in the technical development (IRTA, CTAQUA) and evaluation of the new fish products (HCMR, IRTA, ULL, CTAQUA) and an aquaculture producer association (APROMAR) who will contribute knowledge of the production and marketing issues of the industry. A market research SME (HRH) has also joined the consortium recently, and will take care of the originally sub-contracted activities in WP28 and 29, and will also be involved slightly in WP30. Overall, these Partners in DIVERSIFY are among Europe's best when it comes to applying a socioeconomic/business approach to analyzing the determinants of consumer acceptance of the products resulting from the new species under study. In this way, business and technical expertise will be optimally combined all along WP7 to lead the ideal transformation of raw materials into high added-value end-products that will be valued by consumers, markets and the industry.

A large number of **participating SMEs and large enterprises** representing producers of five of the six selected species (except wreckfish), also joined DIVERSIFY, to ensure optimal expertise, and access to commercial scale aquaculture facilities (especially sea cages) for the species to be studied, in an equal fashion. The participation of SMEs also ensures that the research undertaken is meaningful to the industry and the results will be rapidly absorbed and applied. The involvement of no less than 9 SMEs and 2 large enterprises together with the RTD Partners was an active strategy to ensure strong connections between the research expertise at the universities and research institutes with the applied expertise in companies of different branches and sizes. Therefore, the 9 SMEs and 2 large enterprises are ready to invest in the selected species with business plans based on innovation to increase aquaculture production of these species. This group of companies is best characterised by knowledge of the selected species and a high degree of innovation both to produce new species and new processed products. For example, the 1 SMEs (ARGO) and 1 large enterprise (SARC) working with meagre have a combined knowledge that has been gained from the initiation of meagre culture over 10 years ago and which has overseen the production of over 3000 t of meagre, representing over 30% of the meagre cultured in Europe. One of the large enterprises in the consortium (SWH) is the biggest producer of Atlantic halibut in the world with 1000 t per year (55% of the world's production). The pikeperch SME (F2B substituting ASIALOR) has won an award for innovation in 2012 and works with fully controlled environment in RAS to achieve all-year-round production. The consortium includes a group of 4 SMEs (ARGO, GMF substituting ITICAL, FORKYS and CANEXMAR) and 1 large enterprise (SARC) to work on greater amberjack that demonstrate innovation, foresight and a track record of committing resources to species diversification. FORKYS will collaborate also with meagre and has great interest in wreckfish. The grey mullet SMEs (DOR, GEI and IRIDA) demonstrate high innovation in producing value added products, with involvement in certification schemes and processes such



as smoking, and have high interest in producing high quality grey mullet and grey mullet roe (bottarga) for the southern European and International markets. The feed manufacturer SME who will be responsible for producing the grow out feeds for all grey mullet work (IRIDA), has been producing feeds for various marine fishes (beyond gilthead sea bream and European sea bass), and has participated in national research programmes for the domestication of Atlantic bluefin tuna and meagre. Also, the largest feed manufacturer in the world and with the best feed/nutrition research centre (SARC) has been collaborating with many of the RTD Partners in previous projects and will be involved in DIVERSIFY, in the development of optimal diets that will ensure these species have optimal growth combined with minimal feed costs and environmental pollution. Finally, an SME is participating in GWP Socioeconomics (HRH), in the market research and focal groups.

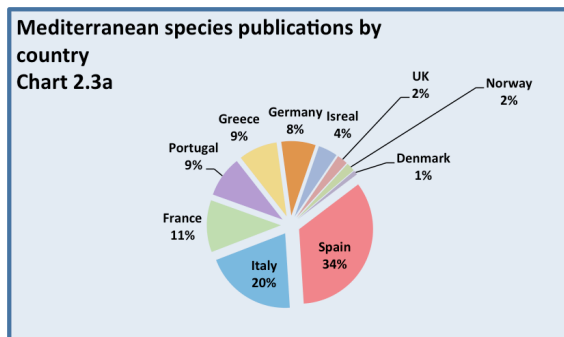
The SMEs and large enterprises will be responsible for the field application of the research carried out in the project as well as for the production of the diets used in the different studies. A true collaboration and active cross-talk between RTD Partners and industrial Partners is one of the key objectives within the DIVERSIFY. This objective will be ensured through active involvement of the SMEs in RTD activities, as well as active and direct dissemination of research results by RTD Partners to the industrial Partners.

The structure of the consortium is well balanced in relation to the objectives of the DIVERSIFY project, the scientific requirements of the specific Call and to ensure efficient transfer of results and outcome of the project to the Industry, mainly SMEs. The composition of DIVERSIFY was selected to ensure adequate transfer of knowledge and techniques between Partners from different geographic locations as well as between the different fish species. It also aims at creating an active dialogue between researchers on the one hand and aquaculture breeders, producers, retailers and aquaculture feed producers on the other. This is in order to ensure transfer of knowledge to the aquaculture sector. To ensure also that the planned deliverables will not be hampered by relying on just one Partner working with a certain species, the activities of DIVERSIFY have been distributed widely among appropriate Partners:

- Meagre: 9 RTDs, 1 SMEs, 1 large enterprises
- Greater amberjack: 8 RTDs, 4 SMEs, 1 large enterprise
- Pikeperch: 8 RTDs, 1 SME
- Atlantic halibut: 4 RTDs, 1 large enterprise
- Wreckfish: 7 RTDs, 1 large enterprise
- Grey mullet: 8 RTDs, 3 SMEs

### 3. Geographical considerations

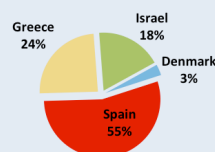
The selection of Partners has ensured a geographical composition of the consortium that will cover northern, central and southern areas of European aquaculture. Focused around the North sea, several Partners with expertise and facilities for Atlantic halibut aquaculture, nutrition and biology are found. Partners in the centre of Europe have a large experience of and will focus on pikeperch aquaculture while Partners from the Mediterranean sea and Atlantic Ocean have the knowledge and expertise associated with farming of meagre, greater amberjack, grey mullet and wreckfish.



The emphasis of DIVERSIFY on Mediterranean species resulted in more Partners (23 Partners or 60%) from Mediterranean countries and fewer (15 Partners or 40%) from Northern countries (including France). A total of 66% of the Total budget has been allocated to Partners in Mediterranean countries working on Mediterranean species, including wreckfish. This can be further broken down to show eight RTD centres,



Recent research projects in the selected Mediterranean species by country (total of 17)  
Chart 2.3b



one SME and one large enterprise (26% of the Partners) are in Spain and receive 32% of the budget. Also, the HCMR from Greece receives 14.6% of the budget. Therefore, the selection of more Partners from the Mediterranean is related to the emphasis on and higher allocation of resources to Mediterranean species, which is in turn related to species culture potential and required tasks to achieve the Calls aim. The allocation of many research tasks for the Mediterranean species also resulted in a high number of Spanish institutions,

which is related to a significantly higher research effort by Spanish institutions in aquaculture. This research effort can be seen in an analysis of a Web of Knowledge search for scientific publications with the species Latin names in the title, in which 34% of all publications (213) were from Spain (Chart 2.3a). These publications are a testament to a higher number of recent and ongoing initiatives with the selected species by Spanish research organizations. Another piece of information underlining the importance of Spanish Partners in DIVERSIFY is the fact that of the 17 recent/ongoing research projects involving one (or more) of the selected Mediterranean species, 55% were Spanish national ones (Chart 2.3b). An advantage of having these Spanish Partners which are placed in completely different locations within Spain (North Atlantic –IEO, CMRM-, South Atlantic –FCPCT, IEO, ULL, CANEXMAR- and Mediterranean –IRTA, APROMAR, CTAQUA), is that through these national projects, they have developed strong synergies among them (FCPCT with ULL, IEO with ULL and CMRM), having worked together for several years with all of the Mediterranean species considered in the project, in different aspects of their culture or their commercial value for consumers.

The PC Partner (HCMR) receives 14.6% and two Spanish RTD centres (FCPCT and IRTA) receive 7.4% and 8.3%, respectively, of the Total budget. This higher allocation is related to a combination of expertise with the selected species and expertise in various scientific disciplines, resulting in a higher allocation of tasks and, therefore, resources. The HCMR (Greece), in addition to being the PC and responsible for management of the consortium and being also directly involved with dissemination activities, **will participate in all 6 RTD WPs** and will contribute to the work planned for **all six selected species**, albeit not with an effort of similar magnitude in all of them. Its participation will be both with large scale facilities for the rearing trials and analytical labs. In particular HCMR will provide access to the AquaLabs, a certified facility on Crete for experiments and rearing of marine organisms (certification codes EL 91-BIO-03 and EL 91-BIO-04). This facility includes a broodstock area (GWP Reproduction and genetics), an intensive and a semi-intensive (Mesocosm type) hatchery with the necessary infrastructures for phyto- and zooplankton cultures (GWP Larval husbandry), a pre growing zone and specialized facility for nutritional and feeding trials (GWP Nutrition and GWP Grow out husbandry) and a pilot sea cage farm (a unique experimental facility in the Mediterranean: GR94FISH0001) for the implementation of experimental trials in GWP Grow out husbandry. Further to the above facilities, HCMR runs a fully equipped Genetics laboratory that will participate in GWP Reproduction and genetics, two fish pathology laboratories (Crete and Athens) that will participate in GWP Fish health and a wide range of state-of-the-art laboratory equipment that will be used in GWP Nutrition. This expertise in the research areas and species is also evident in the recent and ongoing initiatives and analysis of publications. HCMR has been involved in recent and ongoing initiatives and holds broodstocks for the three Mediterranean species, meagre, greater amberjack and wreckfish. In relation to publications, the Web of Knowledge search for scientific publications with the Mediterranean species showed that HCMR was the single European institution with the most publications in the consortiums Mediterranean species. FCPCT (Spain), have ongoing expertise in meagre and greater amberjack and coordinate GWP Nutrition and the combination of expertise in these species, the availability of biological material and expertise in research areas has resulted in the assignment of tasks in all WPs except socio-economics. IRTA (Spain) is a multidisciplinary research institution dedicated to all aspects of food production with a centre specializing in aquaculture and a centre specialized in food technology and quality of processed products (meat and fish). IRTA has ongoing expertise in meagre and leads the consortium's work on meagre (Species leader) and GWP Reproduction and genetics. The combination of expertise in meagre and expertise in all research areas has resulted in the allocation of tasks in all WPs including





socioeconomics. Finally, both FCPCT and IRTA also have large-scale facilities for rearing trials and analytical laboratories.

Notwithstanding the emphasis on the Mediterranean, DIVERSIFY spans as far as Israel to the East and the Canary Islands to the West, and as far South as Crete, and Norway to the North. Strong, functional and long-term relationships also exist among RTD Partners and among them and SMEs or large enterprises in these participating countries. These relationships include the HCMR with the 5 SMEs from Greece, since this RTD performer is the main research organization in the area of aquaculture in Greece, and similar strong long term working relationships exist between IOLR and DOR; IMR with NIFES, SWH and SARC; UL with DTU and FUNDP, and SWR with TU/e and AU.

**4. Suitability of the task assignments, aims and planning:**

Research tasks were assigned on the basis of (a) available expertise with the species, building on ongoing initiatives and/or (b) expertise in a scientific discipline. Thus, DIVERSIFY has assigned tasks that build on past and current national and EU research initiatives (Table 2.3a) in which Partners of the consortium have coordinated or participated often in collaboration with other Partners in DIVERSIFY, hence facilitating the transversal work that will be required in DIVERSIFY. These projects collectively set out the groundwork for future work providing partial or complete solutions to bottlenecks, and indicating future bottlenecks and how to tackle the development of solutions. Background work and relevant information has been obtained by various Partners in the research areas (rows) and for the selected species (columns) as indicated in the following Table.

**Background work done in various scientific disciplines in the selected species by DIVERSIFY Partners**

Background work	Meagre	Greater amberjack	Pikeperch	Atlantic halibut	Wreckfish	Grey mullet
Establish broodstock	HCMR, FCPCT, IRTA	HCMR, FCPCT, IEO	ASIALOR	IMR, SWH	HCMR, IEO, CMRM, MC2	IOLR, DOR
Control of reproduction	HCMR, FCPCT, IRTA	HCMR, FCPCT, IEO	UL, DTU, FUNDP, ASIALOR	IMR, NIFES, SWH	HCMR, IEO, CMRM, MC2	IOLR
Tools for genetic characterisation	FCPCT, IRTA					
Larval rearing protocols	HCMR, FCPCT, IRTA	HCMR, FCPCT, IEO	UL, DTU, FUNDP, ASIALOR	IMR, NIFES, SWH	HCMR, IEO	IOLR
Nutritional requirement studies	HCMR, FCPCT, IRTA	HCMR, FCPCT, IEO, ULL	UL, DTU, FUNDP	IMR, NIFES		IOLR
Reduction of dietary fish meal / oil	HCMR, IRTA					IOLR
Identification of health issues	HCMR	HCMR, FCPCT				
Marketing and new products	CTAQUA					



This research effort in recent and ongoing initiatives by many of the Partners of DIVERSIFY has resulted in key references on the targeted species (See Table below) and demonstrate further the background work that has been completed and taken into consideration for the selection of research activities, as well as the allocation of Tasks to the different Partners, in order to meet the aims of the Call and ensure the success of DIVERSIFY.

**Scientific publications in various scientific disciplines in the selected species by DIVERSIFY Partners. The Reference list is at the end of this Section.** (Peer reviewed in black, non-peer reviewed in grey)

<b>Reviews on species culture</b>	
Meagre	Duncan et al., 2013
Pikeperch	Kestemont, Melard, 2000
<b>Control of Reproduction and Genetics</b>	
Meagre	Fernandez-Palacios et al., 2007; 2009; 2011b; Duncan et al., 2008; 2012; Andree et al 2010; Mylonas et al., 2011; Soula et al., 2011
Greater amberjack	Mylonas et al., 2004; Jerez et al; 2006; Jerez et al; 2007; 2009; 2011; Rodríguez-Barreto et al., 2012
Pikeperch	Kucharczyk et al., 2007; Teletchea et al., 2009; Henrotte et al., 2010; Zarski et al., 2012
Atlantic halibut	Norberg et al., 1991; 1995; 2001; Bjormsson et al., 1998; Finn et al., 2002; Vermeirssen et al., 2003; Babiak et al., 2012
Wreckfish	Papandroulakis et al., 2004; Papandroulakis et al., 2008; Fauvel et al., 2008
Grey mullet	Aizen et al., 2005
<b>Basic husbandry and rearing protocols for larvae</b>	
Meagre	Roo et al., 2007; 2009; Roo et al., 2010a,b; Hernandez et al., 2007; Scabini et al., 2008; Papadakis et al., 2008; 2009; 2013; Abreu et al., 2009; Fernandez-Palacios et al., 2009; Vallés and Estévez, 2013;
Greater amberjack	Papandroulakis et al., 2005; Papadakis at al., 2013
Pikeperch	Hamza et al., 2008; Kestemont et al., 2007; Lund, Steinfeldt, 2011; Steinfeldt et al., 2010; Lund et al., 2012;
Atlantic halibut	Hamre et al., 2008a,b; 2013a,b; Harboe et al., 2009
<b>Nutrition and Grow out</b>	
Meagre	Chatzifotis et al., 2010; 2012; Estevez et al., 2011; Gines et al., 2013
Greater amberjack	Aly et al., 1999; Papadakis et al., 2008
Pikeperch	Steenfeldt, Lund, 2008; Steinfeldt et al., 2010
Wreckfish	Papandroulakis et al., 2004; Peleteiro et al., 2010; 2011; Rodriguez-Villanueva et al., 2011
<b>Health</b>	
Meagre	Katharios et al., 2011
<b>Marketing, new processed products, food (and fish) and consumer behaviour,</b>	
Meagre	Monfort 2010; Grigorakis et al., 2011
General	Arvanitoyannis et al., 2004; Krystallis et al., 2010



The consortium's expertise in the state-of-the-art of the relevant scientific disciplines is also evident through book chapters and review articles published by the Partners:

Research area	Citation
Reproduction and genetics	Mylonas and Zohar 2001; Mylonas et al., 2010a; Duncan et al., 2013
Larval rearing and nutrition	Izquierdo et al., 2001; Koven 2003; Rosenlund et al., 2010; Izquierdo, Koven 2011; Hamre et al., 2013
Grow out	Mozes et al., 2012
Health	Secombes, Ellis, 2012
Marketing, new products	Grunert, Wills, 2007; Monfort, 2010

A point also worth mentioning is that even RTD Partners that have not been involved so far with research of the selected species, have experience in projects aimed at advancing basic and applied knowledge to the aquaculture production for new fish species with similar characteristics as those selected in DIVERSIFY (e.g., UNIBA with Atlantic bluefin tuna). Therefore, the consortium demonstrates high degree of expertise in the species and research areas selected, and tasks have been assigned to the appropriate experts to ensure aims and plans are fulfilled.


**Table 2.3a.** Research Projects on the selected species of DIVERSIFY, where Partners from the consortium were involved.

	Species	Country	Partner	Project title	Funding agency	Duration
1	Greater amberjack	Spain	IEO, ULL	Assessment on the essential fatty acid requirements and carotenoids of <i>Seriola dumerili</i> broodstock (Ref.: AGL2008-05014.C02)	Spanish Government: Ministry of Education and Science/ Canary Islands Government	2009-2011
2	Greater amberjack	Spain	ULL	Fish meal and fish oil substitution by vegetable oils in <i>Seriola dumerili</i> diets. Analytical study: lipids. (Ref.: AGL2011-30547-C03)	Spanish Government: Ministry of Science and Innovation	2011-2013
3	Greater amberjack	Spain	IEO, ULL	Evaluation of the requirements of essential fatty acids and carotenoids of <i>Seriola dumerili</i> broodstock.	Spanish Government: Ministry of Education and Science	2009-2011
4	Greater amberjack	Spain	IEO, ULL	Evaluation of the requirements of essential fatty acids and carotenoids of <i>Seriola dumerili</i> broodstock.	Canary Island Government: Agency for Research, Innovation and Information Society	2009-2011
5	Greater amberjack	Spain	FCPCT, IEO	Pilot project for the production of Carangids.	Canary Island Government: University Foundation of Las Palmas	2011
6	Greater amberjack	Greece	HCMR	KRIPIS-Diversification of aquaculture with new fast growing species.	General Secretariat for Research and Technology	2013-2016
7	Atlantic halibut	Norway	IMR, NIFES	Improving metamorphic success and juvenile quality in Atlantic halibut and white grouper through dietary supplementation of iodine.	Norwegian Government: The Research Council of Norway	2005-2007
8	Atlantic halibut	Norway	IMR	Gutfeeling. New feeding strategies for Atlantic halibut and cod larvae to increase the output of high quality juveniles in production systems.	Norwegian Government: The Research Council of Norway	2009-2012
9	Atlantic halibut	Norway	IMR	LIFECYCLE	EU, 7th Framework	2009-2013
10	Atlantic halibut	Norway	IMR, SWH	Production of all female Atlantic halibut.	Sterling White Atlantic halibut	2010-2014
11	Atlantic halibut	Norway	IMR	PROPHYLHATCH	Norwegian Government: The Research Council of Norway	2006-2008
12	Atlantic halibut	Denmark	HCMR, IMR	PROAQUA	Danish Government: The Research Council of Denmark	2013-16
13	Meagre	Spain	IRTA, FCPCT	Spanish national plan for the culture of meagre ( <i>Argyrosomus regius</i> ).	Spanish Government: Ministry of Agriculture, Food and Environment	2005-2008
14	Meagre	Spain	HCMR, IRTA, FCPCT	Research of the control of reproduction of the meagre ( <i>Argyrosomus regius</i> ) held in captivity.	Spanish Government: Ministry of Science and Innovation	2008-2011
15	Meagre	Greece	HCMR	Development of reproduction and culture methods for meagre ( <i>Argyrosomus regius</i> ) to improve the competitiveness of the aquaculture industry.	Greek Government: National programme KRANIOS	2011-2014
16	Grey mullet	Israel	IOLR	Growing grey mullet in integrated systems to improve bottarga production.	Middle East Regional Cooperation (MERC) Egypt-Israel	2011-2013
17	Grey mullet	Israel	IOLR	Improved management of restocking the Kinneret (Sea of Galilee).	Israel Ministry of Agriculture	2011-2013



Species	Country	Partner	Project title	Funding agency	Duration	
18	Grey mullet	Israel	IOLR	The culture of grey mullet in Israel and Egypt	Middle East Regional Cooperation (MERC) Egypt-Israel	2002-2007
19	Grey mullet, pikeperch	Denmark	DTU	New species in Danish Aquaculture.	European Fisheries Fund (EFF) and Danish Ministry of Food, Agriculture and Fisheries	2011-2012
20	Pikeperch	Denmark	DTU	Development of production techniques and systems for rearing of pikeperch	European Fisheries Fund (EFF) and Danish Ministry of Food, Agriculture and Fisheries	2004-06
21	Pikeperch	Denmark	DTU	Refining intensive rearing methods of pikeperch.	European Fisheries Fund (EFF) and Danish Ministry of Food, Agriculture and Fisheries	2007-2009
22	Pikeperch	Belgium	UL, FUNDP	Improving pikeperch juvenile production by broodstock management, nutrition, sex control and culture techniques (LUCIOPERCIMPROVE).	EU-FP6-COOP-CT-2005-017646	2005-2007
23	Pikeperch	Belgium	FUNDP	Bioeconomic feasibility of pikeperch intensive culture in recirculating system (LUCIOPERCA).	EU-FP5-CRAFT project	2001-2003
24	Pikeperch	France	UL, ASIALOR	Development of a continuous production of pikeperch fingerlings in RAS to supply the Asialor growing units.	French funding (Oséo-innovation grant)	2011-2014
25	Wreckfish	Greece	HCMR	“State of the stocks of European wreck fish ( <i>Polyprion americanus</i> )”.	EU DGXIV, project 98/041.	1998-2002
26	Wreckfish	Greece	HCMR	“Development of methodologies for reproduction, husbandry and larval rearing of the wreckfish, <i>Polyprion americanus</i> – Wreckfish”.	Greek Government General Secretariat for Research and Technology, EURECA	2005-2008
27	Wreckfish	Spain	IEO	The Grouper: An endangered species and how to addressing its disappearance.	Spanish Government: Biodiversity Foundation. Ministry of Agriculture, Food and Environment	2011-2012
28	Wreckfish	Spain	IEO	The culture of large groupers.	Spanish Government: Spanish Institute of Oceanography	2011-2012
29	Wreckfish, Greater amberjack	Greece	HCMR	“Innovative methods for reproduction and larval rearing of fast growers”.	Greek Government Greek Ministry of Agriculture, OPF	2005-2008

### Reference list of Publications on the selected species by consortium Partners (in bold)

- Abreu, N., Socorro, J., Betancor, M., Caballero, M.J., **Fernández-Palacios, H.**, Hernández-Cruz, C.M., **Roo, J.**, Schuchart, D., 2009. New findings in organogenesis in **meagre larvae** (*Argyrosomus regius* Asso, 1801). XII Congreso Nacional de Acuicultura, Madrid, Spain.
- Aizen, J., Meiri, I., Tzchori, Levavi-Sivan, B., **Rosenfeld, H.**, 2005. Enhancing spawning in the **grey mullet** (*Mugil cephalus*) by removal of dopaminergic inhibition. Gen. Comp. Endocrinol. 142: 212-221.
- Aly, T.S., Garcia, A., **Izquierdo, M.S.**, Jover, M., 1999. Utilization of different sources of lipids in extruded diets for ***Seriola dumerilii*** (Risso). VII Congreso Nacional de Acuicultura, Las Palmas, Spain.
- Andree, K.**, Axtner, J., Bagley, M.J., Barlow, E.J., Beebee, T.J.C., et al., 2010. Permanent Genetic Resources Note. Permanent Genetic Resources added to Molecular Ecology. Resources Database 1 April 2010–31 May 2010. Molecular Ecology Resources 10: 1098–1105.
- Arvanitoyannis, I.**, **Krystallis, A.**, **Panayotaki, P.**, **Theodorou, A.I.**, 2004. An investigation of consumer attitudes towards marine fish consumption. Aq. Int. 12: 259-279.



- Babiak, J., Babiak, I., Harboe, T., Haugen, T., van Nes, S. and **Norberg, B.**, 2012. Induced sex reversal using an aromatase inhibitor, Fadrozole, in **Atlantic halibut (*Hippoglossus hippoglossus*)**. *Aquaculture* 324-325: 276-280.
- Björnsson, B.T., Halldórsson, O., Haux, C., **Norberg, B.**, Brown, C.L., 1998. Photoperiod control of sexual maturation of the **Atlantic halibut (*Hippoglossus hippoglossus*)**: plasma thyroid hormone and calcium levels. *Aquaculture* 166: 117-140.
- Chatzifotis, S.**, Panagiotidou, M., Divanach, P., 2012. Effect of protein and lipid dietary levels on the growth of juvenile **meagre (*Argyrosomus regius*)**. *Aquacult. Int.* 20: 91-98.
- Chatzifotis, S.**, Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, I., Mylonas, C.C., 2010. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of **meagre (*Argyrosomus regius*)** juveniles. *Aquaculture* 307: 65-70.
- Duncan, N., Estevez, A.**, Padros, F., Aguilera, C., Montero, F.E., Norambuena, F., Carazo, I., Carbo, R., **Mylonas, C.C.**, 2008. Acclimation to captivity and GnRHa-induced spawning of **meagre (*Argyrosomus regius*)**. *Cybium* 32(2) Suppl.: 332-333.
- Duncan, N., Estévez, A.**, Porta, J., Carazo, I., Norambuena, F., Aguilera, C., Gairin, I., Bucci, F., Valles, R., **Mylonas, C.C.**, 2012. Reproductive development, GnRHa-induced spawning and egg quality of wild **meagre (*Argyrosomus regius*)** acclimatized to captivity. *Fish Physiology and Biochemistry* 38: 1273-1286.
- Duncan, N.J., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C.M., Roo, J., Schuchardt, D., Vallés, R.**, 2013a. Aquaculture production of **meagre (*Argyrosomus regius*)**: hatchery techniques, ongrowing and market. In: Allan, G., Burnell, G. (Eds.), *Advances in aquaculture hatchery technology*. Woodhead Publishing Limited, Cambridge, UK.
- Duncan, N.J., Estévez, A.**, Porta, J., Carazo, I., Norambuena, F., Aguilera, C., **Gairin, I.**, Bucci, F., Valles, R., **Mylonas, C.C.**, 2012. Reproductive development, GnRHa-induced spawning and egg quality of wild **meagre (*Argyrosomus regius*)** acclimated to captivity. *Fish Physiol. Biochem.* 38: 1273-1286.
- Duncan, N.J.**, Sonesson, A.K., Chavanne, H., 2013b. Principles of finfish broodstock management in aquaculture: control of reproduction and genetic improvement. In: Allan, G., Burnell, G. (Eds.), *Advances in aquaculture hatchery technology*, Woodhead Publishing Limited, Cambridge, UK.
- Estévez, A.**, Treviño, L., Kotzamanis, Y., Karacostas, I. Tort, L., **Gisbert, E.**, 2011. Effects of different levels of plant proteins on the ongrowing of **meagre (*Argyrosomus regius*)** juveniles at low temperatures. *Aquacult. Nutr.* 17: 572-582. doi: 10.1111/j.1365-2095.2010.00798.x.
- Estévez, A.**, Treviño, L., **Gisbert, E.** 2007. Initial larval density influences growth but not survival of meagre (*Argyrosomus regius*) larvae. XI National Congress of Aquaculture, Vigo, Spain
- Fauvel, C.**, Suquet, M., Sévère, A., **Mylonas, C.C.**, **Papandroulakis, N.**, 2008. Slow-release GnRHa therapy prevented atresia during vitellogenesis and induced ovulation of captive **wreckfish (*Polyprion americanus*)**. *Cybium* 32(2) suppl: 191.
- Fernández-Palacios, H., Hernández-Cruz, C.M., Schuchardt, D., Izquierdo, M.S., Roo, F.J.**, 2009a. Effect of co-feeding regimes on biological performance and biochemical composition of **meagre (*Argyrosomus regius*)** Asso, 1801) larvae. *Europ. Aquac. Soc. Spec. Publ.* 38: 108-111.
- Fernández-Palacios, H., Izquierdo, M.S., Norberg, B., Hamre, K.**, 2011a. Effect of broodstock diet on eggs and larvae. In: Holt, J. (ed.), *Larval Fish Nutrition*, Wiley – Blackwell, John Wiley and Sons, ISBN: 978-0-8138-1792-7, pp. 153-183.
- Fernández-Palacios, H., Schuchardt, D., Roo, J., Borrero, C., Hernández-Cruz, C.M., Socorro, J.** 2007. Morphometry of **meagre (*Argyrosomus regius*)**, Asso, 1801) during the first month of rearing. XI Congreso Nacional de Acuicultura, Vigo, Spain.
- Fernández-Palacios, H., Schuchardt, D., Roo, J., Hernández-Cruz, C.M., Duncan, N.**, 2009b. Efecto de distintas dosis de GnRHa sobre la calidad de la puesta de **corvina (*Argyrosomus regius*)**. In Libro de Actas, XII Congreso Nacional de Acuicultura, Madrid, España, 24-26 Noviembre 2009, pp. 554-555 (in Spanish, abstract in English).
- Fernández-Palacios, H., Schuchardt, D., Roo, J., Hernández-Cruz, C.M., Sabater, C., Duncan, N.**, 2011b. Efecto de diferentes intervalos de tiempo entre inyecciones con GnRHa, sobre las puestas de reproductores de **corvina (*Argyrosomus regius*)**. XIII Congreso Nacional Acuicultura, Book of Abstracts, Universitat Politècnica de Catalunya ESAB-Castelldefels, 21-24 November 2012, Barcelona, Spain (in Spanish, abstract in English).
- Fernández-Palacios, H., Schuchardt, D., Roo, J., Hernández-Cruz, C.M., Sabater, C., Duncan, N.**, 2011. Efecto de diferentes intervalos de tiempo entre inyecciones con GnRHa, sobre las puestas de reproductores de **corvina (*Argyrosomus regius*)**. Proceedings of the XIII Congreso Nacional de Acuicultura, 21-24 de Noviembre Barcelona. pp. (in Spanish, abstract in English)
- Finn, R.N., Østby, G.C., **Norberg, B.**, Fyhn, H.J., 2002. *In vivo* hydration in **Atlantic halibut (*Hippoglossus hippoglossus*)**; proteolytic liberation of free amino acids, and ion transport, are driving forces for osmotic water influx. *J. Exp. Biol.* 205: 211-224.
- Ginés, R., Robaina, L. Rodríguez-Lozano, A., Domínguez-Montesdeoca, D., **Hernández-Cruz, C.M.**, Romero, J., 2013 (in press). Effect of the different dietary levels of vitamin E on fillet quality of **meagre (*Argyrosomus regius*)**. *Aquaculture* (in press).





- Grigorakis, K.**, Fountoulaki, E., Vasilaki, A., Mittakos, I., Nathanailides, C., 2011. Lipid quality and filleting yield of reared **meagre (*Argyrosomus regius*)** International Journal of Food Science and Technology 46: 711-716.
- Grunert, K.G.**, Wills, J.M., 2007. A review of European research on consumer response to nutrition information on food labels. J Pub Health. 15: 385-399.
- Hamre, K.** Yufera, M., Ronnestad, I., Boglione, C., Conceição, L., **Izquierdo, M.** 2013. (In press). Fish larval nutrition and feed formulation - knowledge gaps and bottlenecks for advances in larval rearing. Reviews in Aquaculture.
- Hamre, K.**, Harboe, T., 2008a. Critical levels of essential fatty acids for normal pigmentation in **Atlantic halibut (*Hippoglossus hippoglossus* L.)** larvae. Aquaculture 277: 101-108.
- Hamre, K.**, Harboe, T., 2008b. Artemia enriched with high n-3 HUFA may give a large improvement in performance of **Atlantic halibut (*Hippoglossus hippoglossus* L.)** larvae. Aquaculture 277: 239-243.
- Hamza, N., Mhetli, M., Khemis, I.B., Cahu, C., **Kestemont, P.**, 2008. Effect of dietary phospholipid levels on performance, enzyme activities and fatty acid composition of **pikeperch (*Sander lucioperca*)** larvae. Aquaculture 275 (1-4): 274-282.
- Harboe, T., Mangor-Jensen, A., Moren, M., **Hamre, K.** Rønnestad, I., 2009. Control of light condition affects the feeding regime and enables successful eye migration in **Atlantic halibut** juveniles. Aquaculture 290: 250-255.
- Harel, M., Ben-Atya, S., Zlotkin, V., **Tandler, A.**, 1998. Mass production of **grey mullet, *Mugil cephalus***: Effect of environmental and nutritional factors on larval performances. Israeli Journal of Aquaculture 50: 91-98.
- Hernández-Cruz, C.M.**, Schuchardt, D., Roo, J., Borrero, C., **Fernández-Palacios, H.** 2007. Optimization of weaning protocol in **meagre (*Argyrosomus regius*, Asso, 1801)**. XI Congreso Nacional de Acuicultura, Vigo, Spain.
- Izquierdo, M.S.**, **Fernandez-Palacios, H.**, Tacon, A.G.J., 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197: 25-42.
- Izquierdo, M.S.**, **Koven, W.**, 2011. Lipids: In: Holt, J. (ed.), Larval Fish Nutrition, Wiley-Blackwell, John Wiley and Sons, ISBN: 978-0-8138-1792-7, pp. 47-82.
- Jerez, S.**, Cejas, J.R., Martín, V., Bolaños, A., Rodríguez, D., Lorenzo, A., 2011. Efecto del tipo de alimento y densidad de cultivo en el engorde de ***Seriola dumerili***. Actas XIII Congr. Nac. Acuicult., Casteldefels, Barcelona (España), 21-24 November, 2011.
- Jerez, S.**, Cejas, J.R., Samper, M., Felipe, B.C., Santamaría, F.J. and Villamandos, J.E. 2007. Crecimiento y maduración sexual en ejemplares de medregal ***Seriola dumerili*** nacidos en cautividad en Canarias. XI Congreso Nacional de Acuicultura, Vigo (España), 24/09/2007 a 28/09/2007.
- Jerez, S.**, Hernández, I., Cejas, J.R., Almansa, E., Samper, M., Santamaría, F.J., 2009b. Efecto de la estrategia de alimentación en el crecimiento del medregal (***Seriola dumerili***) en condiciones de cultivo. XII Congreso Nacional de Acuicultura, Madrid, 24-26 November 2009.
- Jerez, S.**, Hernández, I., Cejas, J.R., Almansa, E., Samper, M., Villamandos, J.E., Felipe, B.C., 2009a. Efectos de la estrategia de alimentación en la hematología y bioquímica sanguínea del medregal (***Seriola dumerili***) en condiciones de cultivo. XII Congreso Nacional de Acuicultura. Madrid, 24-26 November 2009.
- Jerez, S.**, Samper, M., Santamaría, F.J., Villamandos, J.E., Cejas, J.R., Felipe, B.C., 2006. Natural spawning of greater amberjack (***Seriola dumerili***) kept in captivity in the Canary Islands. Aquaculture 252: 199-207.
- Katharios, P.**, Kokkari, K., Papadaki, M., **Papandroulakis, N.**, 2011a. Systemic granulomas in cultured **meagre, *Argyrosomus regius***. In: Aquaculture Europe 11, Rhodes, pp. 537-538.
- Kestemont, P.**, Melard, C., 2000. Chapter 11. Aquaculture. In: Craig, J.F. (ed.), Percid Fish Systematics, Ecology and Exploitation, Blackwell Science, Oxford, UK, pp. 191-224.
- Kestemont, P.**, Xu, X., Hamza, N., Maboudou, J., Imorou, Toko, I., 2007. Effect of weaning age and diet on **Pikeperch** larviculture. Aquaculture 264: 197-204.
- Koven, W.M.**, 2003. Key factors influencing juvenile quality in mariculture: A Review. Israeli Journal of Aquaculture/Bamidgeh 55 (4): 283-297.
- Krystallis, A.**, **Linadrakis, M.**, **Mamalis, S.**, 2010. Implementation and assessment of the Discrete Choice Methodology for New Product Development (NPD). Agribusiness. 26: 100-121.
- Kucharczyk, D., **Kestemont, P.**, Mamcarz, A., 2007. Artificial reproduction of **pikeperch**. Practical manual, Polish Ministry of Science, 80 pp.
- Lund, I.**, Skov, P.V., Hansen, B.W., 2012. Dietary supplementation of essential fatty acids in larval **pikeperch (*Sander lucioperca*)**; short and long term effects on stress tolerance and metabolic physiology. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 162 (4): 340.
- Lund, I.**, Steinfeldt, S.J., 2011. The effects of dietary long-chain essential fatty acids on growth and stress tolerance in **pikeperch larvae (*Sander lucioperca* L.)**. Aquaculture Nutrition 17: 191-199.
- Machias, A., Somarakis, S., **Papandroulakis, N.**, Spedicato, M.-T., Suquet, M., Lembo, G., Divanach, P., 2003. Settlement of the **wreckfish (*Polyprion americanus*)**. Mar. Biol. 142: 45-52.
- Monfort, M.C., 2010. Present market situation and prospects of **meagre (*Argyrosomus regius*)**, as an emerging species in Mediterranean aquaculture, Studies and Reviews, General Fisheries Commission for the Mediterranean No. 89, FAO, Roma, pp. 28.



- Mozes, N., **Papandroulakis, N.**, Vergara, J.M, Biswas, A.K., Takii, K., Ntatsopoulos, A., 2011. Production systems. In Pavlides, M., Mylonas, C.C. (eds.), *Sparidae: Biology and Aquaculture*, Wiley-Blackwell, Oxford, 390 pp.
- Mylonas, C.**, Mitrizakis, N., Sigelaki, I., Papadaki, M., 2011. Spawning kinetics of individual female **meagre** (*Argyrosomus regius*) after treatment with GnRHa implants. *Indian Journal of Science and Technology* 9th ISRPF Issue, 4: No. S8 p. 230-231.
- Mylonas, C.C.**, Fostier, A., Zanuy, S., 2010a. Broodstock management and hormonal manipulations of reproduction. *Gen. Comp. Endocrinol.* 165: 516-534.
- Mylonas, C.C.**, **Papandroulakis, N.**, Smboukis, A., Papadaki, M., Divanach, P., 2004. Induction of spawning of cultured greater **amberjack** (*Seriola dumerili*) using GnRHa implants. *Aquaculture* 237: 141-154.
- Mylonas, C.C.**, Zohar, Y., 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. *Rev. Fish Biol. Fish.* 10: 463-491.
- Norberg, B.**, 1995. **Atlantic halibut** (*Hippoglossus hippoglossus*) vitellogenin: induction, isolation and partial characterization. *Fish Physiology and Biochemistry* 14 (1): 1-13.
- Norberg, B.**, Valkner, V., Huse, J., Karlsen, I., Lerøy Grung, G., 1991. Ovulatory rythms and egg viability in **Atlantic halibut** (*Hippoglossus hippoglossus*). *Aquaculture* 97 (4): 365-371.
- Norberg, B.**, Weltzien, F.-A., Karlsen, Ø., Holm, J.C., 2001. Effects of photoperiod on sexual maturation and somatic growth in **Atlantic halibut** (*Hippoglossus hippoglossus* L.). *Comp. Biochem. Physiol.* 129B (2-3): 357-366.
- Papadakis, I.**, Kentouri, M., Divanach, P., **Mylonas, C.C.**, 2013. Ontogeny of the digestive system of **meagre** *Argyrosomus regius* reared in a mesocosm, and quantitative changes of lipids in the liver from hatching to juvenile. *Aquaculture* (in press).
- Papadakis, I.**, **Chatzifotis, S.**, Divanach, P., Kentouri, M., 2008. Weaning of greater **amberjack** (*Seriola dumerilii* Risso 1810) juveniles from moist to dry pellet. *Aquacult. Int.* 16: 13-25.
- Papandroulakis, N.**, **Mylonas, C.C.**, Syggelaki, E., Katharios, P., Divanach, P., 2008. First reproduction of captive-reared **wreckfish** (*Polyprion americanus*) using GnRHa implants. *Aquaculture Europe* 08, September 15-18, Krakow, Poland, European Aquaculture Society Special Publication 37, pp. 507-508.
- Papandroulakis, N.**, **Mylonas, C.C.**, Maingot, E., Divanach, P., 2005. First results of greater **amberjack** (*Seriola dumerili*) larval rearing in mesocosm. *Aquaculture* 250: 155–161.
- Papandroulakis, N.**, **Mylonas, C.C.**, Syggelaki, E., Katharios, P., Divakaran, S., 2008. First reproduction of captive-reared wreckfish (*Polyprion americanus*) using GnRH implants. *Proceedings of the European Aquaculture 2008*, Krakow, Poland. pp.
- Papandroulakis, N.**, Suquet, M., Spedicato, M.T., Machias, A., Fauvel, C., Divanach, P., 2004. Feeding rates, growth performance and gametogenesis of wreckfish (*Polyprion americanus*) kept in captivity. *Aquacult. Int.* 3: 1-13.
- Peleteiro J.B.**, Rodríguez-Villanueva J.L., Crespo J. Alvarez-Blázquez B., Mariño J.C., Linares F., Pérez-Rial E., Hernández-Urcera J., 2010. First experiences with **wreckfish culture** (*Polyprion americanus*) in Galicia. Behaviour and ongrowing. *Aquaculture Europe 2010*. Porto (Portugal), pp 988-989.
- Peleteiro J.B.**, Saavedra C., Perez-Rial E., Soares E.C., Álvarez-Blázquez B. Vila A., 2011. Diversificación de especies en acuicultura marina. Desarrollo de técnicas de cultivo de la cherna (*Polyprion americanus*). *Actas XIII Congreso Nacional de Acuicultura*. Castelldefels, Barcelona (España) 2011.
- Robaina, L. Rodríguez-Lozano, A., Domínguez-Montesdeoca, D., Hernández-Cruz, C.M., Romero, J., 2013 (in press). Effect of the different dietary levels of vitamin E on the growth, fish composition, fillet quality and liver histology of ongrowing **meagre** (*Argyrosomus regius*). *Aquaculture* (in press).
- Rodríguez-Barreto, D., **Jerez, S.**, Cejas, J.R., Martín, M.V., Acosta, N.G., Bolaños, A., Lorenzo, A., 2012. Comparative study of lipid and fatty acid composition in different tissues of wild and cultured female broodstock of **greater amberjack** (*Seriola dumerili*). *Aquaculture* 360–361: 1–9.
- Rodríguez-Villanueva, J.L., **Peleteiro, J.B.**, Perez-Rial, E., Soares, E.C., Álvarez-Blázquez, B., Mariño, C., Linares, F., Mañanós, E., 2011. Growth of **wreckfish** (*Polyprion americanus*) in Galicia, Spain. *Aquaculture Europe 2011* (EAS), 18-21 October, Rhodes, Greece.
- Roo, F. J.**, **Hernández-Cruz, C.M.**, **Borrero, C.**, **Schuchardt, D.**, **Fernandez-Palacios, H.**, 2010a. Effect of larval density and feeding sequence on **meagre** (*Argyrosomus regius*; Asso, 1801) larval rearing. *Aquaculture* 302: 82-88. doi: 10.1016/j.aquaculture.2010.02.015.
- Roo, F.J.**, **Hernández-Cruz, C.M.**, **Fernández-Palacios, H.**, **Schuchardt, D.**, **Izquierdo, M.S.**, 2009. Effect of rearing system intensiveness on biological features, culture performance and larval quality of **meagre** (*Argyrosomus regius* Asso, 1801) larvae. *Europ. Aquacul. Soc. Spec. Publ.* 38: 371-374.
- Roo, J.**, **Hernández-Cruz, C.M.**, **Borrero, C.**, **Fernández-Palacios, H.**, **Schuchardt, D.**, 2007. Effect of rearing density and feeding regime in the larval rearing of **meagre** (*Argyrosomus regius*, Asso, 1801) during the first month. *XI Congreso Nacional de Acuicultura*, Vigo, Spain.
- Roo, J.**, **Hernandez-Cruz, C.M.**, **Borrero, C.**, **Schuchardt, D.**, **Fernandez-Palacios, H.**, 2010. Effect of larval density and feeding sequence on **meagre** (*Argyrosomus regius*; Asso, 1801) larval rearing. *Aquaculture* 302: 82-88.
- Rosenlund, G.**, Corraze, G., **Izquierdo, M.S.**, Torstensen, B., 2010. The effects of fish oil replacement on nutritional





- and organoleptic qualities of farmed fish. In: Turchini, G., Ng, W. and Tocher, D. (Eds.), *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*, CRC Press, Cambridge, UK, ISBN: 978-1-4398-0862-7, pp. 487-522.
- Scabini, V., Roo, J., Hernández-Cruz, C.M., Schuchardt, D., Borrero, C., Fernández-Palacios, H.**, 2008. Effect of larval density and feeding sequence on **meagre** (*Argyrosomus regius* Asso 1801) larval rearing. XIII International Symposium on Fish Nutrition and Feeding, Florianopolis, Brasil.
- Secombes, C.J.**, Ellis, A.E., 2012. The immunology of teleosts. In: *Fish pathology*. Ed. Roberts, R. Wiley
- Soula, M., Zamorano, M. J., Navarro, A., Sánchez, J.J., Neil, D., Alejandro, G., **Afonso, J.M.**, 2011. Diseño de dos nuevas PCRs múltiplex para **corvina** (*Argyrosomus regius*). XIII Congreso Nacional Acuicultura, Book of Abstracts, Universitat Politècnica de Catalunya ESAB-Castelldefels, Barcelona, España, 21-24 de Noviembre de 2012 (In Spanish, abstract in English).
- Steenfeldt, S., **Lund, I.**, Höglund, E., 2010a. Is batch variability in hatching time related to size heterogeneity and cannibalism in **pikeperch** (*Sander lucioperca*)? *Aquac. Res.* 42(5): 727-732.
- Steenfeldt, S.J., **Lund, I.**, 2008. Development of methods of production for intensive rearing of pikeperch juveniles. DTU Aqua Research Report no. 199-2008, Technical University of Denmark, Denmark (in Danish).
- Steenfeldt, S.J., Vestergaard, M., Overton, J.L., **Lund, I.**, Paulsen, H., Larsen, V.J., Henriksen, N.H., 2010b. Further development of intensive **pikeperch** rearing in Denmark. DTU Aqua Research Report no. 228-2010, Technical University of Denmark, Denmark (in Danish).
- Teletchea, F., Gardeur, J.-N., Psenicka, M., Kaspar, V., Le Doré, Y., Linhart, O., **Fontaine, P.**, 2009a. Effects of four factors on the quality of male reproductive cycle in **pikeperch** *Sander lucioperca*. *Aquaculture* 291 (3-4): 217-223.
- Vallés, R., **Estévez, A.**, 2013. Light conditions for larval rearing of **meagre** (*Argyrosomus regius*). *Aquaculture* 376-379: 15-19.
- Vermeirssen, E.L.M., Mazorra de Quero, C., Shields, R.J., **Norberg, B.**, Kime, D.E., Scott, A.P., 2004. Fertility and motility of sperm from **Atlantic halibut** (*Hippoglossus hippoglossus*) in relation to dose and timing of gonadotropin-releasing hormone agonist implant. *Aquaculture* 230: 547-567.
- Zarski, D., Kucharczyk, D., Targonska, K., Palinska, K., Kupren, K., **Fontaine, P.**, Kestemont, P., 2012. A new classification of pre-ovulatory oocyte maturation stages in **pikeperch**, *Sander lucioperca* (L.), and its application during artificial reproduction. *Aquaculture Research* 43: 713-721.

*i) Sub-contracting: If any part of the work is to be sub-contracted by the participant responsible for it, describe the work involved and explain why a sub-contract approach has been chosen for it.*

### **Subcontracting WPI Management**

**Certificate of the Financial Statements (5,000€, Partner 1. HCMR; 2,000€, Partner 3. IRTA, 2,200€, Partner 4. IOLR; 4,851 €, Partner 7. IMR; 2,500 € Partner 11. AU)**

An audit certificate is required by all Partners receiving more than 325,000€ of EU contribution. The above-mentioned Partners will acquire this certificate through a subcontract of an external auditor.

### **Subcontracting WP2 Reproduction and genetics - meagre**

**Use of Illumina HiSeq genome analyser (10,000€, Partner 1. HCMR)**

Subcontracting is necessary for part of Task 2.5 “Development of Single Nucleotide Polymorphisms (SNP) marker tools for the genetic characterization of fast and slow growers”, since the quantity and quality of genetic information to be obtained nowadays has exponentially increased with the use of equipment such as the Illumina HiSeq genome analyser, which is available in one or two public research institutes. An amount of 10.000 is expected to be spent in libraries preparation and two lanes of sequencing on this apparatus which will generate more than 500 million good quality reads, something that is not achievable with the pyrosequencing equipment that HCMR possesses.

### **Subcontracting WP19 Larval husbandry – grey mullet**

**Participation of the Israeli company Zoopt (15,000 €, Partner 4. IOLR)**



Subcontracting the Israeli company Zoopt is necessary for Tasks 19.3 and 19.4. These tasks focus on determining the effect of co-feeding ciliates (mainstream mesocosm species) and rotifers on digestive tract maturation and enzyme production and to use the selected diet from this task to determine the timing of weaning and diet type according to DT maturation and the shift from carnivorous to omnivorous feeding. Zoopt has developed expertise and unique technology to consistently and dependably mass culture ciliates (>30,000 ciliates/ml) at volumes suitable for larval rearing. Moreover, joint studies carried out at the IOLR with Zoopt in the past have shown that larvae of grouper, blue fin tuna and gilthead sea bream selectively consume ciliates over rotifers at least during the first few days of exogenous feeding. Moreover, ciliates are enriched with essential fatty acids much faster and at higher levels. We hypothesize that these ciliates will also be readily consumed by mullet larvae, resulting in a more rapid maturation of the digestive tract, improve overall performance and become an integral part of the larval rearing of grey mullet. The cost of subcontracting Zoopt involves preparation, maintenance and monitoring of batch cultures of ciliates in a series of culture tank facilities, which is a labour intensive activity requiring 3 workers. These employees use specially designed nutrient and enrichment mixtures to increase ciliate concentration and content of essential fatty acids. The ciliates before packing and transport from north Israel (near Haifa) to the IOLR in Eilat (1 hour flight) will be cleaned, filtered and concentrated (100,000 cells/ml) using Zoopt designed technology and equipment.

### ***Subcontracting WP24 Fish health - meagre***

#### ***Use of Scanning and Transmission Electron Microscope (3,000€, Partner 1. HCMR)***

Subcontracting is necessary for the Tasks 24.1 and 24.2. Since this equipment is not available at the facilities of HCMR in Crete and observation should be made by the principal investigator with his physical presence, it is necessary to outsource the specific task. HCMR has access to the Electron Microscope Lab of the University of Crete, which is located in the same geographic area having already a framework contract which reflects the best value for money principle.

### ***Subcontracting WP28 Socioeconomics – new product development and WP29 Socioeconomics – Consumer value perception and segmentation – Data collection for the face-to-face (f2f) surveys (54,852€, Partner 38. HRH)***

The face to face surveys of WP28 and WP29 rely on the collection of primary data through the following tasks:

- a) Task 28.1 International qualitative research, namely focus groups (at least one per country) and expert interviews (at least 5 per country) in 5 countries (UK, D, ES, F, I) to generate input for new product development;
- b) Task 29.2 Hedonic sensory tests in the 5 countries (n=100 per country or segment at minimum) to investigate consumer sensory perceptions about the new products developed in WP7.2.

To fulfill the objectives of the above tasks, different types of f2f interviews have to be conducted. For this purpose and in the effort to keep the data collection quality on a high quality level, local teams should be used (through small-scale subcontracting) in cooperation and strict supervision of HRH (Partner 38).

The above-described subcontracting is necessary in order to recruit the participants to the focus groups and expert interviews (WP28.1), as well as the eligible interviewees for the participation to the sensory tests (WP29.2). Furthermore, the subcontracting is necessary due to the fact that it is essential, in the frame of marketing research quality issues, that the moderator of the focus groups, as well as the interviewer of a face to face interview to speak the same language (native) with the participants. Under this scope, local teams of moderators and interviewers will be hired by a cooperating research agency in each one of the five involved countries (who for legal issues should be paid from the local agency). HRH will do all the effort to keep this subcontracting to the minimum possible, undertaking all the tasks of briefing, supervising, controlling and processing of the data collected from the local teams



In this respect, subcontracts will only cover the execution of a limited part of the project, as the above-described tasks have to be carried out from local moderators and interviewers. However, HRH will have the full responsibility of the data collection and for this reason expert executives from HRH personnel will be presented to the briefing and training of the local teams, as well as attending or viewing (through one way mirror or closed circuit or focus vision) the focus groups and an adequate part of interviews to secure the data collection according to the project objectives.

The subcontracting amount is broken a number of independent subcontracts, established with different companies from the five selected countries, indicatively as follows:

***Subcontract 1 –UK, (11,000€ Partner 38. HRH)***

***Subcontract 2 –Germany, (11,000€ Partner 38. HRH)***

***Subcontract 3 –Spain, (7,000€ Partner 38. HRH)***

***Subcontract 4 –France, (11,000€ Partner 38. HRH)***

***Subcontract 5 –Italy, (9,000€ Partner 38. HRH)***

Furthermore, the subcontracting is necessary due to that it is essential, in the frame of the marketing research quality issues, the moderator of the focus groups as well as the interviewer of a face to face interview to speak the same language (native) with the participants. Under this scope local teams of moderators and interviewers will be hired by a cooperating research agency in each one of the five involved countries (who for legal issues should be paid from the local agency).

**As part of Amendmnet 4, an additional amount of 6,000 € subcontracting (EU contribution) is assigned for the platform development (for the e-shop) and consumables correspond to the provision of on line consumer panel in the 5 selected countries.**

### ***Subcontracting WP31 Dissemination***

***Print and layout of the project leaflets (5.000€), translations of Food Today articles into 10 languages (2.500€), set-up and updating of a quadrant for project on Eufic.org (1,200€), Partner 37. EUFIC***

Subcontracting is necessary for the following dissemination activities: Print and layout of the two project leaflets, translations of the two FoodToday articles, and set-up and updating of a quadrant highlighting the project on Eufic.org (to drive traffic). The reasons for this are that EUFIC does not have the technical expertise in-house to design and print the leaflet, translate the articles into 10 languages (French, Spanish, German, Czech, Hungarian, Polish, Greek, Slovak, Italian, Portuguese), and the technical expertise to set-up and do the technical updates in order to have a quadrant highlighting the DIVERSITY project on Eufic.org (>600.000 visits/month) with the purpose to drive traffic to the project website. For the leaflets, EUFIC will select a subcontractor according to ECGA rules (at least 3 quotes submitted from different potential subcontractors), for the translations and website technical activities, we have framework contracts with:

Prime Production Ltd (translation agency)  
Unit 15, Mill House, Windmill Business Centre  
2-4 Windmill Lane, Middlesex  
Southall, UB2 4NJ  
T: +44 (0)844 482 0004, F: +44 (0)844 482 0475  
E: judha@primeproductions.org.uk

EUFIC works with Prime Production since 2012 for translations of its core publication Food Today. They offer good quality of translation (reducing the proofreading time by EUFIC staff)

Alligence Communications (website technical activities)

Cogels-Osylei 33

2600 Berchem

Belgium

T. +32 (0)3 270 0 270, F. +32 (0)3 270 0 271

[www.alligence.com](http://www.alligence.com)



EUFIC works with Alligence since 2004 for EUFIC’s website [www.eufic.org](http://www.eufic.org) (proprietary technology)



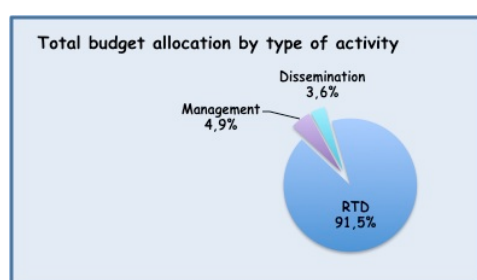
## 2.4 Resources to be committed

*This section will be based on section 2.4 of the proposal, but may require more details than provided in the proposal.*

*In addition to the budget breakdown form (Part A) and the overviews of the staff effort broken down to workpackage level in the relevant Workplan Tables, please provide:*

- A management level description of resources and budget identifying personnel and any major costs, and*
- A description of the resources which are needed to carry out the project (personnel, indirect costs, equipment, etc.) for each beneficiary*

*Show that the project will mobilise the resources necessary to carry out the work for the overall duration, including those resources that will complement the EU contribution. Describe how the resources will be intergrated and used to form a coherent projet withing the overall financial plan.*



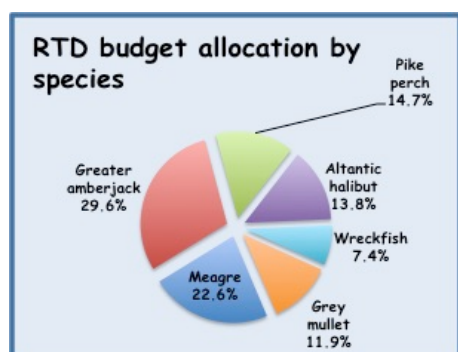
DIVERSIFY will mobilize total financial resources of 11,8 M€, with a **total requested EU contribution of 8,96 M€, allocated to 37 Partners, including 10 SMEs and 2 large enterprises**. A total of 2,879 M€ (24% of the Total budget) is provided by Partners as matching funds. Of the Total budget, **91.5% is allocated to RTD activities**, 3.3% to Dissemination and 5.0% to Management. The **10 SMEs (that includes one Professional Association) have been allocated 15.4%** of the EU contribution, as required by the Call. The involvement of the 12 private

enterprises (aquaculture production and fish feed production) is indispensable for the implementation of DIVERSIFY and the rapid and effective uptake of the project's results by the aquaculture industry. It is expected that the protocols developed for the selected species will make a major impact on the European aquaculture industry, and enable its expansion and profitability.

Of the **Management budget**, 38.6% is specifically allocated to the project coordinator (PC). The budget allocation for management activities is justified by the participation of a large number of Partners representing the four corners of Europe (from Canary Islands, Spain to Israel and from Crete, Greece to Norway) and the long duration of the project (5 years).

For **Dissemination activities**, the EU requested budget has been allocated mainly to the WP31 Dissemination leader, and the PC, as well as the Species leaders (SLs) and other Partners who will be directly involved with the proposed dissemination activities. These include, but are not limited, to setting-up and maintaining a dedicated website, annual presentations of the PC in the EU Forum at the EAS meetings, presentations of the SLs in the EAS meeting in Y2 and Y4, organization of 6 species-specific “Know-how Transfer” seminars in Y5, and organization of Promotional workshops for specialized audiences in the fish market sector (Spain, UK and Italy). The overall allocated budget and staf effort for dissemination has been increased significantly from the proposal stage (an increase of >80%), and is regarded as adequate to promote and disseminate the activities of such diverse project of this magnitude.

### Research, Technology and Development (RTD) budget.

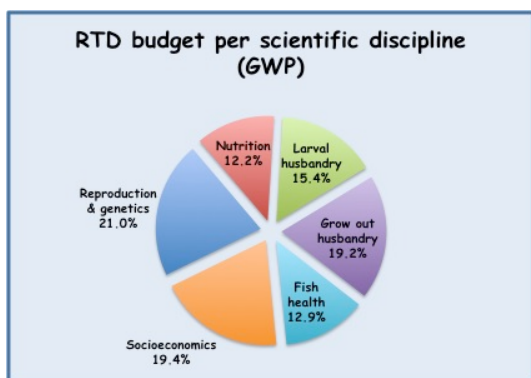


The allocation of resources for each of the identified species and research areas (WPs) reflects (a) the potential of each species to achieve commercial aquaculture production, (b) the degree of difficulty of the various biological and technical challenges, as well as (c) the level of inclusion of each species in the aquaculture industry. DIVERSIFY is in agreement with the European Aquaculture Technology and Innovation Platform (EATIP, 2012), which identified the Mediterranean as requiring and having a greater potential for species diversification. At the same time, DIVERSIFY maintains an inclusive approach with species for all EU aquaculture





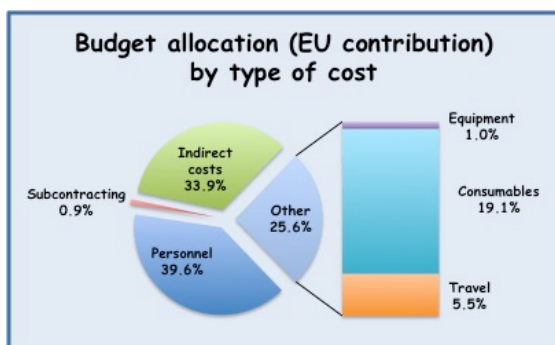
sectors, and including a highly sustainable herbivorous species, the grey mullet. Greater amberjack and meagre offer an excellent potential for Mediterranean cage culture, both coastal and offshore, where expansion is expected. Greater amberjack was allocated a larger percentage of resources to solve the many bottlenecks and capitalise on and promote the high potential and interest from many SMEs (four such SMEs are Partners in DIVERSIFY). Therefore, the choice of species and research type has guided the distribution of funding towards a relatively larger number of Partners from the Mediterranean (61%) and mainly from Spain (29%), than from northern and central Europe. The high number of Spanish Partners is related to the greater number of aquaculture research organizations and research effort in Spain --among EU countries-- and in particular to research in the chosen Mediterranean species. Generally, resource distribution is similar to the geographic distribution of the Partners. The allocation of resources to Partners is related to their involvement in the various selected species and research areas, and their expertise to tackle the identified bottlenecks. Therefore, HCMR with ~15%, and FCPCT and IRTA with ~8.5% each of the Total budget have extensive resources to meet an increased commitment to the proposal based on expertise and ongoing initiatives, particularly for greater amberjack and meagre. Therefore, these Partners will be involved with research in many species and/or research areas. In Particular, **HCMR will participate in all 6 RTD Disciplines** and will contribute to the work planned for **all six selected species**, albeit not with the same effort, utilizing an extensive multidisciplinary infrastructure and expertise. As PC, the HCMR has been allocated resources for WP1 Management and WP31 Dissemination.



The allocation of resources for each WP can be considered as addressing three main aspects towards the overall objective. GWP Reproduction & Genetics and GWP Larval husbandry were allocated ~37% of the budget to reduce the cost of production of high quality juveniles for all species. GWP Nutrition, GWP Grow out husbandry and GWP Fish health were allocated ~46% to reduce costs of grow out and particularly nutrition and feeding that constitute the major production cost of Aquaculture. The budget for GWP Fish health is a little bit more than the one of Nutrition, as it addresses significant identified bottlenecks in this area. The socioeconomic component has been allocated a significant

proportion of the RTD budget (>19%) to demonstrate how the new products with improved production methods can be marketed. Included in the socioeconomic component are costs related to collection and analysis of data and part of the fieldwork, *i.e.*, the recruitment of participants and interviewing that will be subcontracted.

### Budget allocation by type of cost



The majority (39.6%) of the budget will be applied towards **personnel**, which contribute a total of **1602.22 PM** (or **135.3 Person years**). **Consumables** take up 19.1% of the Total budget, and will include acquisition of broodstock from the wild for greater amberjack and wreckfish; fish feed for larvae, juveniles and broodstock; production and enrichment of live food; general consumables for fish husbandry; laboratory consumables and chemicals for laboratory analytical procedures (nutrient, hormone analyses and biochemical analyses, histology, microbiology, immunology, molecular biology, etc.); and office consumables.

**Travel** expenses constitute 5.5% of the budget, and will cover travelling for management purposes (annual coordination meetings), dissemination (conferences, workshops and seminars) and a great many research interactions among various Partners. Since a question arose during the negotiation phase, regarding our



travel budget being high, we would like to point out the following:

1. Most of the experimental facilities where fish are maintained (e.g. NCM in Israel, HCMR in Crete, IRTA in Tarragona, IFREMER in France, FCPCT, ULL and IEO in Canary Islands, etc.) are located away from major cities in the EU. As a result, the scientist traveling from and to these facilities have to include in their travel a domestic flight section. This adds cost for flight tickets, but also room and board since for a day's work, it is often required to travel for three days (one to arrive to destination and one to return home).
2. The work proposed in DIVERSIFY includes many interactions of scientist in the field, and not simply exchange of data and samples. For example, for GWPs Reproduction and Genetics, scientists will be traveling to various sites to implement (a) sampling for reproductive tissues, (b) monitoring and evaluation of reproductive status and sperm quality and (c) spawning induction and cryopreservation. This requires the participation of the highly skilled scientist involved in DIVERSIFY for a period of time and over multiple years during the course of the program. The same holds true for many of the other WPs. Furthermore, the involvement of various SME and large companies (SARC, SWH) in the research, requires multiple personnel exchanges between these Partners and the RTD Partners associated with their tasks.
3. The program will run for 5 years, thus it will require 6 annual coordination meetings (a kickoff and one at the end of each year). At least 1 participant from each organization will attend these meetings, and in the case of the partners with higher involvement (greater budgets), multiple scientists will be attending. As described extensively in WP1 description and Part B 2. Management structure and procedures, the annual meetings will last for 3 days, due to the size of the consortium and multitude of scientific disciplines.
4. The PC will be traveling to every EAS annual meeting during the course of the program to make summary presentations, while the GWP leaders (HCMR, IRTA, IOLR, IEO, UL and IMR) will be making presentations during year 3 and 5 of the project, in the same forum.
5. In the course of DIVERSIFY, 6 “Know-how Transfer” seminars will be organized in different locations, to present the results obtained to the industry. The cost of traveling for all invited speakers (both belonging to DIVERSIFY and from outside) is included in these traveling expenses of the Partners that will be hosting the workshops (HCMR, IRTA, IMR, IEO, UL and UNIBA). The suggestion of the EU officer of having a single workshop for multiple Mediterranean species in four different countries has been adopted.
6. In the course of DIVERSIFY, 4 promotional workshops will be organized in different countries (by APROMAR and CTAQUA). These two partners will also be involved in other dissemination activities (e.g., “Know-how Transfer” seminars, above), which would require extensive traveling.

The minor part of the budget (1.0%) that is forecasted for purchasing new **equipment** underlines the ability of the consortium to undertake the activities proposed using facilities and equipment already purchased and operated by the Partners. The major (>5,000€) equipment to be purchased includes components to build a new commercial automated feeding system for meagre, equipment for the preparation of a large tank for greater amberjack broodstock, while minor equipment includes personal computers and software, submersible cameras for behaviour observations, sensors for monitoring environmental conditions in fish tanks, and small equipment of this sort.

**Subcontracting** accounts for 0.9% of the budget, and it has been reduced significantly from the proposal, as the majority of the activities to implement the consumer studies have been allocated to a new SME Partner (HRH). Still, a small amount needs to be allocated to subcontracting in the 5 different countries where consumer research will take place. In addition, a small amount is allocated to subcontracting to implement some specialized analyses that are either not available in any of the Partners' laboratories, or due to economies-of-scale are less expensive to out-source.

In the following pages, we present a Partner-by-Partner description of the allocation of resources to DIVERSIFY. The amounts mentioned are Total budget, not EU contribution, thus it includes the 25 or 50% own contribution by the Partners.



## Partner 1. HCMR

HCMR will participate in all 6 Groups of scientific WPs in DIVERSIFY and will contribute to the work planned for all 4 Mediterranean species (meagre, greater amberjack, wreckfish and grey mullet) including a minor participation in the Atlantic halibut and pikeperch work. Its participation will be both with analytical labs and large scale facilities for the rearing trials. In particular HCMR will provide access to the AquaLabs, a certified facility on Crete for experiments and rearing of marine organisms (certification codes EL 91-BIO-03 and EL 91-BIO-04). This facility includes a broodstock area (for GWP Reproduction and genetics trials), an intensive and a semi-intensive (Mesocosm type) hatchery with the related infrastructures for phyto- and zooplankton cultures (for GWP Larval husbandry experiments), a pregrowing zone and specialized facility for nutritional and feeding trials (GWP Nutrition and GWP Grow out husbandry). Additionally, DIVERSIFY will take advantage of the certified HCMR's facility in Athens (certification code EL 25 BIO) 037 employed for some of the studies in GWP Nutrition with meagre and greater amberjack. Support will be also provided to GWP Larval husbandry and GWP Fish Health by the equipped laboratory of HCMR in Athens for analysis of biomarkers related to oxidative stress, metabolism (P450) and digestive enzymes. Finally, the pilot cage farm of HCMR (a unique experimental facility in the Mediterranean, Souda Bay, Crete Island: GR94FISH0001) for the implementation of experimental trials in GWP Grow out husbandry is also included in the available infrastructures. HCMR will also contribute significantly to GWP Socioeconomics, by taking full responsibility of the analytical sensory characterization of the produced species and products and by coordinating the end product quality monitoring.

Further to the research rearing facilities, a wide range of state of the art laboratory equipment (HPLC, UPLC/MS/Tof) will be employed for the biochemical analyses and specialized tools will be also used for the implementation such as a Vectrino current meter for studying hydrodynamics in rearing tanks (GWP Larval rearing husbandry) and an echo integrator for studying the fish behavior in cages (GWP Grow out husbandry). Within GWP Socioeconomics, a specially build taste panel room (located in Athens) will be used for analytic sensory evaluation of produced fish and products and Analytic Instruments (Gas Chromatographers: GC-FDI and GC-MS) for chemical evaluation of fish quality. HCMR runs a fully equipped molecular biology lab for routine and more up-to-date genetic analyses. These facilities include a robotic station performing DNA extractions (Qiacube), several PCR and Real-time thermocyclers, a high throughput automatic sequencer (ABI 3730xl) for microsatellite genotyping and sequencing, pyrosequencing facilities (454 GS FLX, Roche) and a TaqMan OpenArray System (AB) for SNP genotyping and expression analyses. Finally, there is a large computer platform for bioinformatics analyses and quantitative genetics (14 nodes of more than 8 cores each, approx., 150 CPUs)

**Personnel cost:** A total of **698,803 €** is allocated to personnel costs. This will cover the cost of existing personnel (researchers, technicians) as well as hiring of new personnel (graduate students, post-docs, technicians) who will be involved in various WPs in the areas of Reproduction and Genetics, Nutrition, Larval husbandry, Grow out husbandry, Fish health and Socioeconomics. In addition, an executive secretary will be hired for the duration of the project, to assist the PC (WP1), together with the administrative staff of the organization. The PC will be also involved in WP31 Dissemination. One post-doc will be hired (12 PM) for the analysis of NGS data generated in meagre, SNP identification and genotyping in WP2. One scientific technician (new personnel) will be hired for 13 PM in total, to support the existing personnel (researcher) in biomarkers analysis of WP14, 15 and WP24 and determination of minerals (Ca, P) in fish health. For fish health the cost also involves 2 new staff (one for the nutritional experiments of WP24 Fish health - meagre and one for the microbiological work in WP24) with a total of 36 PM. A PhD student will be recruited, for 26 PM, for fully conducting (within a PhD project and under supervision of the research scientist) the organization and development of descriptive sensory analysis group (selection & training) and also for assisting in chemical analysis of quality attributes in WP28.

**Consumables:** A total of **300,552 €** is allocated to consumables. This includes feeds (larval, nursery, grow out and broodstock) and relevant products (enriching diets for larvae). Also analytical consumables for GWP Reproduction and genetics (reproductive hormones for the production of GnRH $\alpha$  implants for





spawning induction, ELISA chemicals, HPLC columns and chemical consumables, histological processing, water quality analysis, general lab chemicals, consumables related to DNA and RNA extractions, microsatellite and SNP genotyping), GWP Nutrition (chemicals for the analyses of amino acids, enzymes, and vitamin D), GWP Larval husbandry (enzymes, substrates of enzymes, reagents, inhibitors, related chemicals, and multiwells for the estimation of the oxidative stress of the larvae and the study of the development of the somatotrophic axis and the ontogeny of the digestive system and the visual system), GWP Grw out husbandry (for evaluating muscle quality and the stress status of the experimental groups), GWP Fish health (for histology, microbiology and molecular biology and enzymes, substrates of enzymes, reagents, inhibitors, related analytical consumables for real-time PCR, vit D, Ca, P and multiwells for determination of biomarkers of oxidative stress and metabolism) and GWP Socioeconomics (foodstuff and food-related consumables required for training the taste panel and for analytic descriptions and chemical consumables necessary for the chemical analysis of quality). A cost for libraries preparation for NGS analysis is also considered. General laboratory consumables and office consumables are also included in this category.

**Travel costs:** A total of **115,743 €** is allocated related to coordinating and management activities of the project (WP 1), dissemination activities (WP31), as well as for the implementation of various tasks of the scientific GWPs. Specifically, travel costs are associated with travelling of the PC, WP leaders and scientists to the kickoff and annual coordination meetings. Travel costs are also associated with inviting outside scientists to attend and present their relevant work in the kickoff meeting and the annual meeting organized in the premises of HCMR (Y1 and Y5). The PC will be attending the annual EAS meeting for project dissemination purposes (Y1-5) and WP leaders and scientists will be attending these EAS and other conferences in various disciplines (e.g. Fish Reproduction, Fish nutrition, LARVAE, Fish pathology, Food Science) for dissemination purposes. The PC will also be travelling to the 5 Species State-of-the-Art workshops that will be organized under WP31 Dissemination (Y5). Travel costs are also foreseen for management and coordination activities such as *ad hoc* meetings with Partners and Group WP leaders, as well as with EU DG RTD officers (e.g., mid term review). Finally, travel costs have been foreseen for the implementation of the scientific work in the major GWPs. These include spawning induction experiments in the site of various SMEs and other Partners involved in GWP Reproduction and Genetics, in site presence for various experimental trials of focal groups for collecting samples, travelling for sampling within the meagre pathology task, and participation in focal groups and final product quality evaluations (GWP Socioeconomics).

**Equipment:** A total of **59,384 €** is allocated to the purchase of equipment. These include personal desktop and laptop computers, general software (wordprocessing, data analysis, statistical analysis, graphics preparation, slide preparation, antivirus, etc.), as well as printers/scanners needed for coordination, data management, analysis and presentation. A personal thermal cycler (PCR) system for 96-well plates with factory-installed gradient option will be also purchased within WP2 (4,700 €) and a compound microscope with digital camera for the monitoring of reproductive stage of development and sperm analysis using Computer Assisted Sperm Analysis (CASA) software (2,500 €). Equipment will be purchased to improve the feeding automated system in the hatchery (7,800 €), and a submersible camera in WP14, 15 (14,200 €). Accessory equipments such as chromatography column for nutrient analyses (1,000 €), balances (300 €) and water pumps (800 €) will be purchased for taking measurements and maintaining the functionality of rearing facilities also within the needs of WP20, 21. A specialized sensory analysis software and a statistical software, additional sensory analysis booths (15,395 €) together with PC and screens (2,000 €) will be purchased to be applied in the taste panel room to facilitate application of descriptive analysis tests in WP28.

**Subcontracting:** A total of **18,000 €** is allocated to subcontracting. **Certificate of the Financial Statements (5,000 €).** An audit certificate is required by all Partners receiving more than 325,000 € of EU contribution. The Partner will acquire this certificate through a subcontract of an external auditor. **Use of Illumina HiSeq genome analyser (10,000 €).** Subcontracting is necessary for part of Task 2.5 “Development of Single Nucleotide Polymorphisms (SNP) marker tools for the genetic characterization of fast and slow growers”, since the quantity and quality of genetic information to be obtained nowadays has exponentially increased with the use of equipment such as the Illumina HiSeq genome analyser, which is available in one or two public research institutes. An amount of 10,000 € is expected to be spent in libraries preparation and two lanes of sequencing on this apparatus which will generate more than 500 million good quality reads,



something that is not achievable with the pyrosequencing equipment that HCMR possesses. *Use of Scanning and Transmission Electron Microscope (3,000 €)*. Subcontracting is necessary for the Tasks 24.1 and 24.2. Since this equipment is not available at the facilities of HCMR in Crete and observation should be made by the principal investigator with his physical presence, it is necessary to outsource the specific task. HCMR has access to the Electron Microscope Lab of the University of Crete, which is located in the same geographic area having already a framework contract which reflects the best value for money principle.

#### **Complementary Resources committed**

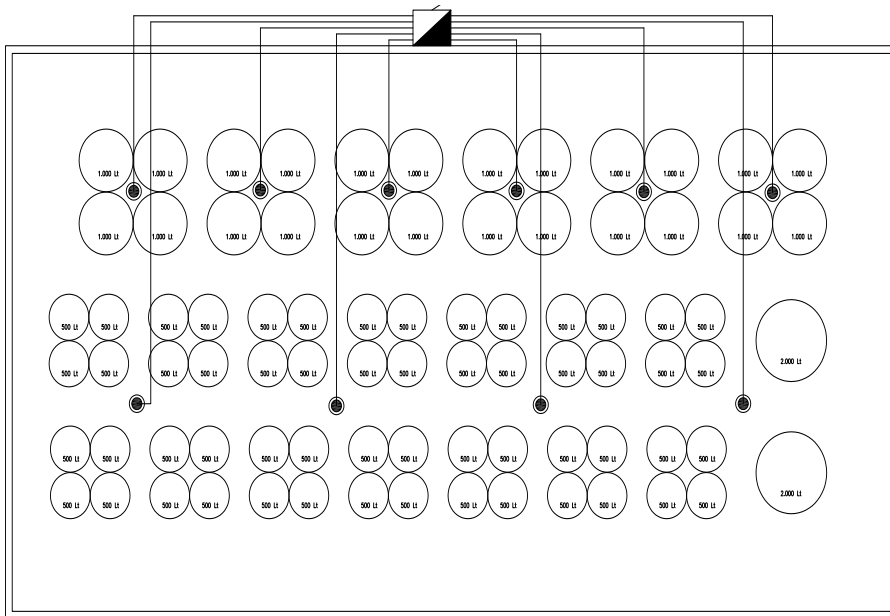
As stated elsewhere, HCMR is currently implementing the research project "KRIPIS-Marine Biology, Biotechnology and Aquaculture", a main topic of which is the Diversification of the Aquaculture production with new fast growing species (the greater amberjack). The research synergies created with DIVERSIFY have been analyzed elsewhere. Furthermore, in the frame of KRIPIS specific research infrastructure will be developed that will be available also for Diversify. In particular, (i) the thermoregulation of the land based broodstock facility, (ii) the cage infrastructure for both broodstock and on-growing trials, (iii) the egg collecting system for cages, and (iv) the improvement of the Mesocosm and the intensive hatchery of the HCMR will be implemented.



**Partner 2. FCPCT**

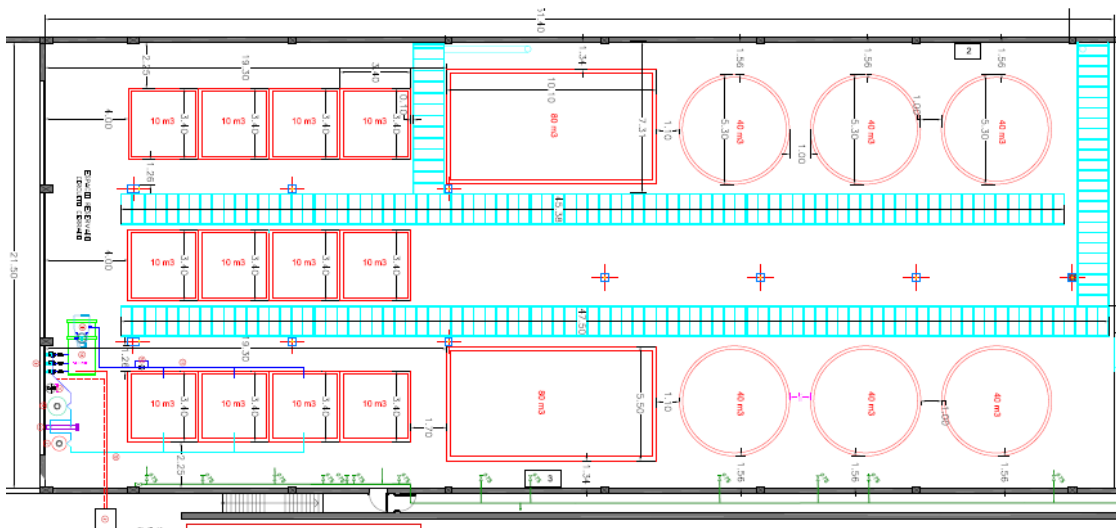
FPCT will participate in the studies of the 4 Mediterranean species (meagre, greater amberjack, wreckfish and grey mullet) and, in a lesser extend, in pikeperch and halibut. FPCT will contribute to the scientific GWPs of Reproduction, Nutrition, Larval Husbandry, Grow-out Husbandry and Fish Health, conducting the studies in the Fast Growing Species Broodstock Management Unit (for GWP Reproduction and genetics), Feed Technology and Testing Unit (FITU) (for GWP Nutrition), Warm Water Species Production Unit (WWSSU) (for larval and grow out husbandry), and Marine Biosecurity Station (MBS) (for Fish health), all of them certified for experimental production of fish and recognized as Aquaculture Facilities for Excellence in Research by Aquaexcel. In addition, FCPCT will participate conducting analysis for FPCT studies and complementing some other partners’ studies in FCPCT laboratories of “Nutrition”, “Chromatography”, “Fish Welfare and Health”, “Ichthyopathology” and “Molecular Biology and Quantitative Genetics”, located in the Institute of Animal Health and Food Security (IUSA) of FCPCT.

The FITU includes an ingredient processing laboratory, a feed production hall, 30 digestibility tanks (200 and 500 litres) and three wet labs with 170 tanks of 100, 200, 500 and 1,000 litres, as well as two lines for commercial scale testing, provided with computer controlled automatic, auto-demand or manual feeding and waste feed collectors (feed intake control), to test diets and ingredients for either larvae (including automated start feeding), juveniles or broodstock of marine fish species, both commercial or new species for aquaculture. Photoperiod control is also available in 100, 200 and 500 litres tanks. This facility will be required for the studies in GWP Nutrition.



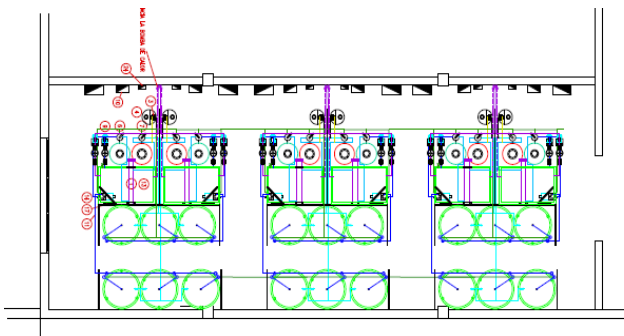
**One of the three units of FITU to be used in GWPs of Nutrition, Larval husbandry and Grow out for larval and juvenile rearing trials**

The WWSSU comprises 45 circular tanks of 1,000 litres capacity with controlled photoperiod and temperature, 6 circular tanks of 40,000 litres and 2 80,000-litres race-ways; 12 square RAS tanks of 10,000 litres; 90 tanks of 500 litres for larval and juvenile rearing, all of them equipped with sensors and automated control of environmental parameters. The large tanks in the picture will be used for broodstock management (GWP Reproduction and genetics) and fast-growing species production (GWPs of Larval and Grow out husbandry).



**Tanks belonging to WWSSU to be GWPs of Nutrition, Larval husbandry and Grow out for larval and juvenile rearing trials**

The MBS, used in GWPs Fish Health comprises three main RAS units (one of them provided in the picture) completely equipped to separately challenge with up to three different pathogens at the same time in all phases of fish life cycle including broodstock, larvae and juveniles of marine fish species. Each of them is provided with automatic and programmable control of flow, oxygen concentration, temperature and salinity and contains 18 circular tanks of different volume (100, 200, 500 and 1,000 litres). The design of the recirculatory units is versatile, which allows a great amount of testing conditions and assays in vivo with any pathogen. These characteristics make the MBS the most versatile and controlled research station in Europe to challenge marine fish with viruses, bacteria or parasites. The MBS is a reference center for disease prevention in the warm East Atlantic. Fish Welfare and Health laboratory is equipped with microbiology and anatomic-pathology techniques.



**One of the three MBS units to be used in Fish health.**

The Molecular Biology and Quantitative Genetics techniques contains manual and automatic sequencers, gel documentation systems, 5 color gene expression equipment, quality quantifier of nucleic acids, design and planning of breeding schemes, development of individual identification systems for physical and molecular reconstruction of genealogy, estimation of genetic parameters and evaluation of players, etc. This laboratory will be used in GWP Reproduction and genetics, GWP Nutrition and GWP Larval husbandry.

Nutrition and Chromatography Laboratories are completely equipped with 3 GLCs, GC-MS, 3 HPLCs, Densitometer, Iatroscan, Kjeldahl, ovens, muffles, etc., where all lipid, protein, aminoacids, fatty acids, lipid classes, vitamins, pigments, toxins, dioxines, PCBs and certain minerals from ingredients, feeds, live preys, and fish can be studied. This laboratory will be used in GWP Reproduction and genetic, GWP Nutrition, GWP Larval husbandry and GWP Grow out husbandry.



The histology laboratory is completely equipped with Immune-histology, confocal microscopy, electron microscopy. The Ichthiopathology laboratory is fully equipped with tools for identification of parasites, bacteria and viruses, vaccine and treatment developments. Both laboratories will be used in the GWPs of Fish health.

**Personnel cost:** A total of **353,344 €** is allocated to personnel costs. This will cover the cost of existing personnel (researchers, technicians) as well as hiring of new personnel (graduate students, post-docs, technicians and research assistants) who will be involved in various WPs in the areas of Reproduction and Genetics, Nutrition, Larval Husbandry, Grow Out Husbandry and Fish Health.

**Consumables:** A total of **176,300 €** is allocated to consumables. This includes ingredients for enrichment products and weaning diets, feeds (larval, nursery, grow out and broodstock), live preys feeds, disinfectants and fish treatment products, other materials for fish husbandry (nets, filters, etc.), hormones, laboratory gasses, glassware and chemical products for genetic studies, optical and electron microscopy histology studies, immunohistochemistry studies, in-situ hybridization studies, biochemical and enzymatic studies, chromatography studies, behaviour studies, microbiology consumables for disease challenges, etc. General laboratory consumables and office consumables are also included in this category. Besides, 2,000 € will be allocated for consumables used in dissemination of the results obtained in the project.

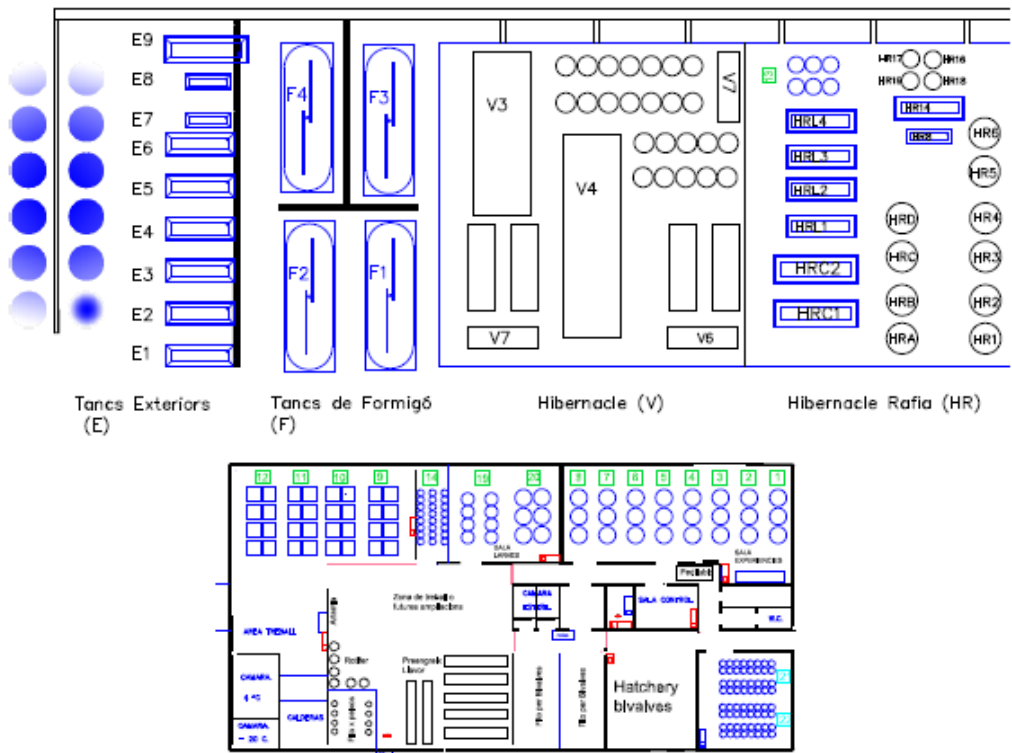
**Travel costs:** A total of **21,025 €** is allocated for travel costs associated with travelling to the kickoff and annual coordination meetings, attending the annual EAS meetings and other conferences for project dissemination purposes and implementation of the scientific work.

**Subcontracting: *Certificate of the Financial Statements (2,000 €)*.** An audit certificate is required by all Partners receiving more than 325,000 € of EU contribution. The Partner will acquire this certificate through a subcontract of an external auditor.



**Partner 3. IRTA**

This Partner will participate in all 6 GWPs designed for implementation and will contribute to the work planned for all 4 Mediterranean species (meagre, greater amberjack, wreckfish and grey mullet) including a minor participation in the pikeperch work. Its participation will be both with analytical labs and large scale facilities for the rearing trials. All the experiments with fish reproduction, larval rearing, grow out and health will be carried out at IRTA Sant Carles de la Ràpita indoor and outdoor facilities, most of them held in RAS (recirculation aquaculture systems) designed at IRTA (IRTAMar™). Indoor facilities will be dedicated mostly to larval rearing (different cylindrical tank sizes: 100, 500 l), grow out (quadrangular tanks 450 l and cylindrical tanks 1,500 l) and health (challenge room), whereas outdoor facilities will be dedicated to broodstock holding and for the grow out of >500-gr fish. A total of 32 meagre broodstock are currently being held in IRTA from two origins, wild (17, mean weight 15 kg) and cultured (15, mean weight 12 kg). Laboratories for (1) biochemistry, equipped with ultraturrax, sonicator, water baths, ovens, analytical balances, gas-liquid chromatographer, densitometer, spectrophotometer (spectro and fluorimetry) (2) microbiology, equipped with incubators (some shaking), fridge, media preparation facilities, autoclaves, class II cabinet, gas installation, (3) molecular biology, equipped with clinical centrifuges, microcentrifuges, three thermal cyclers (one with temperature gradient), one quantitative thermal cycler, spectrophotometer, gel electrophoresis equipment and digital image analysis system, incubators, freezers, and fume hood and (4) histology, fully equipped with an automatic tissue processor, tissue embedding station, 3 microtomes, oven, fume cupboards, staining train and several microscopes will also be available.



New product development will be carried out in the food processing facilities of IRTA-Monells Centre. IRTA is equipped with industrial and pilot size equipment of the most advanced technologies for production of food products, especially meat and fish, including packaging, marination, drying, smoking, storage, etc. IRTA is also equipped with laboratories to carry out physical (NIR, visible and microwave spectrometry, texture analyser, colorimeter equipment), chemical (LC- and GC instruments: UPLC-DAD-MS/MS, HPLC-DAD/FLD, GC-MS/MS and GC-MS) and sensorial assessment of quality of fresh and processed products.

The sensory and consumer behaviour lab at IRTA is equipped with a testing room made up of 10 sensory booths designed according to international regulations. The lab also includes a fully equipped industrial kitchen for sample preparation and three different highly trained tasting panels, one of them specialised in seafood. In addition, IRTA has an internal database of 600 persons all over Catalonia and different rooms





designed and prepared for both qualitative (focus groups, projective techniques, etc.) and quantitative consumer research (hedonic test, willingness to pay, etc.).

**Personnel cost:** A total of **404,441 €** is allocated to personnel costs. This will cover the cost of existing personnel (researchers, technicians) who will be involved in the different WPs of Reproduction and Genetics, Nutrition, Larval husbandry, Grow out husbandry, Fish health and Socioeconomics. In addition, an administrative assistant will be hired to cover just 1 PM to assist in WP 31 Dissemination.

**Consumables:** A total of **341,821 €** will be dedicated to the purchase of consumables. This includes feeds and products (filters, nets, Artemia cysts, etc) for the rearing trials. Also analytical consumables for GWP Reproduction and genetics (reproductive hormones for spawning induction, ELISA chemicals; consumables related to DNA and RNA extractions), GWP Nutrition (chemicals for the analyses of proximal composition –total proteins, carbohydrates and lipids- as well for lipid class and fatty acid composition, immunological serum parameters markers of intestinal inflammation and immunohistochemistry), GWP Larval husbandry (Artemia and weaning diets, double staining for analysis of skeletal deformation and digestive enzyme activity), GWP Fish health (for histology, microbiology and molecular biology) and GWP Socioeconomics (related to training the taste panel for analytic descriptions).

**Travel costs:** **45,600 €** will be allocated to travel costs, mostly related to coordinating and management activities of the project and also for the implementation of various tasks of the GWPs, especially for the travels related to consumer acceptance for GWP Socioeconomics. Travels for the assistance of the researchers involved in the project to different congresses related to the research activities of the project are also considered here, as well as the organization of one of the Species State-of-the-Art seminars (Meagre) in Barcelona and the organization of one of the Annual Meetings are also considered.

**Equipment:** A small amount **10,003 €** is allocated for **equipment** for the purchase of a thermal cycler (6,700 €) and to cover the depreciation of already existing equipment.

**Subcontracting:** *Certificate of the Financial Statements (2,000 €)*. An audit certificate is required by all Partners receiving more than 325,000 € of EU contribution. The Partner will acquire this certificate through a subcontract of an external auditor.



## Partner 4. IOLR

**Description of Organization:** IOLR is a non-profit government research organization that consists of 3 research centers, one of which is the National Center of Mariculture (Eilat), which will participate in this proposal. The IOLR centre is internationally known as a leading research institute in the area of marine aquaculture. It is made up of nine research groups working interactively to domesticate marine species for aquaculture. The main research areas include fish reproduction, larval rearing and physiology, pathology, algal and zooplankton culture, genetic improvement of farmed marine species, development of feeds, research and development of cost effective and environmentally friendly systems for intensive fish growth as well as integrated systems for growth of fish, molluscs and algae. The professional staff at the IOLR in Eilat includes about 50 scientists, research assistants and technicians. The research infrastructure provides a continuous supply of filtered and UV treated seawater to a wide array of experimental and semi-commercial rearing tanks and ponds. In addition, the Eilat facility has a number of well-equipped analytical laboratories carrying out molecular, biochemical and biological research.

The specific Departments involved in this project (Dept. of Reproduction and Dept. of Larval Rearing and Physiology) are fully equipped with analytical instrumentation as well as culture and grow-out facilities to carry out all tasks effectively and efficiently. Analytical instrumentation in these departments include, but are not limited, to GC/MS/MS, HPLC, refrigerated centrifuges, radioactive wet and dry labs, spectrophotometer, lyophilizer, dry and wet fume hoods, microscopes with photographic capability, micro-analytical balances, -70°C freezers, pH meter, beta counter, ELISA reader, laminar-flow hood for tissue culture, CO<sup>2</sup> incubators, PCR cyclers, gel electrophoreses apparatuses for protein and DNA separation, RNA/DNA calculator, dot-blot apparatus, UV/white light table with compatible digital camera, hybridization ovens, dark room with photographic facilities for autoradiography and fluororadiography, electroporator, electron microscope, luminometer, fluorescence microscope equipped with digital camera and luminescence spectrometer. The hatchery is comprised of thirty 400 l V-tanks, eighteen 1,500 l V-tanks, six 6,000 l V-tanks and sixty 20 l aquaria all having full capacity for salinity and temperature control and using filtered (10 µm) and UV treated sea water. There is also broodstock facilities consisting of biopsy rooms, fourteen 4 m<sup>3</sup>, fifteen 1 m<sup>3</sup>, three 25 m<sup>3</sup>, six 40 m<sup>3</sup> and four 30 m<sup>3</sup> tanks as well as a large range (forty-eight 200 l) of outdoor rearing tank facilities for experimentation.

**Main Tasks:** The IOLR is the **Leader of GWP Larval Husbandry** and the **Species Leader for grey mullet**, and is primarily responsible for all studies on the grey mullet in GWP Reproduction and genetics (Reproduction), GWP Nutrition (Nutrition), GWP Larval husbandry (Larval husbandry) and GWP (Grow out husbandry). IOLR will focus on (1) hormone-based treatments for the induction of gonadal development and maturational processes in grey mullet males and females. (2) Dietary effects on grey mullet broodstock egg fecundity, quality and larval first feeding. (3) Phytoplankton and nutritional effect (essential fatty acids, taurine) on grey mullet composition and performance. (4) Determine the effect of co-feeding ciliates and rotifers on digestive tract maturation and enzyme production in grey mullet. (5) Determine weaning time in grey mullet and type of feed according to the shift from carnivorous to omnivorous feeding. (6) Determine changes in the essential fatty acid requirement as a function of developmental stage and ambient salinity in grey mullet. (7) Gene expression of key enzymes in the synthesis of taurine and bile salts in grey mullet. (8) Nutritional effects (essential fatty acids, taurine) on grey mullet roe (bottarga) quality and improving grow out feeds for grey mullet. (9) Evaluate and maximize the dietary incorporation of a non-GMO genetically selected soybean meal that will increase nutrient absorption and reduce DT inflammation, as well as (10) testing this diet at different stocking densities on F1 and wild caught juveniles over a range of environmental conditions. In addition IOLR is involved in studying reproductive characteristics of the greater amberjack (WP2).

**Personnel cost:** A total of **166,311 €** is allocated to personnel costs. This will cover the cost of existing personnel (technicians) as well as support for graduate students. The technicians and students will be involved in various WPs including GWP Reproduction and genetics, GWP Nutrition, GWP larval husbandry) and GWP Grow out husbandry and will participate in fish feeding and maintenance as well as analytical work in the laboratory.





**Consumables:** A total of **165,000 €** is allocated to consumables. This includes algae, rotifer and *Artemia* production as well as feeds (larval, nursery, grow-out and broodstock). Consumables such as filters, columns, fittings, ferrules and chemicals for HPLC and GC/MS/MS fatty acid and amino acid (taurine) analyses including standards and reagents for all the molecular biology aspects (e.g. primers, enzymes, DNA purification kits, DNA ligation kits, sequencing services, synthetic genes, etc), protein analyses (SDS-PAGE, antibodies), ELISA kits for hormonal analyses, real-time quantitative PCR (enzymes and other PCR-related supplies) and disposables (gloves, pipet tips, tubes, etc) are also included.

**Travel costs:** The IOLR researcher as scientific responsible, GWP Larval husbandry and species leader has been allocated a budget of **23,125 €** for coordinating and managing activities associated with these duties as well as travelling to conferences and scientific meetings, for annual project meetings for the two IOLR researchers responsible for the research.

**Subcontracting: *Certificate of the Financial Statements (2200 €)*.** An audit certificate is required by all Partners receiving more than 325,000 € of EU contribution. IOLR will acquire this certificate through a subcontract of an external auditor.

**Complementary resources committed:** IOLR is committing to this project the time of other researchers and technicians involved in the running costs of maintaining broodstock, algae, rotifer and *Artemia* production as well as feeding fish during grow-out and maintenance of infrastructure equipment and UV systems.



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**Partner 5. UNIABDN**

The University of Aberdeen (Scottish Fish Immunology Research Centre - SFIRC) will participate in WP 1 and GWP Fish health, with work confined to immune studies in meagre and greater amberjack. SFIRC UNIABDN? is the world leader for research on fish cell-mediated immunity and the elucidation of the cytokine network has been ongoing for nearly 30 years. Major facilities that will be utilised in this programme include state of the art molecular and fish cell culture laboratories, including FACS and clean room facilities, where cloning of fish immune genes, analysis of their expression and production of recombinant proteins for bioactivity testing is routine. SFIRC will have a major role in cloning key immune genes in both species, developing qPCR assays for expression analysis, and raising antibodies to the main immunoglobulin isotypes present. These assays and reagents will be used to monitor the immune responses post-vaccination (eg against *Nocardia*) or pathogen/PAMP exposure, and to assess the health impact of feeding different diets.

**Personnel cost:** A total of **91,152 €** is allocated to hire a PhD student to undertake the immune studies at Aberdeen. The student will be trained to clone fish immune genes and to undertake expression analysis. In addition, 3% of Professor Secombes' time (**14,852 €**) is allocated for supervision, GWP Fish health coordination and report writing.

**Consumables:** A total of **70,883 €** is allocated to consumables. This includes the costs of molecular biology reagents for gene cloning, expression analysis by real time RT-PCR, purchase of PAMPs and serum antibody analysis. In addition, costs of **10,000 €** for production of anti-meagre IgM/T monoclonal antibodies are also included.

**Travel costs:** A total of **16,785 €** is allocated to attend coordinating and management activities of the project (WP 1), and travel costs associated with visiting Partners 1-3 to help with in vivo and in vitro experiments and to collect samples.



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**Partner 6. SWR**

SWR (more specific LEI) will participate in GWP Socioeconomics. SWR is the GWP Socioeconomics leader and will participate in all WPs within GWP Socioeconomics (WP27 to WP30). SWR will participate with the fisheries specialist of the department Natural resources and the consumer research specialists of the department Consumer and Behaviour. SWR will bring in the knowledge about the economics of the fisheries and aquaculture sector and consumer research knowledge. For the socioeconomic research no facilities are necessary.

For the desk research and fieldwork in WP27, the SWR network as well the network of the other partners in this WP will be used to fulfill the competitive and macro and meso economic analysis. For the fieldwork in consumer behavior (WP29) local representative databases of consumers will be used. These respondents will be recruited by subcontraction paid by our partner in GWP Socioeconomics AU, who pays all subcontraction.

SWR will bring in knowledge about consumer research and more specific the development of consumer questionnaires, desk research, data analysis and analysis of large databases in both macro and meso economic analysis and consumer research. This knowledge is state of the art, given the recent publications of SWR in internationally recognised journals. The management tasks of SWR will both be used to participate in the overall management team and to lead GWP Socioeconomics. SWR is also the task leader of the overall concluding report of GWP Socioeconomics.

**Personnel cost:** A total of **248,140 € (including own contribution of 25%)** is allocated to personnel costs. This will cover the cost of existing personnel (researchers) who will be involved in GWP Socioeconomics. This personnel will be used to do the WP leadership and to fulfill the tasks in the WP.

**Consumables:** A total of **8,750 €** will be used for other direct costs.

**Travel costs:** A total of **12,984 €** is budgeted as travel costs. These travel costs are mainly used for attendance of the yearly coordination meetings and for coordination meetings of the WPs 27 to 30. For the fieldwork in WP27 and WP29 also travelling costs have to be made for fieldwork (WP 27) and the instruction of the fieldwork companies (WP29) in the several countries.

**Equipment:** For the SWR contribution in GWP Socioeconomics use of equipment is not relevant.



## Partner 7. IMR

IMR will participate in the scientific GWP Reproduction and genetics, GWP Nutrition GWP Larval husbandry and GWP Fish health in DIVERSIFY and will contribute, as major research partner and species leader to the work planned for Atlantic halibut. Its participation will be both with analytical labs and large scale facilities for the rearing trials. In particular, IMR will provide access to the Austevoll Research Station. This facility includes a broodstock area (for GWP Reproduction and genetics trials), and intensive rearing facilities for marine larvae and juveniles, with the related infrastructures for live feed production, and a special laboratory for experimental production of formulated feed (for GWP Nutrition and GWP Larval husbandry experiments). The Austevoll Research Station also has analytical laboratories equipped for hormone assays (ELISA, TRF, RIA) and basic molecular biology (RNA and DNA isolation and evaluation). Additionally, DIVERSIFY will take advantage of the laboratories at IMR's main office in Bergen, which holds state-of-the-art laboratories for molecular biology, with full facilities for cloning, transfection, protein isolation, QPCR, histology and immunohistochemistry and a laboratory for fish pathology.

**Personnel cost:** A total of **183,552 €** is allocated to personnel costs. This will cover the cost of existing personnel (researchers, technicians) who will be involved in various WPs in the areas of Reproduction and Genetics, Nutrition, Larval husbandry and Fish health. In addition, specialized administrative staff within the organization will be made available to DIVERSIFY. The Species leader will be also involved in WP31 Dissemination.

**Consumables:** A total of **218,928 €** is allocated to consumables. This includes feeds (larval, nursery, grow out and broodstock) and relevant products (enrichment of diets for larvae). Analytical consumables/chemicals will be covered for GWP Reproduction and genetics (reproductive hormones for the production of GnRH $\alpha$  implants for spawning induction, ELISA reagents, histological processing, general lab chemicals; consumables related to DNA and RNA extractions, probes and other reagents for qPCR). It also includes consumables in GWP Nutrition (production of experimental diets for early weaning), GWP Larval husbandry (probiotics, general consumables for *in vitro* experiments and microbiology, general consumables for construction and maintenance of the RAS and FT rearing systems, and for ongrowing *Artemia* culture systems) and GWP Fish health (for cloning, transfection, protein isolation, QPCR, histology, immunocytochemistry, microbiology and molecular biology). General laboratory consumables and office consumables are also included in this category.

**Travel costs:** A total of **48,000 €** is allocated to travel. Travel costs are related to coordinating and management activities of the project (WP 1), dissemination activities (WP31), as well as for the implementation of various tasks of the scientific GWPs. Specifically, travel costs are associated with travelling of the Species leader and scientists to the kickoff and annual coordination meetings. Travel costs are also associated with inviting outside scientists to attend and present their relevant work in the species seminar on Atlantic halibut (Y5) and the coordination meeting organized in the premises of IMR (Y2). The scientists will be attending EAS and other conferences in various disciplines (e.g. Fish Reproduction, LARVI, Fish pathology) for dissemination purposes. Travel costs are also foreseen for the implementation of the scientific work in the major GWPs associated with Atlantic halibut. In particular, these include spawning induction and larval husbandry experiments at the site of SWH (Partner 22), which is located in northern Norway.

**Equipment:** No major equipment will be purchased within the project.

**Subcontracting:** *Certificate of the Financial Statements (4,851 €)*. An audit certificate is required by all Partners receiving more than 325,000 € of EU contribution. The Partner will acquire this certificate through a subcontract of an external auditor.

**Complementary funding:** The IMR will contribute with 25% of the total costs. At present, there is no other complementary funding available for the project.



## Partner 8. IEO

The participation of the IEO is mainly focused on two species: greater amberjack and wreckfish, with a budget allocation of 57% and 37% of total budget, respectively. Several studies will be performed for both species on 5 of the WPs designed for implementation: GWP Reproduction and genetics, GWP Nutrition, GWP Larval husbandry, GWP Grow out husbandry and GWP Fish health (34%, 9%, 34%, 13%, 4% of the total budget respectively). In greater amberjack, budget allocation will be 23% for reproduction, 10% for nutrition, 36% for larval rearing, 23% for grow out and 8% for pathology studies. In wreckfish, budget allocation will be 55% for reproduction, 10% for nutrition and 35% for larval rearing studies. The infrastructure that the IEO will provide (free-of-charge) to carry out this proposal includes the Marine Culture Units of IEO-Vigo and IEO-Canarias, two experimental culture facilities fully equipped to conduct the planned experiments with wreckfish and greater amberjack, respectively. Both facilities include breeding area (for GWP Reproduction and genetics and GWP Nutrition trials), hatchery, including areas for phyto- and zooplankton cultures (for GWP Nutrition and GWP Larval husbandry trials), and growing zones (for GWP Grow out husbandry and GWP Fish health trials). In addition, the fully equipped laboratories of IEO-Vigo, IEO-Canarias and IEO-Murcia, will also be available without any cost for the development of the proposed studies. This includes the necessary equipment to carry out the histological, hematological, biochemical and immunological analysis and studies to be done in the different WPs, such as spectrophotometer, multi-mode microplate reader, laminar flow cabin, microscopes with photographic capability and other general laboratory equipment (analytical balances, centrifuges, -80°C freezers, ice machine, muffles, stoves, autoclave, thermostatic bath, fluorometer, lab water purification system, pH meter, heating magnetic stirrer, vortex, microwave, computer equipment, etc.). Thanks to the implementation of previous research projects funded by national programs, the IEO will also provide free-of-charge broodstock groups of wreckfish and greater amberjack acclimated to captive conditions.

**Personnel costs:** A total of **139,867 €** (25% of total budget) is allocated to personnel costs. This will cover the cost of existing personnel (researchers, technicians) who will be implicated in several studies of various WPs for both species in the areas of Reproduction and Genetics (39%), Nutrition (12%), Larval husbandry (31%), Grow out husbandry (11%) and Fish health (4%). The personnel will be also involved in Management (WP 1) and Dissemination (GWP 31). Finally, a technician will be hired for the duration of the project, to develop several tasks in wreckfish, together with the research staff of the IEO.

**Consumables costs:** A total of **183,143 €** (32% of total budget) will be allocated to consumables cost of wreckfish (40%) and greater amberjack (60%). The largest budget allocation will be in GWP Reproduction and genetics (34%) and GWP Larval husbandry (37%), while in GWP Nutrition, GWP Grow out husbandry and GWP Fish health the budget will be 9%, 15% and 5% of the total consumables cost, respectively. This comprises fish feed including experimental diets for the rearing trials in GWP Reproduction and genetics and GWP Nutrition (broodstock), GWP Nutrition and GWP Larval husbandry (larvae), GWP Grow out husbandry and GWP Fish health (grow out) as well as supplies to maintain the necessary seawater quality (filtration) and fish manipulation (drugs and anesthetics, nets, plastic materials, etc.). Also general and specific lab consumables for the histological processing (gonad maturation, larval skeletal deformities) and blood samples obtained for sexual hormonal levels and hematological analysis. In addition, chemical reactivities for the determination of several plasmatic indicators of health and welfare (enzymes, hormones, electrolytes, etc.), larvae nutritional condition (RNA/DNA), as well as oxidative stress and humoral parameters of the immune system in larval and juveniles.

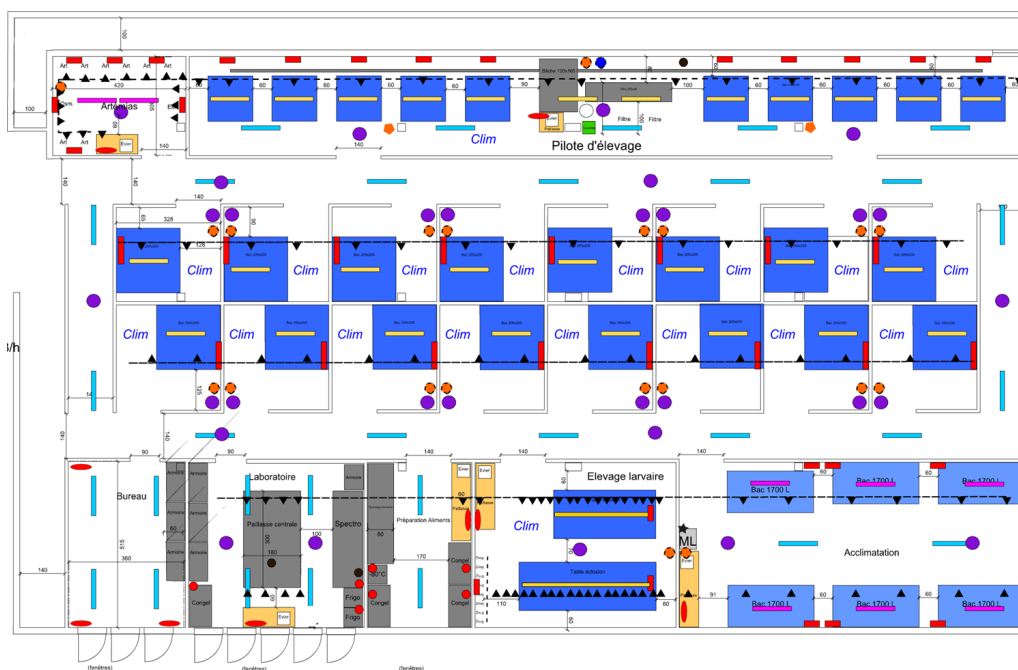
**Travel costs:** A total of **31,263 €** (6% of total budget) is allocated to coordinating and management activities of the project (WP 1) and dissemination activities (GWP 31). Travel costs are also foreseen for management and coordination activities such as *ad hoc* meetings with Partners and Group WP leaders. Finally, travel costs are also associated with the assistance to congresses to present relevant scientific results.

**Equipment costs:** The IEO has the necessary facilities to carry out the tasks for both species in the different WPs, so no budget will be allocated for equipment.



## Partner 9. UL

UL will be only implicated in tasks concerning pikeperch, and more specifically GWP Larval husbandry (larval husbandry) and GWP Grow out husbandry (grow out). We will also have a minor participation in the GWP Reproduction and genetics (reproduction, supply of fin samples and contact pikeperch farmers). UL will be the species leader for pikeperch and the main interlocutor with the SME Asialor (partner 29). The distance between UL laboratory and these SMEs is only 50 km. The UL participation will concern some analytical aspects (especially the use of specific multifactorial methodology such as fractional or complete factorial designs) and large-scale facilities for the rearing trials. New experimental facilities (Experimental platform for aquaculture, 800 m<sup>2</sup>, investment of 2,500,000 €, see the plan below) are now built on the site of the “Faculty of Sciences and Technologies” in Nancy for our Laboratory (Unit of Research on Animal and Functionalities of Animal Products, UR AFPA). These facilities, mainly compounded by independent recirculated aquaculture systems (RAS) with a very precise regulation of water temperature and lighting conditions, will be available in February 2014. This experimental platform for aquaculture has been specifically designed to allow experiments at pilot scale for fish rearing and multifactorial experiments ( $2^3 = 8$  or  $2^4 = 16$  experimental units for larval rearing and grow out respectively).



**Personnel cost:** A total of **115,556 €** is allocated to personnel costs (34 PM). This will cover the cost of existing personnel (researchers and technicians, 10 PM) as well as hiring of new personnel (24 PM, PhD student) who will be involved in various WPs in the areas of Management (WP1), Reproduction (GWP Reproduction and genetics, WP4), Larval husbandry (GWP Larval husbandry, WP16), Grow out husbandry (GWP Grow out husbandry, WP22) and Dissemination (WP31).

**Consumables:** A total of **60,000 €** is allocated to consumables. This includes feeds (larval, nursery, grow out and broodstock) and specific consumables (biofilter media, UV lamps) used for experimental RAS. It concerns also chemical products for water quality analysis and fish sampling. General laboratory consumables and office consumables are also included in this category.

**Travel costs:** A total of **11,915 €** is allocated (4%) related to coordinating and management activities of the project (WP1) and dissemination activities (WP31), as well as for the implementation of various tasks of the scientific GWPs. Specifically, travel costs are associated with traveling of scientists to the kickoff and annual coordination meetings. Travel costs are also foreseen for management and coordination activities such as *ad hoc* meetings with Partners and Group WP leaders. Specific travel costs will be related to



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scientists travel according to collaborations with partners 3 and 21 (WP16) and with partner 16 (WP22). This will also concern many local travels (exchanges with the SME Asialor, partner 29). As pikeperch Species Leader, specific travel costs will concern the organization of a “pikeperch workshop” (WP31 Dissemination) that will be held at Y5.

**Equipment:** No equipment purchase is foreseen.





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**Partner 10. TU/e**

TU/e will participate in the GWP Socioeconomics in DIVERSIFY, particularly WP27 and WP28 and will lead WP30 regarding business model development for the species, as well as marketing strategy and test markets for the new products developed. It will take advantage of previous involvement in studies on business models for startups and other firms, as well as expertise for marketing of innovations. It will cooperate closely with LEI to approach additional SMEs to participate and contribute to the test markets in the identified markets.

**Personnel cost:** A total of **189,525 €** is allocated to personnel costs. This will cover the cost of existing personnel (researchers) as well as hiring of new personnel (graduate students, and PhD candidate) who will be involved in various parts of the GWP Socioeconomics.

**Consumables:** A total of **3,000 €** is allocated to consumables. This includes software and mock-ups for test markets.

**Travel costs:** A total of **19,525 €** is allocated to travel costs. Specifically, travel costs are associated with travelling of the scientists to the kickoff and annual coordination meetings and for the research involved in GWP Socioeconomics. WP27 involves interviews with the trade, WP28 involves coordination and meeting with the team. Further a serious amount is reserved for setting up and coordinating the test markets of WP30. Additional travel is anticipated for developing assumptions for the diffusion studies for international market development. Travel costs are also associated with inviting outside scientists to attend and present their relevant work in the kickoff meeting and the annual meeting organized in the premises of TU/e and for the scientists to attend the annual EAS meeting for project dissemination purposes (Y1-5) and other relevant conferences in various disciplines (e.g. strategy, food marketing). Travel costs are also foreseen for *ad hoc* meetings with Partners and Group WP leaders related to the general progress and particularly GWP Socioeconomics.

**Equipment:** A total of **2,000 €** is allocated to the purchase of equipment. These include laptop computers needed for data management, analysis and presentation.





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**Partner 11. AU**

AU will participate in all socioeconomics WPs in DIVERSIFY (i.e. GWP Socioeconomics), as well as in WP 1 (i.e. project management), and will contribute to the work planned in relation to new product development, consumer testing and acceptance, as well as the development of marketing strategies and entire business models for the companies involved. AU's participation will be mainly in the form of experience and expertise provided in relation to survey implementation and data collection methodologies, as well as analytics and empirical testing of conceptual models developed, and empirical results coming from consumer-centered experimental settings. In particular, AU will participate in the following tasks: a) the analysis of the institutional and organizational (i.e. business-related) contexts within which the new fish species' products will be developed (WP27); b) the mere new product concept development, mainly in relation to its consumer-driven part (WP28, task 28.1); and c) the development of relevant business models and marketing strategies for the companies involved (WP30). However, AU's contribution mainly lies in its leading role regarding the analysis of consumer perceptions about the expected value associated with the new products and the potential and relevant communication means that can bring about a behavioural change in favour of the new species and overall fish categories (i.e. WP29).

For all the above tasks, AU will mobilise the time and expertise of its staff members (existing and newly hired within the frame of the project), who will supervise and take full responsibility of the surveys and analytics' tasks, namely the data collection in the multi-country environment selected appropriate for the project, as well as the data analysis and the development of valuable input for the devising of resulting business models and marketing strategies.

Moreover, AU will contribute with the provision of fully equipped work station/office facilities necessary for hosting the existing and new staff members involved in the implementation of the project, as well as with all types of statistical software necessary for the analytical work done after the collection of the data.

For the above tasks, AU guarantees timely delivery and high quality outputs from the planned work through the wise exploitation of the total resources (i.e. requested and provided) anticipated for personnel and data collection costs within the frame of the project.

**Personnel cost:** A total of **233,811 €** is allocated to personnel costs. This covers the cost of existing personnel (8.7 PM at the Professor-level), as well as hiring of new personnel (a PhD candidate), who will be involved in the various WPs in the area of Socioeconomics (GWP Socioeconomics). More specifically, a PhD student will be recruited for 36 PM, for fully conducting (within a PhD project and under supervision of the existing academic staff) the organization and development of the work within GWPs 27-30, as described above. In addition, secretarial support (i.e. 0.3 PM) will be provided by existing administrative staff of the organization, while a small additional contribution (i.e. 0.2 PM) is requested.

**Travel costs:** A total of **9,000 €** is allocated, related to coordinating and management activities of the project, as well as for the implementation of various tasks of the scientific GWP Socioeconomics on socioeconomics, and mainly WP29. Specifically, travel costs are associated with traveling of the WP29 leader and scientists to the kickoff and annual coordination meetings. The WP29 leader will be attending the annual EAS meeting for project dissemination purposes (Y1-5) and other conferences in various disciplines for dissemination purposes. Other travel costs are also foreseen for management and coordination activities such as *ad hoc* meetings with partners and other GWP Socioeconomics leaders.

**Equipment:** A total of **1,500 €** is allocated to the purchase of equipment. These include personal tablets/laptop(s) and any additional statistical software that might be needed.

**Subcontracting: *Certificate of the Financial Statements (2,500 €)*.** An audit certificate is required by all Partners receiving more than 325,000 € of EU contribution. The Partner will acquire this certificate through a subcontract of an external auditor.



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**Partner 12. APROMAR**

APROMAR will participate in WP31 (Dissemination), GWP Socioeconomics and WP 1 (Management).

In WP31 Dissemination APROMAR will be involved in the organisation of workshops, in reaching the European aquaculture industry, in optimizing the link with the food industry and consumers, in the creation and revision of documents and actions and in the production of documents. Regarding GWP Socioeconomics, APROMAR will work in WP27 on the tasks External competitive analysis, Internal competitive analysis and Opportunities for growth; in WP30 in the tasks Business model innovation, New product marketing strategy development, and Recommendations regarding industry development. And finally, in Management, APROMAR will work on its part of best management of the project.

**Personnel cost:** A total of **92,762 €** is allocated to personnel costs offering technical expertise, management and administrative tasks. Three persons will be involved.

**Consumables:** A total of **4,000 €** is allocated to consumables on Dissemination (general office supplies, poster presentations, etc.).

**Travel costs:** A total of **17,757 €** is allocated to travelling for WP1, GWP Socioeconomics and WP 31, which includes participation in the annual coordination meetings, Promotional workshops, State of the art species seminars and EAS meetings.

**Equipment:** No specific equipment will be acquired by APROMAR for the realization of this project.



**Partner 13. UNIBA.** Two UNIBA Departments will be involved in the project: the Department of Emergency and Organ Transplantation, section of Veterinary Clinics and Animal Production, and the Department of Bioscience, Biotechnology and Pharmacological Sciences.

UNIBA will participate to the proposal with a study on male and female gametogenesis (WP3 Reproduction and Genetics - greater amberjack, task 3.1 and WP7 Reproduction and Genetics - grey mullet, task 7.4) and nutrition (WP13 Nutrition - grey mullet, task 13.4). UNIBA work will involve histological, immunohistochemical, biochemical and molecular biology analyses, as well as *In Situ* detection of apoptotic cells.

- Task 3.1 - Wild-caught amberjack broodstocks maintained in captivity at ITTICAL will be sacrificed at different times during the reproductive season. Blood, brains, pituitaries, gonads, muscle and liver will be sampled to study the reproductive cycle, and spines will be collected for age determination. Wild fish will be sampled at the same time as a reference for evaluating reproductive function of captive fish. Proliferation and apoptosis of germ cells during spermatogenesis will be examined using Proliferating Cell Nuclear Antigen (PCNA) and the TUNEL method. A comparison of liver vitellogenin (Vg) and ovary Vg receptor (VgR) gene expression between captive and wild females will be assessed by cDNA sequencing and real time-PCR (qPCR); Vg plasma level will be determined by ELISA; an analysis of oocyte yolk accumulation will be performed on histological sections using image analysis. UNIBA will be in charge of the sampling of all the biological material (brain, gonad, pituitary, plasma,) necessary for the implementation of the activities of the other partners involved in greater amberjack reproduction (HCMR and IOLR). These samples will be properly stored until their shipment to the relevant laboratory.
- Task 7.4 – Grey mullet gonad samples will be analysed histologically and oocyte yolk accumulation will be monitored using image analysis software.
- Task 13.4 - UNIBA will be responsible for designing adequate feeding regimes for broodstock to optimize reproduction success. In particular, specific requirements of protein, TAU, ARA, DHA and carotenoid sources to optimize spawn quality in mullet will be determined through the analysis of liver Vg gene expression, oocyte Vg receptor gene expression and yolk accumulation under different dietary conditions.

All the equipments necessary for the analyses is already available in the two Departments of UNIBA that will be involved in the proposal and the project will not be charged for their use. The main equipment consists of, but is not limited to: automatic embedding machine, paraffin oven, microtomes, cryostat, freezers, light microscopes with computerized image analysis systems; stereoscopes, Elisa microplate reader, Thermocycler, Real Time PCR Sequence Detection System. University lecture and conference rooms will be available to host project meetings with no charge.

**Personnel cost:** A total of **85,205 €** is allocated to personnel costs. This will cover the cost of existing personnel (researchers, technicians, administrative officers) as well as hiring of external personnel (graduate students, biologists/veterinarians) who will be involved in GWP Reproduction and Genetics, GWP Nutrition and GWP 31 Dissemination. The external personnel will be involved in field work (participation to alive fish capture campaign; fish sampling from rearing tanks and cages), in laboratory work, in scientific data elaboration (biostatistics) as well as in the presentation of salient project results in scientific Conferences.

**Consumables:** A total of **41,000 €** is allocated to consumables. This includes: alive fish that will be purchased and transferred to the rearing site (the cost of alive fish capture will be shared with the Participant 24 ITTICAL); dead fish that will be purchased from fishermen soon after their capture in order to compare reproductive parameter between the wild population and the fish reared in captivity; plastic and glass tubes, bottles, beakers, paper, scalpels, etc.; reagents for histology (formalin, picric acid, ethanol, xylol, paraffin, dyes); antibodies for immunohistochemistry and Elisa; kits for immunohistochemistry and for Elisa; kits for *In Situ* cell death detection (TUNEL); RNA later for storage of samples destined for RNA analysis; kit for RNA extraction; reverse transcription reagents; primers and kits for PCRs.



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**Travel costs:** A total of **24,367 €** is allocated for travel and subsistence costs. These costs will include travels related to: alive fish capture and transfer to ITTICAL facilities, sampling of fish caught from the wild and fish reared in ARGO (Greece) for Task 3.1 and ITTICAL for Task 3.2; participation to the kickoff and annual coordination meetings; attendance of conferences of Aquaculture and/or Reproduction Associations for dissemination purposes.

**Equipment:** No equipment purchase is foreseen since all the instruments necessary for the implementation of UNIBA activities are already available in its laboratories.



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**Partner 14. IFREMER**

IFREMER will participate only in GWP Reproduction and genetics in DIVERSIFY and more specifically on Mediterranean endemic species (meagre, greater amberjack, wreckfish and grey mullet). Its participation will mainly involve sampling and cryopreserving of sperm, recording sperm data and then training end-users for field assessment of gamete quality in the different large-scale facilities of Mediterranean partners. Finally analytical treatments will be processed in Ifremer laboratories and offices.

**Personnel cost:** A total of **69,407 €** is allocated to personnel costs. This will cover the cost of existing personnel (1 researcher and 1 technician) which will be occasionally reinforced through exchanges of trainees (COST network such as Aquagamete) without any complementary cost for the project.

**Consumables:** A total of **5,000 €** is allocated to consumables. This includes low cost laboratory products (mainly salts), disposable tubes and counting cells, ATP assay kits and external disk for video storage.

**Travel costs:** A total of **17,000 €** is allocated of which 5,000 € are dedicated to coordinating and management activities of the project and travelling to the annual coordination meetings (WP 1 Management) and 12,000 € for the implementation of reproduction experiments in GWP Reproduction and genetics, which will take place in other Partner facilities needing travels (different sites in Spain, Italy, Greece).

**Equipment:** No equipment budget was programmed in the frame of this project. The experiments will use already existing equipment, such as microscope camera and laptop, provided by IFREMER.



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**Partner 15. ULL**

ULL will participate in all 6 Groups of scientific WPs in DIVERSIFY and will contribute to the work planned for all selected species at different stages of their life cycle (eggs, larvae, juveniles or broodstock), primarily focusing on nutritional aspects. Within GWP Socioeconomics Socioeconomics, ULL will contribute to Task 28.3 Monitoring technical quality of the products. A wide range state-of-the-art laboratory equipment including analytic instruments such as ovens, analytical balances, GC, GC-MS, HPLC, scanning densitometers and NIR spectroscopy will be used for the implementation of ULL actions related to the determination of biochemical composition within GWPs and for the quality evaluation of final developed products in GWP Socioeconomics. The lab facilities also include all the necessary tools for the measurements of digestive enzymes, ATPase, and other enzymatic activities acting as biomarkers of oxidative stress.

**Personnel cost:** A total of **161,462 €** is allocated to personnel costs. This amount will cover the cost of the contribution of highly trained and experienced existing personnel of the organization committed to the project, including senior researchers that will be directly involved in the organization of labours, interpretation and publication of results, a pre-doc fellow and a senior technician that will actively help with the necessary laboratory work. In addition, a new post-doc will be hired for three years as the basis for the implementation of research activities within the scientific WPs.

**Consumables:** A total of **28,081 €** is allocated to consumables. This category is mostly linked to the acquisition of analytical consumables, primarily chemicals, for the analyses of proximal composition, lipid, fatty acids and carotenoids profile of biological samples (GWP Reproduction and genetics, GWP Nutrition and GWP Larval husbandry), measurements of digestive enzymes and ATPases activities (GWP Nutrition and GWP Larval husbandry), monitoring of oxidative stress status of experimental groups (Task 21.3), cell isolation and cellular viability and integrity of osmoregulatory epithelia under monogenean parasitism (Task 25.5), evaluation of chemical quality of developed products (Task 28.3), as well as for the purchase of several C<sup>14</sup>-radiolabelled fatty acids to assess dynamic aspects of lipid nutrition through metabolism assays (Task 10.2).

**Travel costs:** Part of the total **8,265 €** allocated in this category will be used to cover costs associated with travelling of the Scientific Responsible to the kickoff and annual coordination meetings. The Scientific Responsible and/or the hired post-doc will also attend EAS or other national or international conferences on the fields of Fish Nutrition or Food Science for dissemination purposes. Travel costs also include costs brought about from shipment of biological material (e.g., eggs, larvae, tissues or final product samples) received from other Partners of the consortium with rearing facilities where the experimental design of the project will take place. ULL will receive biological samples from FCPCT, IRTA, IEO-Vigo, HCMR and IMR for detailed biochemical composition and enzymatic analysis. Finally, this budget is also foreseen for short-term travel activities from collaborative effort among partners, as those derived from the implementation of the scientific work proposed in WP3, including studies on lipid metabolism related to stress tolerance through radiotracing of C<sup>14</sup> fatty acid metabolism in specific pikeperch tissues, at DTU facilities in Denmark.

**Equipment:** No new equipment is needed to be purchased, the current laboratory facilities are sufficiently equipped for the implementation of ULL's tasks within DIVERSIFY.

**Complementary Resources committed:** If needed, additional analytical consumables and office consumables will be purchased with complementary fundings from other regional or national research projects.



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## Partner 16. FUNDP

FUNDP will participate in two Work Packages of the Diversify project, namely GWP Nutrition and GWP Grow out husbandry, with a special emphasis on Pikeperch. In GWP Nutrition, FUNDP will be involved in task 10.1 dealing with the improvement of larval nutrition of pikeperch, including enzymatic analyses and histology of target tissues (using photonic and, if needed, electronic microscopy), transcriptomic analyses of target genes expression and without *a priori* approach based on 2D-DIGE proteomics on specific organs (principally liver). Resistance of larvae/juveniles will be assessed by bacterial and challenge tests. In GWP Grow out husbandry, research will focus on tasks 22.1 to 22.3 including the effects of husbandry practices and environmental factors on pikeperch growth, immune and physiological status under experimental and farm conditions, as well as the effects of fish domestication level and geographical origin on growth and stress sensitivity.

**Personnel cost:** A total of **137,480 €** is allocated to personnel costs. This amount will cover the cost of the contribution of highly trained and experienced existing personnel of the Research Unit in Environmental and Evolutionary Biology (URBE) committed to the project, including senior researchers that will be directly involved in the experimental design, daily organization of labours, interpretation of data and publication of results, as well as the partial cost of a PhD fellow who will conduct the experiments and analyse the samples with the help of a technician. This category also includes the cost for project management.

**Consumables:** A total of **40,840 €** is allocated to consumables. This category includes the costs for chemicals and disposable tubes for enzymatic analyses, primers and chemicals for expression analysis of selected genes as well as cyanins for 2D-DIGE proteomics and mass spectrometry analyses. Cost for stress hormone analyses (ELISA kits) and consumables for hematological and immunological markers will be included in this category.

**Travel costs:** Most part of the **8,900 €** allocated in the travel costs category will be used to cover the travels of the researchers for participating in joint experiments with other partners involved in pikeperch nutrition and grow out WPs, namely with UL and ASIALOR in France and DTU in Denmark. During these travels, samplings will be done and analyses will be performed by FUNDP. A part of the travel costs will also be used by the Scientific Responsible for attending the kickoff and annual coordination meetings.

**Equipment costs:** The available budget does not allow any additional equipment to be purchased. Research will be conducted with the existing equipment present in FUNDP.

**Complementary Resources committed:** Institutional funds will be allocated to support the costs of some experiments and related analyses. Grants for PhD fellow will also be searched through Belgian government fundings completing the budget allocated to the personnel.



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**Partner 17. NIFES**

*This Partner has been taken over universally by P7. IMR, who is from now on responsible for the implementation of the work originally assigned to NIFES, and has also received its remaining budget.*

NIFES' main participation is in WP11 Nutrition - Atlantic halibut (6 PM), with minor participation in WP5 Reproduction and Genetics - Atlantic halibut and WP17 Larval husbandry – Atlantic halibut (1 PM each). The institute is a National Reference Laboratory within nutrient analysis and has a well developed laboratory infrastructure, with certified analyses run on a routine basis, of all nutrients and many contaminants. We also have state of the art labs for biochemistry, cell biology, microbiology, histology and molecular biology, including genomics and proteomics. The rearing experiments in which we are involved will be performed by IMR (partner 7).

**Personnel cost:** A total of **81,815 €** is allocated to personnel costs. This will cover the cost of existing personnel, ca 4 PM for technical work and 4.5 PM for researchers.

**Consumables:** A total of **28,123 €** is allocated to consumables. This includes 25,000 € for general laboratory consumables (chemicals, salts, plasticware, etc.), 3,400 € for a transcriptome library for halibut and the rest for unforeseen costs.

**Travel costs:** A total of **8,060 €** is allocated to travel. Some of these costs (2,447 €) are for travelling locally in connection with sampling and internal meetings within the halibut group. The rest will contribute to covering the annual meetings in the project.

**Equipment:** No equipment purchase is foreseen for the project.





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**Partner 18. CTAQUA**

CTAQUA will participate in 3 Groups of scientific WPs in DIVERSIFY. Its contribution will focus on the WP13 Nutrition and WP23 Grow out of grey mullet. CTAQUA will contribute to the nutritional work with its wet lab facilities and in the grow out of grey mullet with a large-scale trial. The wet lab includes several recirculation systems mostly dedicated to nutritional and feeding trials (GWP Nutrition). For large-scale trials, CTAQUA members are fish producer companies and fish farmers, most of them using pond culture as fish cultivation method (GWP Grow out husbandry). CTAQUA participates also in GWP Socioeconomics, with the development of new products from the DIVERSIFY species, the study of the consumer sensory perceptions and the development of new market strategy. Its facilities include a processing room where physical prototypes can be developed and their basic quality characteristics can be evaluated. **CTAQUA is WP31 Dissemination leader.** The institution has a broad experience in communication and transfer of scientific activities to the aquaculture sector as well as a large network of contacts within the aquaculture industry, stakeholders and the responsible administration for fisheries and aquaculture.

**Personnel cost:** A total of **128,139 €** is allocated to personnel costs. This amount covers the cost of existing personnel (researchers, technicians) as well as hiring of new personnel (graduate students and technicians) who will be involved in various WPs in the areas of Nutrition, Grow out husbandry, Socioeconomics and Dissemination (WP31).

**Consumables:** A total of **43,666 €** is allocated to consumables. This includes feeds (grow out diets and chemicals for water quality control) for GWP Nutrition and GWP Grow out husbandry and consumables related to the processing and development of new fish products for GWP Socioeconomics. General laboratory consumables, maintenance of recirculation systems and office consumables are also included in this category.

**Travel costs:** A total of **19,151 €** is allocated to travel costs; these are related to attendance to the annual project meetings (WP 1), the implementation of various tasks of the GWPs Nutrition, Grow out husbandry and Socioeconomics, and mostly to the dissemination activities (WP31). The WP31 leader will be very much involved in the organization of the Promotional Workshops to be held in Spain, Greece, Italy and UK. Travel costs are also associated with inviting relevant speakers from specialized consumer organizations and/or professional associations to address consumer and market related topics. The WP Dissemination leader will be organizing the presence of DIVERSIFY at the European Seafood Exposition, as well as attending the annual EAS annual meeting for project dissemination purposes (Y1-5). Travel costs are also foreseen for meetings with main European Associations of fish processors, consumers, supermarkets to establish collaboration agreements with the purpose of broadcasting project activities.

**Equipment:** None.



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## Partner 19. CMRM

CMRM will be involved in the development of the culture of wreckfish, it will participate in 3 Groups of scientific WPs in DIVERSIFY (Reproduction and Genetics, Nutrition and Larval husbandry) and it has some resources that will be made available free of charge to the DIVERSIFY project in an effort to reduce the overall cost of the proposal. So, some facilities can be available for the DIVERSIFY project: breeding tanks with a wreckfish broodstock, which has been acclimated to captive conditions for four years, tanks for larval culture in RAS system, small-sized sea cages in order to develop the on-growing of juveniles. It has also a laboratory with some equipment (spectrophotometer, analytical balance, gas chromatographer provided with automatic injector, TLC Scanner etc) available to perform the biochemical analysis needed for the project (WP6, WP12 and WP18).

The budget requested to the EU for personnel and other direct costs has been reduced to the minimum and will be used for the maintenance of installations, the capture of wild fish to increase the broodstock, feeding for larval and broodstock wreckfish, some basic equipment as well as fungible material needed for the biochemical analysis.

**Personnel cost:** A total of **55,000 €** is allocated in the budget for new personnel and this will cover hiring a technician with total dedication to the project. The CMRM existing personnel involved in Diversify consists of two researches and a technician. They will be involved in various WPs in the areas of Reproduction and Genetics, Nutrition and Larval husbandry.

**Consumables:** A total of **40,666 €** is allocated to consumables. This includes feeds (larval, nursery, and broodstock) and relevant products (enriching diets for larvae, fresh and commercial dry food for wreckfish broodstock). This category includes also analytical consumables for Reproduction and Genetics (water quality analysis, general lab chemicals; consumables related to disinfection and prevention pathology products, canvas to cover the tanks of wreckfish broodstock, anaesthetic), Nutrition (chemicals for the analyses of lipids, proteins and fatty acids), and Larval husbandry (water quality analysis, probes maintenance products, calibration probes of RAS system, cartridge filter, ultraviolet lamps).

**Travel costs:** A total of **15,313 €** is allocated and will be related with management activities of the project (WP 1) and with the implementation of various tasks of the scientific WPs (Reproduction and Genetics, Nutrition and Larval husbandry). Specifically travel costs are associated with travelling of the scientific responsible to the Kick off meeting and if it is required to the annual coordination meeting. Furthermore, travel costs are also associated with attendance of the EAS annual meeting by the scientists.

**Equipment:** A total of **10,000 €** is allocated to the purchase of equipment. These include a multiparameter meter to measure temperature, dissolved oxygen and salinity, a flujometer, water level probes, alarm control, alarm systems, alarm headquarters. The justification would be the need to have controlled parameters as oxygen, temperature and salinity as well as the flow and the water level in the tank of wreckfish broodstock. The function of the alarms is to notify us of any difficult situation or critical values. This control is essential to avoid risks in the broodstock tanks.

This equipment will be used 100% of the time in the broodstock tank control for the five years of the project. The amortization will be 20%/year, so at the end of 5 years, the equipment will be totally amortized (100%).



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**Partner 20. SARC**

Skretting Aquaculture Research Center (SARC) will participate in the GWP Nutrition and GWP Fish health Pathology. The main tasks will be to formulate and to produce the experimental feeds to be used by the other partners in 8 experimental research trials. Formulation and discussion with the other partners of the experimental diets involves one Principal researcher and one Senior researcher.

The feed production of the experimental diets, requires the procurement of raw materials, their analysis and approval, in order to make sure that all diets produced in this project will be made with raw materials of equivalent quality and composition.

The analysis of raw materials prior to feed production as well as the quality analysis of the final feed involves personnel at the laboratory of SARC and the use of necessary equipment and consumables.

The experiment involves renting the Feed Technology Pilot Plant of SARC. This includes the personnel at the pilot plant and the use of the machinery (mixers, extruders, coaters dryers, etc) to produce the extruded diets at experimental level.

Finally, the costs also include the shipment fees of the experimental feeds to each partner involved.

**Personel:** A total of **93,950 €** is allocated to personnel costs, which includes the two researchers and the technicians involved in the feed production and laboratory analysis.

**Consumables:** A total of **22,000 €** is allocated to consumables, which includes the procurement of raw materials for the production of the experimental diets, like vegetable raw materials (soya bean meal, corn and wheat gluten soya concentrate, wheat, etc), fish meals, fats and oils (fish oil and vegetable oils) and the minerals, vitamins and amino acids necessary for the dose response trials planned.

**Travel costs:** A total of **17,000 €** is allocated to travelling, which includes the travelling for the regular meetings of the project and management.



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**Partner 21. DTU**

DTU (DTU Aqua) mainly participates in work on pikeperch (WP10 Nutrition – pike perch, WP16 Larval husbandry – pike perch, WP22 Grow out and husbandry – pike perch), but will also contribute in nutritional work on meager (WP8 Nutrition – meagre). Its participation will be both with analytical labs, advanced RAS facilities for the rearing trials on larvae, juveniles and ongrowing fish, as well as facilities for behavioural studies and respiratory physiology at DTU Aquas facilities at the North Sea Research Centre.

Further to the research rearing facilities, state of the art laboratory equipment and analytical equipment (HPLC, GC/MS) for analyses of amino acid, hormones and fatty acid analyses will be used.

**Personnel cost:** A total of **130,717 €** is allocated to personnel costs. This will cover the cost of existing personnel (researchers, technicians) as well as co-funding of 1 post-doc, who will be involved in nutritional and behavioural related aspects (GWP Nutrition).

**Consumables:** A total of **14,750 €** is allocated to consumables. This includes feeds (larval, nursery, grow out and broodstock, and relevant products (enriching diets for larvae) and running costs of RAS systems incl. water, heat. Lab chemicals for HPLC and GCMS and for protein, lipid, cortisol analyses etc are also included.

**Travel costs:** A total of **13,777 €** is allocated to implementation of various tasks of the scientific WPs management and dissemination activities. Specifically, travel costs are associated with travelling of task scientific responsables to kickoff and annual coordination meetings.

**Equipment:** A total of **1,800 €** is allocated to the purchase of ingredients and chemicals and chemicals for analytical purposes, including part of tracking system software for behavioural examinations of fish juveniles.



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**Partner 22. SWH**

This Partner is a commercial halibut producer with a full-scale juvenile production facility at Rørvik in northern Norway. Resources committed are broodstock tanks, personnel and fish for hormone induction trials and larval feeding trials.

**Personnel cost:** A total of **96,047 €** is allocated to personnel costs. This will cover the cost of existing personnel who will be involved in various WPs: WP Reproduction and Genetics, GWP Nutrition and GWP Larval husbandry and some costs connected with report preparation in WP1 Management.

**Consumables:** A total of **72,285 €** is allocated to consumables. This includes costs connected with holding experimental broodstock tanks (water supply, oxygenation etc), feeds (larval, nursery, and broodstock), and relevant products (enrichment of diets for larvae). General laboratory consumables and office consumables are also included in this category.

**Travel costs:** A total **22,770 €** is allocated to travel. Travel costs are related to dissemination activities (WP31), as well as for the implementation of various tasks of the scientific GWPs. Specifically, travel costs are associated with travelling to the kickoff and annual coordination meetings. Travel costs have been foreseen for the implementation of the scientific work in the GWPs, as the broodstock holding facility and hatchery are located in Northern Norway and travel is required for the scientific responsible to get there and supervise the activities. These include spawning induction experiments involved in GWP Reproduction and Genetics, as well as larval nutrition and rearing trials within GWP Nutrition and GWP Larval husbandry.

**Complementary funding:** As SWH is an enterprise, 50% of the total costs will be covered by own funding. There is at present no other complementary funding available for the project work.



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**Partner 23. ARGO**

ARGO will participate in WP3 Reproduction and Genetics-greater amberjack, in Task 3.2 Development of an optimized spawning induction protocol for captive greater amberjack in the Mediterranean and Task 3.5 Spawning induction of greater amberjack and egg collection in cages. The company is in charge of obtaining mature breeders from the wild and maintaining them in cages in the facility for the duration of the project. The broodstock will be used for spawning induction experiments, using hormone treatment if necessary and for the development of egg collection methods from sea cages.

ARGO also participates in WP20 Grow out husbandry – meagre in Task 20.2 Effect of rearing environment, where the effect of light intensity will be examined on fish distribution and growth performance.

The owner of ARGO and Scientific Responsible Mr. Anastasios Raftopoulos has expertise in the field of fish farming since 1987, when ARGO was founded. Since then, ARGO has grown into a modern, vertically integrated unit with a hatchery, a modern unit for fry pre-fattening, feeding units in the open sea, as well as a modern packaging and standardization unit. In 2007, ARGO participated in the European Programme ALFA (FP6), for the continuous production of live feed in aquaculture hatcheries.

Due to a total mortality of the broodstock obtained for the activities of Task 3.1 (WP3) and the difficulties faced by the company to acquire another stock, this Task was moved to another Partner (P23. ARGO), who succeeded in acquiring an adequate broodstock and offered to give this to the project. Therefore, a significant part of the original budget from ITTICAL (75,000 EU contribution) was transferred mainly to ARGO. Smaller budget transfers were also made to partners HCMR, IOLR, UNIBA, IFREMER and UL, to cover the additional cost incurred due to the samplings that were now going to be done in Greece, in partner ARGO.

**Personnel cost:** A total of **149,000 €** is allocated to personnel costs. This will cover the cost of existing personnel (technicians, workers, guards, accountant) as well as hiring of new personnel (an ichthyologist, technician) who will be involved in the areas of development of an optimized spawning induction protocol for captive greater amberjack in the Mediterranean (led by HCMR) and in the area of grow out husbandry – meagre.

**Consumables:** A total of **135,000 €** is allocated to consumables. These include fish feeds, acquisition and grow out of broodstock. Also included are nets and ropes for the rearing cages, as well as fuel for boats and for the transfer of personnel at the plant site.

**Travel costs:** A total of **9,125 €** is allocated for travel to the annual coordination meetings.

**Equipment:** A total of **9,833 €** is allocated to the purchase a variety of equipment. These include personal desktop and laptop computers needed for data collection, management and transmissions, as well as underwater cameras for continuous recording of the movements and behavior of fish into the cages. Due to the development of a method to train fish to accept dry, commercial pellets during the first year of the project, the purchase of a small fish feed machine (approximately 200 Kg/h) for the production of moist food used for the rearing of wild amberjack broodstock (commercial feed mixed with small fish) is no longer considered necessary, and part of the money originally planned for equipment will go to the purchase of additional commercial feeds. On the other hand, due to the transfer of Task 3.1 to ARGO and the addition of spawning induction experiments (Task 3.2) there is a need for the purchase of a compound microscope and a stereoscope for the evaluation of gametes and eggs (5,500 €) and a low-temperature incubator (2,000 €) for the incubation of eggs and the evaluation of embryonic development and larval survival. This equipment will also be used during all the reproductive seasons to evaluate the quality of the produced eggs from the induced spawning experiments, prior to their shipment to the various larval rearing sites.



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**Partner 24. ITICAL:**

ITICAL exited the consortium on 31 May 2016. Up to that time, it has been approved for a total of **90,372 €** according to the financial statement of the 1<sup>st</sup> and 2<sup>nd</sup> Periodic Report.



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**Partner 25. DOR**

DOR will participate in WP7 (Reproduction and Genetics – grey mullet) in the task optimization and scale-up of a breeding protocol for grey mullet in captivity. In WP19 (Larval husbandry – grey mullet) DOR will evaluate an improved grey mullet larval rearing protocol, based on work carried out in WP13 (Nutrition – grey mullet) and WP19, in one of three commercial systems (depending on the number of eggs) that include nineteen 10 m<sup>3</sup> tanks, eight 7 m<sup>3</sup> tanks or four 5 m<sup>3</sup> tanks (19.5). In addition, DAG will participate in WP23 (Grow out husbandry – grey mullet) where it will grow over 1 year F1 grey mullet fingerlings in two 6000-m<sup>2</sup>, which will differ in their stocking density (0.5 and 1 juvenile m<sup>-2</sup>). The fish will be fed an improved grey mullet grow-out diet and DOR staff will evaluate fish performance in terms of FCR, PER, SGR, overall weight gain and survival. An economic evaluation of the trial will also be calculated. These results will be compared to (1) growing F1 juveniles in cement bottom tanks in Eilat, Israel (IOLR), as well as (2) growing wild juveniles in earthen and cement bottom tanks in Greece (GEI) and Spain (CTAQUA).

**Personnel costs: 28,400 €** will be allocated to cover the cost of DOR technician time (18.5 PM) that will be carrying out the assigned tasks of WP7 (4 PM), WP19 (4 PM) and WP23 (10 PM) tasks as well as WP1 (management; 0.5 PM) and WP30 Socioeconomics (0.1 PM).

**Consumables: 11,875 €** will be allocated to purchase GnRH and MT implants, enrichment preparations, *Artemia* cysts, broodstock feed and the rearing of algae and rotifers.

**Travel costs:** Travel costs of **8,00 €** is allocated for attending the annual consortium meetings and for attending scientific conferences and the State of the art species seminars, as well as for a meeting with the Socioeconomics team for the preparation of the feasibility study.

**Complementary resources committed:** DOR is committing to this project the time of the hatchery manager as well as the running costs for utilities and the maintenance of infrastructure, equipment and UV systems.





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**Partner 26. GEI**

**Personnel cost:** A total of **20,000 €** is allocated to personnel. This will cover the cost of existing technicians and specialized labor workers who will be involved in the implementation of the project for a total of 16 PM (WP23 Grow out husbandry-grey mullet).

**Consumables:** A total of **2,500 €** is allocated to consumables such as plastic water and air pipes, purchase of animals, nets, general laboratory and office consumables.

**Travel costs:** A total of **1,000 €** is allocated to cover the costs of travelling mainly associated to the transport, food, lodging and registration fee of the scientific leader to participate to the planned international meeting and workshops that will be periodically organized by the consortium for dissemination and coordination purposes (WP1). In addition, company personnel will participate in aquaculture conferences in Europe.

**Equipment:** A total of **5,000 €** is allocated to purchase of equipment such as water pumps, water oxygenation system and probe for ammonia measurement.

**Complementary Resources committed:** GEI will activate all the required resources to conduct the research in the manner and within the time limits established, involving internal skilled and specialized personnel. An adequate number (6) of grow-out cement ponds (20 m<sup>2</sup>), already present in the farm, will be made available to maintain the animals in a flow-through water regime and natural photoperiod. The water temperature in the tanks will be equipped with an oxygen monitoring system and the temperature of water will be monitored daily.



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**Partner 27. FORKYS**

**Forkys Aquacultures SA** is a private enterprise located in Chios Island, specialized in the reproduction and grow-out of marine fish species. It is active since 1994. Its main activity is the production of European sea bass and gilthead sea bream in sea cages. Moreover, new species such as common sea bream, common dentex, sharpnose sea bream and meagre are being cultivated at Forkys net cages. Recently, FORKYS acquired a hatchery and a fish farm in Crete, Greece and started with hatchery production of both species. Forkys employs more than 120 persons involved in reproduction, grow-out and packing. FORKYS has also begun recently the production of meagre. The company's goal is to increase the annual production of hatchery to 15 million fry production and the production of grow-out farms to 10,000 metric tones. In addition, FORKYS is interested in diversifying its production with new/emerging species such as meagre, the greater amberjack and wreckfish. FORKYS will be involved in Larval husbandry and Grow out husbandry. In WP15 Larval husbandry - greater amberjack incubation of eggs and rearing of larvae for the optimization of a husbandry protocol will be done in 2 periods of 4 months each at the facilities of Forkys hatchery in Crete. In WP21 Grow-out husbandry - greater amberjack, in a period of 12 months, the optimum grow-out depth will be determined at net cages in the grow-out farm of Forkys in Crete.

**Personnel:** A total of **17,100 €** is allocated to personnel. This will cover the cost of existing personnel (scientists, technicians, divers and specialized worker) who will be involved in the project for a total of **30.5 PM**. In particular the personnel effort will be shared in the WP 1 Project management for 0.5 PM for financial management and administration of the project; WP3 Reproduction and genetics – greater amberjack for husbandry of the broodstocks, spawning induction, egg collection, evaluation of egg quality and shipment to partners and in the WP21 Grow out husbandry – greater amberjack for the sea cage rearing of juveniles.

**Travel costs:** A total of **8,900 €** is allocated to cover the costs of travelling as needed for the collection of wild fish, and for attending the annual coordination meetings.

**Equipment:** A total of **3,000 €** is allocated to the purchase of equipment. These include microscope, computer, laptop for data recording.

**Consumables:** A total of **16,230 €** is allocated to consumables. Main items will cover: the amberjack broodstock capture; the purchase of commercial feeds, fresh or frozen raw seafood for the daily feed of greater amberjack broodstocks; reagents and chemical products to assure the right water parameters (pH, alkalinity) of fish tanks; general laboratory and office consumables.



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**Partner 28. CANEXMAR**

CANEXMAR will participate in WP1 Management and WP 9 Nutrition-greater amberjack, including Task 9.2 Development of diets for grow-out of amberjack to maximize growth (led by HCMR). In particular CANEXMAR will be responsible of Sub-task9.2.2, where the grow-out diet developed within Sub-task9.2.1 will be tested at an SME level, in order to assay its efficiency to maximize growth potential and enhance fillet quality. Survival, growth feed utilization and fillet quality will be determined.

CANEXMAR also participates in WP 21 Grow out husbandry – greater amberjack in Sub-task21.1.2 effect of cage type on performance. A comparison of surface and submerged cages will be performed in trials with commercial cages of 12 m diameter; 10 m depth, for 2 successive rearing periods of 12 months each. The final stocking density will be kept at 15 kg m<sup>3</sup>. Growth performance and health status will be estimated every second month. The tasks will include: a) maintenance and feeding of fish during the experimental period, (b) monitoring and recording parameters as the daily oxygen levels and temperature and (e) support to the FCPCT staff during the experimental period. Amberjack juveniles will be stocked in regular cages (12-m diameter) where and optimized diets will be tested in replicates over 1 year.

**Personnel cost:** A total of **72,000 €** is allocated to personnel costs. This will partially cover cost of existing personnel (technicians, divers, workers, guards, accountant) as well as hiring of new personnel who will be involved in the areas of development of an optimization greater amberjack grow out operations for both tasks.

**Consumables:** A total of **37,150 €** is allocated to consumables. This includes all consumables needed to carried out grow out of greater amberjack juveniles. Also included are, adaptation of submersible rearing cages, nets and ropes for the rearing cages, as well as fuel for boats and for the transfer of personnel at the plant site.

**Travel costs:** A total of **11,000 €** is allocated to cover the costs of travelling for attending the annual coordination meetings.

**Complementary Resources committed:** CANEXMAR SL will support DIVERSIFY with the whole staff of employees (administrative, divers, technicians, mechanics, biologist) and facilities including mooring structure for 10 cages, 1 boat and 2 zodiacs.



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## **Partner 29. ASIALOR**

ASIALOR exited the consortium on 31 May 2016. Up to that time, it has been approved for a total of **90,372 €** according to the financial statement of the 1<sup>st</sup> and 2<sup>nd</sup> Periodic Report.



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**Partner 30. CULMAREX**

CULMAREX exited the consortium on 20 March 2015. Up to that time, it has been approved for a total of **10,040.16 €** according to the financial statement of the 1<sup>st</sup> Periodid Report



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**Partner 31. IRIDA**

IRIDA S.A. is a dynamic and independent company that produces and supplies aquafeed to the Mediterranean aquaculture industry. The products include high quality fish feeds manufactured with a diameter range from 1.0-12.0 mm for species such as gilthead sea bream, European sea bass, meagre, sharpnout seabream and red porgy. IRIDA is the first fish feed company in Greece that has been certified for Organic Fish Feed production.

IRIDA invests in research and innovation in order to support in the best way the Mediterranean aquaculture industry. Main research areas for the R&D efforts include improvement of taste and quality for farmed fish, development of sustainable fish feeds and species diversification.

In DIVERSIFY, IRIDA will formulate and produce the experimental diets for the growing experiments of grey mullet in WP23 Grow out husbandry – grey mullet. IRIDA will take care of the raw material, as well as the feed analysis and quality.

**Personnel:** A total of **14,993 €** is allocated to personnel costs. This will cover the cost of **Mr Nikos Papaioannou** (Scientific Responsible), who is the technical manager of the company and will oversee the feed formulation. Salaries will also be paid for the technicians producing the feed in the company factory.

**Consumables:** A total of **22,000 €** is allocated to consumables, which includes raw materials for fish feed production, including fishmeal, fish oil, vitamin mixes, soya, carotenoids, krill meal, etc.

**Travel costs:** A total of **8,600 €** is allocated to travelling expenses for attending the annual coordination meetings.



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**Partner 32. MC2**

The participation of the MC2 is exclusively focused on Wreck-fish, with a total budget of 51.552,00 €. Studies will be performed for implementation of WP2 and WP4. Budget allocation will be 66% for reproduction and 34% for larval husbandry.

The infrastructure that the MC2 will provide (free-of-charge) to carry out this proposal includes a stock of 26 individuals of wreckfish most of them over 20 kg in weight, spontaneous egg laying with males natural sperm fecundation. They are mainly held summer and winter in a 3,500 m<sup>3</sup> exhibit tank, iving among many other species representatives of our shore. A 75 m<sup>3</sup> isolation tank is directly connected to the main exhibition tank trough a water corridor provided with two floodgates. This makes fish handling very easy and as stress-free as possible to wreckfish. Wreckfish are now used to enter into the isolation tank by themselves. Beside the main exhibit tank “Nautilus”, a quarantine area has least one 33 m<sup>3</sup> cylindrical, 1,8 m deep fiber glass tank provided with an eeg collector.

Aquarium Finisterrae facilities not only includes isolation main tank and quarantine, but also a full laboratory equipped in order to manage fish and invertebrate pathology, phytoplankton and zooplankton culture, water parameters and quality. At present an exclusive 9 m<sup>2</sup> area is used for a wreckfish hatchery rearing project, but one spacious area situated nearby the mentioned quarantine tank will be used and adapted for the project. Both facilities include breeding area for wreckfish, a hatchery area and general areas for phyto- and zooplankton cultures.

**Personnel costs:** A total of **13,504 €** (26% of total budget) is allocated to personnel costs. This will cover the cost of existing personnel (researchers, technicians) who will be implicated in several studies of various GWPs for the wreckfish in the areas of Reproduction and Genetics and Larval husbandry. The personnel will be also involved in Management (WP1). There is other personnel that will be involved for the duration of the project, but this personal belongs to the actual subcontracting companies, which are working with our Institution.

**Consumables costs:** A total of **10,216 €** (20% of total budget) will be allocated to consumables cost for wreckfish maintenance, with the largest budgets allocations in WP6 (55%) and WP18 (45%). This consumables budget includes feeds (larval, grow out and broodstock), and relevant products (enriching diets for larvae and broodstock). Also analytical consumables for different WP's such as lab consumables required for determination of maturational stage and obtaining of blood samples as well as chemical reactivates to hematological and plasmatic determination of several indicators related with health and welfare (enzymes, hormones, electrolytes, etc.). In addition, general and specific lab consumables will be allocated in WP18 for histological processing, oxidative stress and humoral parameters of the immune system of the larvae and juveniles.

**Travel costs:** A total of **8,500 €** (16% of total budget) is allocated to coordinating and management activities of the project (WP1). Finally, travel costs are also associated with the assistance to international and national meetings to present relevant scientific results. Travel costs are projected for the invitation of outside scientists to attend and present their relevant work in the kickoff meeting and the annual meeting organized in the HCMR (Y1 and Y5). WP leaders and scientist will be attending the EAS and other conferences in various disciplines (e.g. Fish Reproduction, Fish nutrition, Larvae, Fish pathology). Travel costs are also forseen for management and coordination activities such as *ad hoc* meetings with Partners and Group WP leaders.

The MC2 has necessary infrastructure to carry out the tasks assigned for wreckfish in the different WP's, so no budget will be allocated for equipment.



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**Partner 33. FGM**

FGM will participate in the Management section providing support, presence in meetings and contribution in all relevant levels. In the WP31 Dissemination, FGM will contribute by making available its list of members, non-members of the sector, as well as the wide range of contacts in the public and private sector with which a strong and long time collaboration has been built.

**Personnel cost:** A total of **6,900 €** is allocated to personnel costs. This will cover the cost of existing personnel (manager and secretariat) who will be involved.

**Consumables:** A total of **600 €** is allocated to consumables. Office consumables are included in this category.

**Travel costs:** A total of **8,500 €** is allocated for travel costs. These are related to coordinating and management activities of the project (WP1) and dissemination activities (WP31). Travel costs are also foreseen for management and coordination activities such as *ad hoc* meetings with Partners.





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#### **Partner 34. BVFi**

BVFi will participate in the WP31 Dissemination and will monitor all other WPs.

**Personnel cost:** A total of **5,213 €** is allocated to personnel costs. This will cover the cost of existing personnel.

**Consumables:** A total of **5,200 €** (20%) is allocated to consumables. This includes costs for preparing and distributing the project brochure.

**Travel costs:** A total of **9,250 €** is allocated to coordinating and management activities of the project (WP1) and dissemination activities (WP31). Travel costs are associated with the kick-off and annual coordination meetings.



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**Partner 35. MASZ**

*This Partner has been taken over universally by the HUNGARIAN AQUACULTURE AND FISHERIES INTER-BRANCH ORGANISATION (MA-HAL) MAHAL, who is from now on responsible for the implementation of the work originally assigned to MASZ, and has also received its remaining budget.*

MASZ will participate in the WP31 Dissemination and will follow up the other WPs of DIVERSIFY.

**Personnel cost:** A total of **6,500 €** is allocated to personnel costs. This will cover the cost of existing personnel.

**Consumables:** A total of **2,500 €** is allocated to consumables. This includes costs for preparing and distributing the project leaflets.

**Travel costs:** A total of **7,500 €** is allocated to the travel effort for WP1 coordination and management activities of the project (annual coordination meetings) and WP31 dissemination activities.

**Equipment:** none.



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## **Partner 36. ANFACO**

**Personnel cost:** A total of **8,516 €** is allocated to personnel costs. This will cover the cost of existing personnel.

**Travel costs:** A total of **8,700 €** is allocated to management activities of the project (WP1) and dissemination activities (WP31). Travel costs are associated with the kick-off and annual coordination meetings.



## Partner 37. EUFIC

EUFIC will participate in the WP31 Dissemination and provide the following:

- 2 leaflets (one at the beginning to create awareness on the project and one at the end to communicate final results)
- 2 Food Today articles (one at the beginning to create awareness on the project and one at the end to communicate final results)
- Attend the annual meeting of the project (1/year during 5 years)

**Personnel cost:** A total of **19,500 €** is allocated to personnel costs. This will cover the cost of existing personnel who will be involved mainly (18,500 €) in dissemination activities (WP31). In addition (1,500 €), the administrative staff of the organisation shall work on the financial reports (WP1).

**Travel costs:** A total of **6,150 €** travel costs are associated with travelling to attend the kickoff meeting and the annual meetings.

**Subcontracting:** *Print and layout of the project leaflets (5.000 €), translations of Food Today articles in 10 languages (2.500 €), set-up and updating of a quadrant for project on Eufic.org (1,200 €).* Subcontracting is necessary for the following dissemination activities: Print and layout of the two project leaflets, translations of the two FoodToday articles, and set-up and updating of a quadrant highlighting the project on Eufic.org (to drive traffic). The reasons for this are that EUFIC does not have the technical expertise in-house to design and print the leaflet, translate the articles in 10 languages (French, Spanish, German, Czech, Hungarian, Polish, Greek, Slovak, Italian, Portuguese) and the technical expertise to set-up and do the technical updates in order to have a quadrant highlighting the DIVERSITY project on Eufic.org (>600.000 visits/month) with the purpose to drive traffic to the project website. For the leaflets, EUFIC will select a subcontractor according to ECGA rules (at least 3 quotes submitted from different potential subcontractors), for the translations and website technical activities, we have framework contracts with:

Prime Production Ltd (translation agency)

Unit 15, Mill House

Windmill Business Centre

2-4 Windmill Lane, Middlesex

Southall, UB2 4NJ

T: +44 (0)844 482 0004

F: +44 (0)844 482 0475

E: [judha@primeproductions.org.uk](mailto:judha@primeproductions.org.uk)

EUFIC works with Prime Production since 2012 for translations of its core publication Food Today. They offer good quality of translation (reducing the proofreading time by EUFIC staff)

Alligence Communications (website technical activities)

Cogels-Osylei 33

2600 Berchem

Belgium

T. +32 (0)3 270 0 270

F. +32 (0)3 270 0 271

[www.alligence.com](http://www.alligence.com)

EUFIC works with Alligence since 2004 for EUFIC's website [www.eufic.org](http://www.eufic.org) (proprietary technology).



## Partner 38. HRH

HRH is an independent SME with extended experience of consumer surveys as well as of coordinating the data collection in various countries worldwide. HRH, a company of Greek ownership is providing its services to local and International clients since 1992.

HRH will participate in the Socioeconomics WPs 28, 29 and 30, and will assume WP29 leadership by the beginning of month 36 (November 2016), in the area of the execution of the consumer and expert surveys. More specifically HRH will coordinate and be responsible for all the data collection of the surveys described in WPs 28 and 29 using its own resources, infrastructure and experience in similar projects. According to the research program described the surveys will cover five countries (UK, ES, I, FR, D) and will be either qualitative (Focus Groups or In Depth Interviews) or quantitative (Hall tests and On line surveys). More specifically, WP28 and WP29 rely on the collection of primary data through the following tasks:

- a) Task 28.1 An international qualitative research, namely focus groups (at least one per country) and expert interviews (at least 5 per country) in 5 countries (UK, D, ES, F, I) to generate input for new product development;
- b) Task 29.1 An international online consumer survey in 5 countries (UK, D, ES, F, I) with an n=500 at minimum per country (nationally representative samples) to investigate consumers' associations with and perceptions of the new products developed and identify consumer segments within and across countries;
- c) Task 29.2 Hedonic sensory tests in the 5 countries (n=80 per country/segment at minimum) to investigate consumer sensory perceptions about the new products developed in WP28;
- d) Task 29.3 A number of on-line experimental set-ups (i.e., Conjoint or Discrete Choice models) in the 5 countries (n=80 per country/segment at minimum) to test the product concepts (mock-ups) developed in WP29; and
- e) Task 29.4 A second round of experimental set-ups with samples similar to above in terms of size and structure to test for communication effects accompanying the product concepts developed in WP29.

In addition, HRH is responsible to coordinate the entire set of operations in WP29 by month 36, specifically involving the design, implementation and completion of the above-described tasks 29.3 and 29.4.

For the fieldwork of the focus groups, the expert interviews and the hall test (hedonic sensory tests) HRH will cooperate with local teams for the data collection in each one of the countries covered and will be responsible for the quality of the data collected. It is essential in marketing research data collection the interviewer or moderator to speak the same language (native) with the respondents. For this reason HRH will use local interviewers and moderators in each country recruited through cooperating local research agencies but briefed, supervised and controlled for the quality of the collected data from HRH. HRH's experienced executives will be presented during the most of the focus groups, expert interviews and sensory tests or will watch those using viewing facilities (one-way mirror or closed circuit system with camera and monitor in a back room. Focus vision system will be used for mobile viewing, in the case that the HRH executives would not be in the specific country at the time of the group or interview). Additionally for the sensory tests HRH will program the survey questionnaire using a specialized to marketing research surveys platform. The questionnaire will be web based (CAWI or CAPI interviewing) and the data collected will be uploaded to the system used in real time or after the daily field work. Under this procedure HRH and any other authorized partner will have access to the data collected using a unique account number and password. Quality control and basic statistics will be carried out from HRH during the field work in order to secure the final data quality.

For the on line surveys HRH will use its own resources for the web written questionnaires and the panel management (including invitations, reminders and rewards' system). HRH has official license to use the GMI's ([www.gmi-mr.com](http://www.gmi-mr.com)) platform for on line surveys since 2000 and has a great experience of data collection as well as data dissemination through internet.

**Personnel cost:** A total of **119,734 €** is allocated to personnel costs. This will cover the cost of existing personnel as well as hiring of new personnel (translators, coding and data entry, quality control and data validation) who will be involved in the implementation of the research procedures (project specifications,



interviewers' technical manual, questionnaire, discussion guides, decoding of the group discussions and in depth interviews, coding of the open questions) in the five different languages. In addition, the above budget will cover personnel cost for WP29 leadership assumed by HRH by month 36 (November 2016).

**Consumables:** A total of **40,966 €** is allocated to consumables and other direct costs. This includes costs a) to have access to local on line panels in the five countries involved and reach the eligible consumers to fulfill the objectives of consumers surveys in WP29.1 and WP29.3 and 4, and b) to rent the equipped with viewing facilities venue for the focus groups for the WP28.1 as well as to rent the proper equipped venue for the sensory hedonic tests included in the WP29.2, also in light of the 25% increased sample of consumers in the sensory tests agreed.

**Travel costs:** A total of **8,000 €** is allocated related to the travel effort for WP1 coordination and management activities of the project (annual coordination meetings) and the travelling for the implementation of the tasks of WP28 and WP29 in the five selected countries for the included surveys.

**Equipment:** none.

**Subcontracting: *Consumer value perception and segmentation – Data collection for the face-to-face (f2f) surveys (54,852€)***

A total of **54,852 €** is allocated to subcontracting in relation to the face to face surveys of WP28 and WP29 that rely on the collection of primary data through the following tasks:

- a) Task 28.1 International qualitative research, namely focus groups (at least one per country) and expert interviews (at least 5 per country) in 5 countries (UK, D, ES, F, I) to generate input for new product development;
- b) Task 29.2 Hedonic sensory tests in the 5 countries (n=100 per country / segment) to investigate consumer sensory perceptions about the new products developed in WP7.2.

To fulfill the objectives of the above tasks, different types of f2f interviews have to be conducted. For this purpose and in the effort to keep the data collection quality on a high quality level, local teams should be used (through small-scale subcontracting) in cooperation and strict supervision of HRH (Partner 38).

The above-described subcontracting is necessary in order to recruit the participants to the focus groups and expert interviews (WP28.1), as well as the eligible interviewees for the participation to the sensory tests (WP29.2). Furthermore, the subcontracting is necessary due to the fact that it is essential, in the frame of marketing research quality issues, that the moderator of the focus groups, as well as the interviewer of a face to face interview to speak the same language (native) with the participants. Under this scope, local teams of moderators and interviewers will be hired by a cooperating research agency in each one of the five involved countries (who for legal issues should be paid from the local agency). HRH will do all the effort to keep this subcontracting to the minimum possible, undertaking all the tasks of briefing, supervising, controlling and processing of the data collected from the local teams

In this respect, subcontracts will only cover the execution of a limited part of the project, as the above-described tasks have to be carried out from local moderators and interviewers. However, HRH will have the full responsibility of the data collection and for this reason expert executives from HRH personnel will be presented to the briefing and training of the local teams, as well as attending or viewing (through one way mirror or closed circuit or focus vision) the focus groups and an adequate part of interviews to secure the data collection according to the project objectives.

The subcontracting amount is broken into a number of independent subcontracts, established with different companies from the five selected countries, indicatively as follows:

***Subcontract 1 –UK, (11,000 € Partner 38. HRH)***

***Subcontract 2 –Germany, (11,000 € Partner 38. HRH)***

***Subcontract 3 –Spain, (7,000 € Partner 38. HRH)***

***Subcontract 4 –France, (11,000 € Partner 38. HRH)***

***Subcontract 5 –Italy, (9,000 € Partner 38. HRH)***



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Furthermore the subcontracting is necessary due to that it is essential, in the frame of the marketing research quality issues, the moderator of the focus groups as well as the interviewer of a face to face interview to speak the same language (native) with the participants. Under this scope local teams of moderators and interviewers will be hired by a cooperating research agency in each one of the five involved countries (who for legal issues should be paid from the local agency). HRH will do all the effort to keep this subcontracting to the minimum possible undertaking all the tasks of briefing, supervising, controlling and processing of the data collected from the local teams.

As part of Amendmnet 4, an additional amount of 6,000 € subcontracting (EU contribution) is assigned for the platform development (for the e-shop) and consumables correspond to the provision of on line consumer panel in the 5 selected countries.



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## Partner 39. F2B

Fish 2 Be (F2B) is a fish hatchery in Belgium specialised in the out-of-season juvenile production of pikeperch. The company was established in 2013 by founder Ir. Jiri Bossuyt, a seasoned aquaculturist with 10 years of experiences in cultivating Mediterranean species, such as Sea bream (*S. aurata*) and sea bass (*D. labrax*), and freshwater species of the sturgeon (*Acipenseridae*) and percids family (*Percidae*). F2B has done research in the past on breeding stock nutrition. This was partly subsidised by the Flemish government (IWT 140895). Recently we applied for a continuation of this project and to improve also on bio-security and fish health.

F2B will be taking over the role of ASIALOR in diversify. ASIALOR has changed its strategic focus away from research in pikeperch and notified the consortium on their decision. As a result F2B was contacted and is willing to perform the tasks which have not yet been fulfilled within the work packages dedicated to research on pikeperch. The remaining budget that was allocated to the SME ASIALOR will be transferred to F2B.

**Personnel cost:** A total of **70,050 €** is allocated to personnel costs. This will cover the cost of all personnel involved in the experiments described in the work-packages. An additional technician will be hired to help with the practical implementation of the work.

**Consumables:** A total of **19,900 €** is allocated to consumables. This is mainly for feed and breeding stock/larvae acquisition. Also costs related to water-analyses tests, disinfectants, salts, probiotics, nets should be covered by this budget.

**Travel costs:** A total of **5,000 €** is allocated for travel to the annual coordination meetings.

**Equipment:** A total of **3,900 €** is allocated to the acquisition of continuous life feed dispensers, which is required to perform the evaluative tests from WP 10 and 16. Also 6 new controllable tank lights with a different colour spectrum have to be acquired to perform the evaluation tests of WP 16 and WP 22.

**Sub-contracting:** A total of **15,000 €** is allocated for renting systems to perform the grow-out trials from WP 22. F2B is constructed as a fish hatchery with limited grow-out facilities and thus, has not the capacity to hold fish of over 50 g. Part of the evaluation test of WP 22 will be performed at F2B but the continuation of the grow-out trials will be at a fish farm with a recirculating system that can cope with a substantial higher biomass. This budget allows for a 7 month grow-out period in 6 tanks at the allocated site. F2B will provide extra staff for the periodic handling of the fish, such as sorting and sampling. But day to day operations will be performed by the staff at the site.





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**Partner 40. GMF**

**GMF** will participate in WP3 Reproduction and Genetics - greater amberjack, in Task 3.2 Development of an optimized spawning induction protocol for captive greater amberjack in the Mediterranean and Task 3.5 Spawning induction of greater amberjack and egg collection in cages. The company has obtained mature breeders from the wild and maintains them in cages in the facility for the duration of the project. The broodstock will be used for spawning induction experiments, using hormone treatment and for the development of egg collection methods from sea cages. Eggs obtained from these experiments will be used for the rearing of the larvae and the production of juveniles for the other WPs of the project.

**Personnel cost:** A total of **57,400 €** is allocated to personnel costs. This will cover the cost of existing personnel (scientific, technicians, workers, accountant) who will be involved in the areas of development of an optimized spawning induction protocol for captive greater amberjack in the Mediterranean (led by HCMR).

**Consumables:** A total of **33,100 €** is allocated to consumables. These include fish feeds, chemicals, nets and ropes for the rearing cages, as well as fuel for boats for the transfer of personnel at the plant site. Also, buckets, waders, etc.

**Travel costs:** A total of **6,500 €** is allocated for travel to the annual coordination meetings.

**Indirect Costs:** A total of **7,360 €** is allocated for indirect costs. These include electricity, office & telephone expenses etc.



### 3. Impact

#### 3.1 *Expected impacts listed in the work programme*

*(Maximum length for the whole of Section 3 – ten pages)*

*Describe how your project will contribute towards the expected impacts listed in the work programme in relation to the topic or topics in question. Mention the steps that will be needed to bring about these impacts. Explain why this contribution requires a European (rather than a national or local) approach. Indicate how account is taken of other national or international research activities. Mention any assumptions and external factors that may determine whether the impacts will be achieved.*

*When appropriate (relevant for the topic):*

*With regard to the innovation dimension, describe the potential areas and markets of application of the project results and the potential advantages of the resulting technologies/ solutions compared to those that are available today.*

*(Maximum length for the whole of Section 3 – ten pages)*

In contrast to the strong increase of aquaculture worldwide, EU aquaculture production is still limited and far from achieving its real potential. Today, only 20% of seafood consumption in the EU originates from aquaculture and only 10% is produced locally. Aquaculture in the EU has been steady or even declining in the last decade, while imports are increasing, currently at 65% of all seafood consumed. Contributing to this condition is the small variety of aquaculture species, leading to intense competition and reductions in price, as soon as production increases. The **existing market for EU aquaculture fish products is limited presently** and close to saturation for the already well-produced species, such as Atlantic salmon, gilthead sea bream and European sea bass. **Innovative market-oriented diversification** encompassing **higher variety of species and products**, bearing in mind **changes in societal demands and consumer's perceptions**, are necessary to increase the demand for cultured fish and the capacity of European markets to absorb new aquaculture products, and hence cope with the expansion of this sector.

The main expected impact of DIVERSIFY is the **identification of the most appropriate new/emerging fish candidates for the future growth of the European marine and inland aquaculture** and the **improvement of production technologies** for the selected species. Some of these species are produced to a certain extent and have great potential for expansion, while others are **new species with high economical and biological potential**, but need more research to enter commercial production. Furthermore, DIVERSIFY goes well beyond the classic biological and technical approach to species diversification and production increases. Having a significant Socioeconomic component (19.0% of the RTD budget), DIVERSIFY is expected to have also a significant impact on removing bottlenecks in markets and consumer's perception and preferences. This will be achieved through identification of innovative opportunities for growth of the industry and increase of the EU consumption of aquaculture products through **diversification of products and marketing approaches** directed to **improve consumer perception** of aquaculture and **develop new markets**. Such an integrated combination of biological, technological and socioeconomic activities will lead to a reduction in the dependence of the EU on imports from third countries of questionable, at times, production, health, environmental and social standards.

DIVERSIFY builds on past and current research initiatives in which DIVERSIFY Partners have participated, either alone or in collaboration with each other. These projects collectively set out the groundwork for future work and helped in the identification of species-specific bottlenecks that will be addressed in DIVERSIFY, ensuring that 1) effort and resources will focus on filling in gaps in pre-existing biological knowledge and technology and 2) the main institutions with experience in the selected species are part of the consortium, leading to a true magnification of potential impact through synergistic work.

Therefore, to ensure a rapid and substantial impact of DIVERSIFY on the growth of EU Aquaculture a **top-down (bottleneck-based) approach** was employed in defining the objectives for each selected species. Besides collecting information on the “state-of-the-art” for each species, resulting from previous/current projects of DIVERSIFY Partners, this was also achieved by (a) consulting key industrial Partners on the



main obstacles in the production and marketing of each selected species, and (b) taking also into account the different stages of development of the industry (new or emerging). The identified bottlenecks have been prioritized according to the magnitude of economical and productive advancement that can be made by removing each of them. Since the research was designed on the documented obstacles preventing the mastering of the biological cycle of the selected species, DIVERSIFY is expected to **provide solutions to real problems faced by the industry**, guaranteeing that **the results will be implemented readily and enhance rapidly the aquaculture sector, to provide future success stories in EU aquaculture**.

This project represents a truly **global European concerted effort**. It includes marine and freshwater species with wide geographical and ecological distribution range (warm, temperate and cold water marine, as well as freshwater) in Europe. The involvement of RTD centres and SMEs from 10 countries of different European regions from the Aegean Sea (Greece) to the North Sea (Norway), and from the Levantine Sea (Israel) to the Eastern Atlantic (Spain), which are generally exposed to different culture, environmental, industrial and market requirements, guarantees the development of the industry at a truly European level. Furthermore, a strong **participation of SMEs** (a total of 10 Partners) and large commercial operations (SARC and SWH) has been instrumental in the preparation of this proposal and will undoubtedly ensure the **successful and immediate exploitation of the results**, in a way to have a very significant **impact in the further development of the aquaculture sector**. Expansion of this sector, is expected to be both at the level of production and employment, with subsequent downstream effects in the processing and commercialization/distribution industry. According to evaluations of the Spanish Organization of Aquaculture Producers (P12. APROMAR), an increase in production of 1,000 t per fish species would result in (a) the creation of 20 new direct and 10 indirect jobs, (b) a wholesale value of 5€ million potentially leading to (c) 10€ million in new added-value processed products, (d) the development of species-specific technology (machinery, diets, vaccines, etc) and (e) opening of new R&D lines dedicated to the optimization of culture methodologies for these species. This applies for most of the species included in this project, with the exception of grey mullet that has a particular growing production system (extensive polyculture) and market. DIVERSIFY will provide the conditions --both technical and marketing- to enable the EU aquaculture to produce 1000s of t of the selected species, in order to meet the EATIP vision of providing 4.5 million t of sustainable food products by 2030, employing directly more than 150,000 people.

The expected **Specific impacts** of DIVERSIFY are addressed below in terms of their contribution towards the development of (1) different European aquaculture types in the sector, (2) the market and consumer value of aquaculture product and (3) state-of-the-art of aquaculture science.

## **1. Impact on the European aquaculture production sector**

### ***1.1 Mediterranean (warm water) cage culture (meagre, greater amberjack and wreckfish)***

DIVERSIFY has focused mainly (61.3% of RTD budget) on three species that can be cultured in sea cages in the temperate waters of the Mediterranean and eastern Atlantic Ocean, since this is the sector with the highest potential for growth (EATIP, 2012). New sites for aquaculture production are expected to be offshore and larger species would be more appropriate for these new sites. DIVERSIFY selected species that are large, fast growing and with a wide --even cosmopolitan-- distribution, and can be grown in different areas of the Mediterranean and Atlantic coasts, ensuring a larger and widespread impact in the global EU aquaculture industry and economy.

From these three species, only meagre is produced at an industrial level. DIVERSIFY covers different stages of its production (*e.g.*, improvement of broodstock management and setting the basis for selective breeding), as well as optimizing culture conditions, nutrition (*e.g.*, development of specific and balanced feeds) and species-specific feeding strategies (*e.g.*, feeding protocols for enhancing fish growth potential and diminishing size dispersion), and investigating the development of new products and EU-global markets for meagre. This will no doubt boost the meagre aquaculture industry. Some of the same and some different aspects compared to meagre will be studied in greater amberjack and wreckfish. The specific research activities were dictated by the current availability of knowledge in their biological life cycle, the documented bottlenecks in their production and the lack of an industry producing them in Europe. Collectively, the achieved biological knowledge and aquaculture technology development in DIVERSIFY for these three



species is expected to have a strong impact fuelling the future growth of the warm water, cage aquaculture in Europe.

### **1.2 Production of freshwater fish using RAS**

European freshwater aquaculture is dominated by the intensive production of trout and the extensive to semi-intensive production of carp. However, considering not only the aquaculture sector, but also the fishery one, pikeperch is one of the leading species in the European freshwater market. Yet, the overall fisheries have declined from about 40,000 t in the early 70s to less than 20,000 t in 2010. The overall aquaculture production (~400 t) is far from compensating this decline. There are currently 13 farms producing pikeperch --mainly in RAS-- in six European countries and more are at the design stage in France, Poland, Italy, Hungary and Estonia.

The two main bottlenecks that have been identified in pikeperch are (a) high mortalities at the larval stage, due to cannibalism and (b) sudden and unexplained death at the grow out stage. Therefore, the first main impact of the present project will be to reduce these mortalities, through a better understanding of their causes. Obviously, this will improve significantly commercial production, by securing the production process and increasing the economical profitability (reduction of production costs). Furthermore, the activities planned in DIVERSIFY for pikeperch would be potentially helpful for the diverse relative species that are currently at the onset of domestication in Europe. Similar to pikeperch, these new species (Eurasian perch, pike and burbot) are expected to be sensitive to stress (particularly in RAS). So the information obtained on pikeperch could be helpful for these species, and will promote diversification of European freshwater aquaculture beyond the targeted species.

Another task in DIVERSIFY is to characterize the genetic variability of pikeperch stocks farmed today and compare this variability with their wild counterparts. This work is a prerequisite for establishing selective breeding, and thus will be helpful in the coming years by providing a clear picture of the available variability of pikeperch. In addition, it can be a powerful tool to help improving current practices and thus avoid inbreeding and consanguinity that are often observed in other farming operations.

### **1.3 Cold water marine aquaculture (Atlantic halibut)**

The first attempts at aquaculture of Atlantic halibut were made in the 1980's and 1990's, but development of a commercially viable industry has been hindered by the complicated biology and early life stages of this species, and slow growth in the early maturing males. Over the last 10 years, a few commercial hatcheries have achieved a slow but steady increase in number of juveniles produced, and "all-female" production is currently increasing. However, current world-wide production remains stable at ~2,000 t year<sup>-1</sup>, well below market demand. An increase in production requires a higher and more stable production of juveniles. To achieve this, a few major remaining bottlenecks remain to be solved: 1) a stable supply of gametes from F1/F2 broodstock; 2) increase in survival by improved rearing environment, and development of RAS for first feeding of halibut larvae and 3) improved nutrition by using on-grown *Artemia* instead of *Artemia* nauplii in the later phases of first-feeding. These are the objectives of DIVERSIFY, and their impact will be strong improvements in management of broodstock and early life stages in Atlantic halibut. In turn, these improvements will lead to a higher supply of juveniles for grow out and production of market-size fish, which will improve production for existing farms as well as promote establishment of new grow out farms possible. Overall, solving the remaining bottlenecks in juvenile production will contribute to a higher market availability of Atlantic halibut in Europe and world-wide.

### **1.4 Extensive-pond-lagoon culture of a herbivore species (grey mullet)**

As part of the general reasoning of potentiating a truly global impact in terms of aquaculture production in EU and EEA member states, different culture systems have been considered in DIVERSIFY. These include pond or extensive culture, for which some areas of the Mediterranean have traditional activities already implemented or a high natural potential to develop them. The grey mullet is a rapidly growing euryhaline herbivore that is suitable for Integrated Multi-Trophic Aquaculture in earthen ponds and coastal lagoons



across the wide geographical and temperature range of the Mediterranean basin. In addition, it is a cosmopolitan species that has the potential to supply the global market and not just the EU. This refers mostly to the fish roe “bottarga” that can be marketed as a luxury commodity (worth >100 € kg<sup>-1</sup>), although a market for the whole fish also exists in the North of Africa and Middle East (but in this case higher production costs in the EU zone might constrain its export). In addition, even though a strong marketing effort is required and hence is anticipated in this project, this species offers an excellent opportunity to produce a more acceptable product to an increasingly aware consumer that demands “sustainability”, “traceability”, and “reduced environmental impact”. Still, even without significant marketing effort, the European market demand for grey mullet is likely to increase in the coming years, due to the demand from immigrant families originating from North Africa, Middle East and Asia. In sum, this species fits perfectly the new challenges with which aquaculture is being faced worldwide and its successful introduction into the market, both as whole fish or as bottarga, would contribute enormously towards changing consumer perception of aquaculture and bringing innovative and “out of the box” thinking into the sector. Solving the main bottlenecks that have been identified, such as (a) control of the reproductive cycle and improvement of egg quality, (b) establishment of a larval rearing protocol and (c) development of a sustainable and effective fishmeal-free grow out diet will pave the way for transforming this local and traditional activity into a profitable, industrial-scale one, with potential to expand within the whole Mediterranean area.

## **2. Impact on the market and consumer value of aquaculture product**

Seafood supply varies greatly by country and within some large countries by region. Consumption in Poland and Hungary is only 10 kg per capita per annum, whereas in Spain it is well above 40 kg and in Portugal there's a record consumption of over 55 kg. Not only the volume, but the type of product purchased, varies greatly by country as well. In southern European countries, the assortment for sale offers a wide range of species whereas in northern countries it is limited. In Germany, for example, four species (Alaska pollock, herring, salmon and tuna) total over 60% of the overall consumption, whereas in Spain and France the four most consumed species total only 30% of total consumption. In Mediterranean countries, purchase of whole fish sold in bulk is still common, whereas in north European markets, prior to be sold to end buyers, fish products are usually processed, in most cases into a portion-sized packaged item. In the UK 90% of all fish is packaged and branded, whereas in Spain this proportion drops down to 35%. In this latter country seafood is still dominantly sold in bulk, though this share is declining. Consequently, boosting the competitiveness by developing value added products to the current assortment of cultured fish for the selected species in the European market is the core goal in the socio-economic component of DIVERSIFY. However, the protein market in the countries with purchasing power for proteins within the EU is saturated, which means that boosting demand for cultured fish with value added products either has to diminish demand from fisheries or diminish demand for meat products or meat replacements (such as soy substitutes). The first step in doing this is to gain market insights in relation to competitive products, to be able to position well the new selected species in relation to the range of products from fisheries, cultured fish and other protein sources as meat products and meat replacements. The second step is to gain insight in consumer demands regarding proteins and more particular in relation to fish products as a base for the physical prototypes of new value added products to be developed. Earlier research shows that fish is perceived by the consumer as difficult to prepare for a meal. These new value added products have to be healthy, safe, easy to prepare, highly innovative, recognisable as a protein source for a meal and not one of many kinds already in the market. All these aspects will be tested in DIVERSIFY.

A communication and marketing strategy and development of business plans (including positioning, pricing and certification) to implement market introduction will support the involved SMEs to make the step of introducing the value added products of the new species. The buying factors of professional buyers, also to be analysed in DIVERSIFY, will also be the base to make these plans.

So full coverage from farm (technically) to fork (consumer tests) is covered in DIVERSIFY and it is expected that the knowledge acquired will have a significant impact on the perception of aquaculture and its products by the consumer, and on the business strategy needed to be implemented by new or existing operations for expanding their production with these and other new species.





### 3. Impact on the State of the art of aquaculture sciences

DIVERSIFY is expected to advance the current knowledge beyond the state-of-the-art and impact on the current and near future activity of the European aquaculture industry. The diverse and complementary nature of the consortium will allow a number of key basic questions of various fields such as reproduction, development, growth, nutrition, adaptation and immunity to be addressed for a multitude of species. DIVERSIFY is designed to solve the main bottlenecks identified by the sector with regards to the incorporation of new fish species. These improvements will be set up on the conjunction of two sources of information: i) basic knowledge on biological processes affecting fish culture and ii) applied knowledge on the development of species-specific protocols for fish culture optimization. In particular, outcomes with an impact on aquaculture science include:

**Reproduction:** The controlled availability of gametes is imperative for sustainable aquaculture. DIVERSIFY will provide improved understanding of the regulation of reproduction, as well as define optimal broodstock management conditions and broodstock diets in order to assure optimal gamete quality, and will develop species-specific spawning induction protocols. Improved reproductive function may, in turn, reduce the occurrence of skeletal deformities and poor larval and juvenile performance. In addition, specific tools such as ELISA assays for reproductive hormones will be developed with multiple scientific and industrial applications.

**Genetics:** DIVERSIFY will focus on the genetic characterization of actual broodstocks of meagre and pikeperch, the two species with current relevant industrial production, in order to overcome future inbreeding problems in these two species and solve current problems with variable growth rates (meagre) and stress sensitivity (pikeperch). Thus, the genetic characterisation of fast and slow growing meagre (SNP approach) and the genetic diversity and stress sensitivity in pikeperch (microsatellite approach) will provide useful tools for improvement of actual culture practices, and establishing the basis for selective breeding programs that will serve to scale-up and improve their production in terms of quantity and quality.

**Nutrition:** The cost of feeding in aquaculture production is around 40-70% of total production cost. New species in aquaculture are fed with available diets designed for other species, which may constraint their growth performance and general condition. For this reason, it is important to develop species-specific feeds that consider the nutritional requirements of each species at different stages of development and that can improve their performance (*e.g.*, FCR, growth rate), quality (*e.g.*, morphology, fillet yield and product composition, and egg viability) and health condition (*e.g.*, prevention of metabolic disorders). To achieve this goal, DIVERSIFY will establish the unknown nutritional requirements of several macro- and micronutrients and dietary energy needs for most of the species considered in the proposal. In addition, in order to fit larval needs, specific live prey enrichment products will be developed. Development of specific formulated feeds, live prey enrichment products and feeding protocols will result in new products that may be commercialized worldwide.

**Growth and husbandry:** A larval and juvenile rearing system is a complex environment, with numerous factors influencing larval development and performance, as well as behaviour and survival. These factors can be environmental (light intensity, temperature, salinity, tank colour, water current), nutritional (feed composition and availability, feeding frequency), social (fish density) and genetic. For species such as meagre, pikeperch, grey mullet and Atlantic halibut, improvements in terms of fish growth and husbandry will be addressed to refine the existing protocols (*e.g.*, weaning schedule), procedures and facilities (*e.g.*, semi-intensive and intensive rearing procedures, cage culture, RAS and flow-through systems) in order to solve existing bottlenecks (*e.g.*, large size dispersion and high variable growth rates in the case of meagre, high cannibalism rates in pikeperch, low survival and larval quality in halibut and grey mullet). In contrast, emphasis will be given to developing new species-specific larval rearing protocols in the case of greater amberjack and wreckfish, since these are species with important knowledge gaps in these areas. This approach will increase our knowledge on the development of fish larvae in greater amberjack, wreckfish, Atlantic halibut, grey mullet and pikeperch that will serve to synchronize the state of development of the fish under different rearing conditions with the new or existing rearing technology. Finally, the output of these tasks will be the development and refining of rearing protocols for selected species that will result in the improvement of current practices, and an increase in production yields.



**Health:** Fish health is a key trait to be optimized in cultured fish. The effect of the developmental stage, rearing conditions and nutrition on the capacity to modulate specific immune responses will help predict vaccine responsiveness and fish health. DIVERSIFY will characterize the immune system of meagre and greater amberjack to identify key immune molecules as potential markers of immune system development, and induction of antiviral and antibacterial responses in preparation for vaccine development for disease management. In addition, potential solutions for specific bacterial infections and parasitoses will be investigated, providing means to prevent and/or minimize these issues at an industrial scale.

Also related, transport of live animals across large distances and introduction of allochthonous species to new geographic regions is a continual source of introduced and emerging diseases, and potentially zoonotic ones as well. A primary goal of this project - the improved efficiency of fish culture for the food sector - means that autochthonous species can be produced locally and reduce market drives for other aquatic species, and thereby eliminate the risks associated with the introduction of allochthonous species for cultivation that may do harm to local species diversity in the region of introduction. While preventing emerging or introduced aquatic diseases is not a goal or the focus of this project, it is an added positive impact that may have on the sector.

### **Sustainability**

Sustainability of aquaculture production has a strong uphold in DIVERSIFY and has been considered from different points of view, most of them already mentioned above. To summarize, these concerns have been addressed by i) supporting the growth and expansion of the sector based on different production systems that can be regarded as more sustainable (cage culture – no competition with land resources; RAS- ecologically friendly, with efficient use of water; extensive pond-lagoon culture, with very low environmental effects and in some cases even contributing to the restoration of ecosystems – *e.g.*, abandoned “Salinas”/salt marshes); ii) introduction of a herbivorous fish into the aquaculture sector, with positive influence in the environment where it is cultured (improving sediment quality, avoiding oxygen depletion and reducing ammonia levels) and requiring low or close to none input from marine-based feedstuffs; iii) an important focus of the proposal resides on the determination of species-specific dietary requirements, including the investigation of more sustainable ingredients, as well as feeding behaviour, which will result in less waste of diets and nutrients into the environment; iv) research will be conducted that will hopefully enable anticipated potential disease problems to have veterinary solutions prepared in time and, finally, v) considering consumer requirements, including changes in societal and ethnic demands, to enable a market-orientated growth of the aquaculture sector. Altogether, these factors will ensure a sustainable growth and expansion of aquaculture within the EU and EEA member states.

### **Assumptions and external factors**

Several external factors are likely to affect the anticipated impacts described above: i) negative environmental effects of cage culture; ii) spreading of disease/parasites (both while in the cage and after escape); iii) escapes and genetic impact on wild populations/stocks; iv) environmental load/pollution (surplus feed, N, P, contamination, chemicals, pharmaceuticals) effects on wild stocks (*e.g.*, wild fish being attracted to the sea cages to find “easy meals”); vi) animal welfare aspects; vii) feed utilisation. These are aspects that are commonly attributed to aquaculture activities, but as in other farming activities, it is the maturation of the sector as a business that will provide solutions. In DIVERSIFY these concerns are kept in mind and several aspects have been taken into account when designing the WPs. For example, improvement of knowledge on nutritional requirements of selected species and feeding behaviour will minimize nutrient and food waste into the environment by improving FCR and nutrient concentration in the feed. Study of the immune system will obtain health markers in order to improve general wellbeing of the fish population in culture. Development of tools for disease control and treatments will diminish disease and chemical/pharmaceuticals spreading to the natural environment. In addition, it should be mentioned that the choice of other culture systems, such as RAS (where environmental conditions and wastes can be more tightly controlled) and extensive or semi-intensive pond systems and the selection of a herbivorous species, has some of these issues in mind and in these cases such factors are less likely to have a negative effect on the likely outputs of the project.



### 3.2 Dissemination and/or exploitation of project results, and management of intellectual property

*Describe the measures you propose for the dissemination and/or exploitation of project results, and how these will increase the impact of the project. In designing these measures, you should take into account a variety of communication means and target groups as appropriate (e.g. policy-makers, interest groups, media and the public at large).*

*Describe also your plans for the management of knowledge (intellectual property) acquired in the course of the project and, when relevant, the question of open access when submitting articles for scientific publication.*

*When appropriate (relevant for the topic): With regard to the innovation dimension, describe the measures you propose to increase the likelihood of market uptake of project results, such as: verification, testing, and prototyping; supporting the development of technical standards; identifying and collaborating with potential users; identifying potential partners and sources of finance for commercialisation.*

Work Programme 2013 supports strongly the dissemination of research results and technology, and knowledge transfer activities, so that innovative knowledge can lead to innovative applications. A special emphasis is given towards SMEs, and the particular proposal is targeted towards SMEs. Therefore, DIVERSIFY is dedicating a substantial effort to dissemination activities and has allocated a budget of 354.753 € of EU contribution (4.0% of the project) for various activities directed towards (a) aquaculture and food scientists, (b) the commercial aquaculture sector, including SMEs, (c) the food processing industry, (d) relevant regulators and (e) consumers.

DIVERSIFY includes a strong effort not only in species diversification but also in the diversification of the farmed product available to the consumer. In this regard, retailers play a much stronger role in forming consumer preferences and are an indispensable link between producer and consumer. Furthermore, effective communication to policy makers and standard bodies will also be very powerful and beneficial for the industry. A preliminary plan to reach each target audience has been designed (Table 2, next page).

From the start of the project a dedicated programme web site will be set up (fish DIVERSIFY.com), which will include (a) information on the objectives and main tasks, (b) a free-subscription newsletter with regular updates on the project, (c) downloadable documentation on the project for the general public, video podcasts and/or radio podcasts with interviews of project scientists and/or authorities or experts in the project scope, (d) links to abstracts and presentations made in scientific conferences, and to scientific articles, and (e) a blog page for interactions with scientists and aquaculturists, for the acquisition of feedback on DIVERSIFY. Content development will be done by the WP 31 Dissemination leader in collaboration with the WP Species leaders. A project brochure will be prepared, including the project's logo, web site, and detailed datasheets on the objectives, specific issues and WPs addressed by the project. An intranet will also be developed within the website, to provide a means exchange of data and documents among the project's Partners. The means of evaluating the success of the dissemination actions carried out for each target audience will be established via web (on line questionnaire) and via email, to allow the consortium to rely on indicators that will help to measure the effect of the communication efforts.

Partners APROMAR, FGM, BVFi, MASZ, ANF, EUFIC and CTAQUA have the resources and capacity to access main stakeholders in the European aquaculture sector. These organizations agglomerate members from the production sector (fingerling producers and grow out farmers), feed manufacturers, equipment suppliers, fish and shellfish processors, distributors and consumers. The partners have regular contacts with governmental organizations, regional and national policy decision makers, fishing industry representatives, NGO's and the media, which allow the broadcasting of the project advances and results.





During the **annual coordination meetings**, dissemination of results will be made to relevant parties outside DIVERSIFY, who will be invited to attend these meetings. This practice was undertaken in previous EU FP 5 and 7 projects (REPRODOTT and SELFDOTT) and proved very successful for the rapid dissemination of knowledge to the relevant stakeholders. The first two days of the meetings will be attended by aquaculture researchers and stakeholders interested in the selected species, both from Europe and world-wide --the total number of participants not exceeding 60 individuals, in order to allow for easy and fruitful interactions. The first day and the morning of the second day, there will be presentations by the WP leaders and selected researchers in charge of the various tasks of the projects, of the work accomplished and results obtained in DIVERSIFY during the previous 12-month period. In the afternoon of the second day, there will be presentations from invited speakers of international acclaim in the field of research and culture of some of the selected species. The presentations made during these two days will provide both Partners and invited guests with an overview of the work carried out in each species (and relatives from around the world), giving everybody the opportunity to influence and benefit from each others' work, and ensuring rapid dissemination of the project's results. This bottom-up approach will allow the forming of a consensus in the development of new scientific advances.

**Table 3.2a.** Dissemination tools to be used to reach various target audiences.

Dissemination tools	Target audience
Project website, set up project page on social media platforms (facebook, linked-in, twitter, etc.)	Farmers, distributors
Project leaflet and newsletter. Podcasts in the web and video clips in Youtube. Powerpoint presentation in web	Industry multipliers: sectorial associations, consumer associations and retailers' associations
Visibility on aquaculture related web portals	Partners personal and professional contacts
Trade magazines (Intra Fish, Eurofish magazine, Fish Farming International, Aquafeed, etc.)	Scientists, Fish producers and processors
Scientific conferences (European Aquaculture Society, WAS, Field specific conferences)	Researchers and scientists
Scientific Journal articles (Aquaculture, Aquaculture Research, Journal of Fish Nutrition, Fish physiology and Biochemistry, etc.)	Researchers and scientists
Conferences, seminars and workshops: participation with papers, oral presentations, posters (Annual World Food and Innovation Forum).	Producer organizations, consumer organizations, scientists, professional contacts
Involvement in clusters/networks (European Fish Processors Association (AIPCE), European Association of Supermarkets (COOPERNIC), EUROCONSUMERS and BEUC).	Consumers and general public
Inclusion in EU documents and platforms (EATIP, FEAP.)	Government and policy makers; innovation programs

At the end of the project (Y5), **full-day “Know-how Transfer” seminars on the aquaculture of the species** studied in DIVERSIFY will be organized by each Species Leader (IRTA-meagre, HCMR-greater amberjack, UL-pikeperch, IMR-Atlantic halibut, IEO-wreckfish, UNIBA-grey mullet). A total of eight 30-min presentations by DIVERSIFY Partners, as well as invited authorities working with the specific species from outside the consortium will be included. These presentations will cover particular aspects related to



areas such as broodstock management, larval rearing, grow out husbandry, nutrition, fish health, final product quality and socioeconomics, focusing on results obtained in the course of DIVERSIFY. With these seminars, the relevant information obtained from DIVERSIFY will become available to the different sectors involved directly in aquaculture, such as producers, aquaculture support companies (feed, pharmaceutical, equipment, engineering, etc.). Researchers and educators, and government organizations will also be invited to attend these meetings. The seminars will be organized in countries with the biggest potential to culture the particular species, or in countries with close proximity to other countries where the species may have a good potential (See Deliverables D31.29 to D31.34).

To disseminate the project's activities and results to specific audiences --such as fish producers, processors and retailers, consumer organizations, and fisheries and aquaculture authorities-- **promotional workshops** will be organized during the course of DIVERSIFY by CTAQUA and APROMAR and with the collaboration of the partners representing the associations of fish producers and fish processors (FGM, BVFi, MASZ, ANF) . In addition to DIVERSIFY Partners, relevant speakers from specialized consumer's organizations and/or professional associations will be invited to address among others the following topics: consumer perception of aquaculture fish, general trends in farmed fish markets, value added products from farmed fish, labelling and certification, imbalance in the market value chain of aquaculture products due to the dominance of large retailers/buyers, processing and freshness and food safety. The workshops will take place in countries considered strategic for the aquaculture industry (production, distribution and consumption), such as Spain, Greece, UK and Italy (Deliverables D31.16, D31.18, D31.23 and D31.27).

It has been considered important to present the project to the European Seafood Exposition and Seafood Processing Europe, since it is the world's largest seafood trade fair. In the past year, more than 25.000 buyers, suppliers, seafood industry professional and media from more than 120 countries were present to meet and do business. For DIVERSIFY it has been contemplated that Y4 will be the most suitable moment to present the achievements and advances the project would have reached. CTAQUA and APROMAR, in collaboration with the partners representing the associations of fish producers and processor (FGM, BVFi MASZ and ANF) will organize a stand where the brochure and leaflet of the project will be on display together with the most representative scientific results obtained during the first three years (Deliverable D31.21).

During the last two years of the project, the results of the scientific work developed with the DIVERSIFY species will be compiled in the practical form of technical leaflets, one per each species included in the project. These leaflets will be translated to the language of the different associations included in the WP, Greek (FGM), German (BVFi), Hungarian (MASZ) and Spanish (ANFACO). Also the SMEs partners will assist in the design and translation of these technical leaflets, in their own language (French, Norwegian, Italian, Hebrew) (Deliverable D31.24). The diagram below presents the different components of the project



Dissemination activities and their interdependencies. Taking into account the amount of partners in DIVERSIFY, the flow of information is expected to be very significant. The project web will be the main tool for broadcasting information, results and achievements. Eufic partner, as European Consumer Association, will assure that DIVERSIFY outputs will reach the public at large, via two dedicated Press-releases that will be translated into 10 languages (French, Spanish, German, Czech, Hungarian, Polish, Greek, Slovak, Italian, Portuguese) and the inclusion of a DIVERSIFY quadrant in Eufic.org.



## Exploitation of results

The DIVERSIFY project has been designed so that **its results will address the main bottlenecks** that are presently limiting the development of aquaculture in the EU and will benefit the whole value chain of farmed fish: from the fish farm down to the consumer. The fish farming industry will be provided with a way to diversify their business with the production of alternative fish species, avoiding the risk of monoculture, buffering the economic volatility of global fish markets and, finally, enhancing its competitiveness. Suppliers to the aquaculture industry will have the opportunity to broaden their sales by offering equipment, feeds, veterinary material and services tailor-made to the particularities of the new species. Fish processors should be able to benefit by producing new fish products that would be innovative and could be of unexpected interest. Retailers will be able to offer their clients a broader range of products. Finally, consumers will see a widening in their choice of tasty, healthy and nutritious food at affordable prices.

Furthermore, some of the results could be technically applicable to presently produced fish (and wild), for both production and commercialisation phases. In total, the results of DIVERSIFY could improve the business edge of the EU seafood industry all along its value chain. At the same time, they will help reducing the dependency on imports of fish to the EU and improve food security in Europe while offering new business opportunities and employment.

## Intellectual Property Rights

DIVERSIFY will follow the default regime in terms of Intellectual Property rules for Collaborative Projects, where the Partners need to agree and define the IPR terms. The Consortium Agreement (CA) will establish IPR regulations. **Background (pre-existing) information** and knowledge held by the participants prior to their accession to the EC grant agreement, as well as any intellectual property rights that might be needed for carrying out the project, or for using foreground held by the consortium, will be defined in the preparation of the Grant Agreement. SME participants will have royalty free access to the RTD background for the implementation of the project and use of the foreground, under reasonable and fair conditions established in the CA. **Foreground (new) information** and knowledge from the project will belong to the Partner that has generated it, if the Partner was acting alone. In case foreground is generated jointly and different parts of the result cannot be attributed to different Partners, joint ownership will be accepted, unless the Partners concerned have already agreed on a different solution or otherwise stated in the CA. In the absence of an agreement, a default joint ownership regime will be applied (Article 40.1 RfP – Article II.26.2 of GA)<sup>1</sup>. All technical deliverables and reports will be available to all Partners via the intranet. Members will have full access to the project developments. The Steering committee will handle all legal issues of the consortium.

## Publications

The work carried out for the different species in the various work packages will be presented at relevant **European or international conferences**, and when appropriate the work will be prepared for submission to **international ISI-indexed scientific journals**. Each Partner may publish or allow the publication of data, on whatever medium, of knowledge it generated under work carried out within the project, provided that this does not affect the protection of that knowledge. SME Partners will consent access rights to the RTD Partners to use the results for further research, as long as those results are not identified as confidential.

The **involvement of the European Commission** will be acknowledged by adding the following sentence to each publication: “This study has been carried out with financial support from the Commission of the European Communities, 7<sup>th</sup> FP Collaborative Project; KBBE-2003-1-2-09, contract #, under the acronym DIVERSIFY”. It does not necessarily reflect its views and in no way anticipates the Commission’s future policy in this area.”

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<sup>1</sup> Rules of participation and grant agreement. Regulation No 1906/2006 of the European Parliament and of the Council of 18.12.200



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### **Innovation and likelihood of market uptake of project results**

The innovations resulting from DIVERSIFY will be delivered to the whole value chain of the aquaculture sector, in an easily applicable format and will contribute effectively to remove the obstacles presently faced by science, production, markets and consumer perception/preferences that hinder aquaculture development in the EU. These results will allow the industrial production of new/emerging aquaculture species of great potential by European companies and will provide them with a competitive edge that should enhance their sustainability. Aquaculture production in the EU has been stagnant in the last decade. Species-specific husbandry and technology protocols, new products and business models and marketing methods developed within DIVERSIFY will provide the insight for industry expansion and the means for diminishing the dependency of the EU on imported fish products. It is anticipated that through the **direct and substantial involvement of a large number of leading SMEs** in the aquaculture sector, **the above project results have a high likelihood to be** taken up by the industry and market.

The European aquaculture industry is presently struggling to satisfy their clients' and consumers' demands. The knowledge to produce in an effective way, and sell, alternative fish species is sought by the industry. Most companies produce only one or two species, making them extremely vulnerable to the boom and bust cycles that periodically hit the aquaculture industry. However, producing a new species involves a large economic investment for a company and means a risky bet on its future. For this reason, the results of DIVERSIFY will address at the same time all presently known bottlenecks and offer maximum certainty for the producers that could adopt them. Another important innovation considered in this project, is the analysis of the aquaculture production business model as a whole and the formulation of marketing strategies for the newly developed products, aiming to develop a market that is as large and profitable as possible. As a result of this analysis, new policy and strategy recommendations will be available for the market expansion of the sector. At the same time, European fish processors are presently touring the world in search for fish raw materials for their activity, have them be wild or farmed. Providing a new source of quality fresh fish produced at the right price and able to be processed efficiently should attract their interest in the European aquaculture market. Except for Atlantic salmon, which benefits from the availability of a wide range of processed products, the rest of EU aquaculture fish are sold mostly as whole fish, which limits the shelf-life and the product range offered to the markets. One of the main objectives of DIVERSIFY is to improve marketing of fish processed products (fillets, steaks, smoked, battered "fish finger" type products and various other value added products). Furthermore, the aquaculture sector should be able to benefit by producing new and innovative fish products as offered by the project. And finally, retailers should be interested in placing in their shops a broader range of products for consumers that will be attracted by a larger variety of tasty, healthy, easy-to-prepare and nutritious fish.



## 4. Ethics Issues

*Describe any ethics issues that may arise in the project. In particular, you should explain the benefit and burden of the experiments and the effects it may have on the research subjects. All countries where research will be undertaken should be identified. You should be aware of the legal framework that is applicable and the possible specific conditions that are relevant in each country (EU and non-EU countries alike). It is strongly advised that when drafting the research proposal, the local ethics committee or/and relevant competent authorities (Data Protection, Clinical Trials etc) should be contacted for information and, when applicable, guidance. You may also address specific questions to the FP7 Ethics Help Desk (see page 2 in this Annex).*

*Include the Ethics issues table below. If you indicate YES to any issue, please identify the pages in the proposal where this ethics issue is described. Answering 'YES' to some of these boxes does not automatically lead to an Ethics Review. It basically enables the independent experts to decide if an Ethics Review is required. If you are sure that none of the issues apply to your proposal, simply tick the YES box in the last row.*

*(No maximum length for Section 4: Depends on the number of such issues involved)*

### Common statement

The experimental work will be undertaken on six different fish species of great interest for European aquaculture. Experimentation will include work with **fish larvae, juveniles and mature broodstock** maintained in captivity in appropriate and authorized research facilities or in licenced aquaculture production companies. Project activities will be undertaken within national and EU legal frameworks and standards, specifically those related to experimentation with animals and animal welfare, as well as within specific national directives and regulations of each Partner organization as regards research with finfish. The Directive 2010/63/UE has been used as baseline for the experimental planning and design in DIVERSIFY, which encourages the development, manufacture, quality, effectiveness and safety of foodstuffs as well as the protection of human and animal health, and of the environment. Particular attention has been invested by the responsible researchers on the application of the principle of **Reduce-Refine-Replace** (the 3 Rs principle). **Reduction** refers to the use of the absolute minimum of animals to the fewer necessary scientific procedures. **Replacement** refers to substitution of experiments with animals by other methods not entailing the use of live animals whenever possible. **Refinement** refers to the enhancement of protocols to diminish stress imposed on the animal that have to be used.

Experimentation involving fish will be undertaken in Greece, Spain, Norway, Denmark, Israel, Italy, France, Belgium and United Kingdom, whereas in the Netherlands research will be focused exclusively on the socio-economics topic. In DIVERSIFY, each experimenter is strongly requested to promote the welfare of live animals used by raising the standards for their protection in line with the new scientific knowledge available on the capacity of animals to sense and express pain, suffering, distress and lasting harm. Operators are experienced in the handling and restraint of the fish to minimize distress, fear and anxiety, and are aware of the primary criteria for euthanasia in terms of specimen welfare. They are conscious that euthanasia should be painless, achieve rapid unconsciousness and death, require minimum restraint, avoid excitement, and appropriate for the age, species, and health of the fish, and must minimize animal fear and psychological stress.

The number of fish used along the project will be reported to the annual or periodical questionnaires requested by the appropriate authorities of each country. During the research with adult fish, small batches of individuals ( $n \leq 6$  per sex) will be sampled for blood and gonadal biopsy, or sacrificed for collecting of various tissues. This is the absolute minimum number to allow robust and meaningful statistical analyses. In most species, batches of thousands of viable eggs and larvae will be used to stock the experimental facilities according to the experimental design dedicated to husbandry and scientific topic issues. When eggs or post-hatched larvae have to be delivered locally or transported by airplane to the final destination, shipping conditions will be defined to secure the highest survival rate and further rearing success potential based on the collective egg/larval shipping experience of the Partners, who have done so successfully in earlier EU FP programmes (See SELFDOTT). At the end of each experimental phase, surviving individuals will be maintained for further rearing in tanks or transferred to cages. In those experiments where the collection of fish from the wild is necessary for the project (e.g., greater amberjack, wreckfish and grey mullet), fish will





be captured by professional fishing vessels, and if necessary to be sacrificed, this will be done with stunning and/or pithing before sampling.

In **WP7 Socioeconomics**, all consumer tests will be conducted anonymously. Identifying characteristics of consumers will be used in recruiting. In the analysis and reporting, only demographic characteristics will be presented in an aggregate way.

### **Information specific to various Partners involved with rearing and experimenting with fish**

The Institute of Marine Biology, Biotechnology and Aquaculture of **HCMR** has registered facilities for culturing and performing experiments in fish according to the Greek National and the European Law. The facilities are registered under the code EL91-BIO-04 of the regional Veterinary Authority. The personnel participating in the project is adequately trained and experienced for the design and performance of experimental studies with aquatic animals and to avoid or minimize animal pain and suffering. All experimental protocols are submitted for approval at the Regional Veterinary Authority.

All experiments with animals at the **FCPCT** are regulated by the Bioethical Committee of the University of Las Palmas Gran Canaria (Canary Islands, Spain). The research will be performed according to the Spanish legislation (RD1201/2005) and the European Directive 2010/63/EU for the protection of animals used for experimentation.

All experimental protocols in **IRTA** are developed under the supervision and approval of an ethics committee for animal experimentation (Comitè d'Ètica de l'IRTA) operating according to European and national law (European Directive 2010/63/EU and RD1201/2005), and experiments are conducted by authorized personnel. IRTA is registered as a centre for animal experimentation (No. T990002) and all fish installations are approved for rearing, maintaining and experimenting with fish. The number of fish used in experimentation is the minimum possible, and all fish are sacrificed humanely. An effort is made to always use the finest analytical techniques to enable the use of the minimum number of experimental animals.

All feeding trials and experiments carried out at **IOLR** will be according to animal welfare regulations stipulated by the Israeli Ministry of Health and the National board for animal experimentation. Experimental fish will be maintained in aerated, flowing water systems to guarantee their well-being and will be fed daily according to the specific protocol of the experiment underway or 1-2 times per day when being maintained. Daily observations will be made to detect external or behavioural signs of possible disease or infection and if suspected then the fish will be sampled and treated by the IOLR fish pathologist. Daily monitoring of feed provided, mortalities if any, and measurements of environmental parameters will be documented. Fish that are sampled during the experimental trials to determine biological parameters will be sacrificed humanely using a high dose of anesthetic (clove oil) followed by immersion in ice. This method of humane sampling of fish is recommended to recipients of grants supported by the Ministry of Agriculture of Israel. It should be noted that there are no fish welfare guidelines for the rearing of fish larvae prior to the juvenile stage in Israel. As a result no formal request needs to be submitted to conduct studies. However, in all larval and grow-out studies researchers will minimize the number of fish sampled in order to provide statistically meaningful results.

**UNIABDN** has a UK Home Office Project Licence for work with fish, that complies with the “Animals (Scientific Procedures) Act 1986” and UK Home Office guidance (PPL60/4013- Studies on the immune system and disease resistance of fish). It has been ethically reviewed locally, and is running for five years from 12/5/09.

At **IMR**, fish will be maintained under standard aquaculture conditions. Sampling and tagging (PIT tags) will be conducted under anaesthesia. Approval will be obtained from the local or national authority for use of experimental animals for hormone injection trials and samplings involving biopsies. All experiments will be performed according to Norwegian legislation and the European Directive 2010/63/EU for the protection of animals used for experimentation and other scientific purposes. All personnel involved in the experiments have acquired FELASA B (technicians) or FELASA C (scientists) certification for work with animal research.



All the experiments involving animals held at Spanish Institute of Oceanography (**IEO**) units from IEO-Vigo (wreckfish) and IEO-Canarias (greater amberjack) are supervised and approved by the Ethical Committee CEIBA-IEO (Comité de Ética de la Investigación y Bienestar Animal del Instituto Español de Oceanografía). The experimentation will be performed according to the Spanish legislation (RD1201/2005) and the European Directive 2010/63/EU for the protection of animals used for experimentation and other scientific purposes. The personnel participating in the project are suitably trained and experienced for the design and performance of experimental studies with aquatic animals and to avoid or minimize animal pain and suffering.

All experiments that will be done at the **UL** on animal manipulation will obtain an agreement from the “Regional Ethical Committee for Animal Experimentation”. The experimental protocols will be carried out in the total respect of the rules described by Council Directive 86/609 EEC regarding the protection of animals used for experimental and other scientific purposes. Persons carrying out or supervising the development of the experiments have received instruction in a scientific discipline relevant to the experimental work being undertaken and are capable of handling and taking care of laboratory animals. They have satisfied the authority that they have attained a level of training sufficient for carrying out their tasks. All experiments will consider the welfare of fish so as to avoid unnecessary pain. At the end of an experiment, fish will be killed rapidly using a human method. All experimenter have adhered to the ‘Guidelines for the use of Animals in research’ published by Animal Behaviour 55: 251-257.

**UNIBA and ITTICAL** will carry out gametogenesis studies on greater amberjack and mullet. These studies will require the capture of wild adult fish (about 36 amberjack and 50 mullet) and their rearing in captivity until sacrifice. During rearing in captivity, the fish will be treated according to the “Council Directive 86/609 EEC for the protection of animals used for experimental and other scientific purposes” (EEC, 1986) and the “Ethical justification for the use and treatment of fishes in research” (Anonymous, 2006). For comparison, a similar number of adult wild fish will be sampled. These wild fish will be captured by professional fishing vessels, killed by fishermen as usual for commercially-caught fish and sampled by UNIBA researchers only after their death.

**IFREMER** facilities will not maintain any fish for experimentations. IFREMER personnel experimenting in the other facilities of the consortium have received individual animal experimentation agreement (established according to European rule 86/609 CEE and French legislation) from the French authority DPP (Direction de la Protection des Populations).

All the experiments involving animals held at **ULL** have to be supervised and approved by the Ethical Committee CEIBA (Comité de Ética de la Investigación y Bienestar Animal) whose current rules of procedure were placed by the Governing Body of 14 May 2010. A major ethical issue addressed by CEIBA in terms of the use of animals in scientific research is that all protocols should implement the principal of the 3Rs. The experimentation will be performed according to the Spanish legislation (RD1201/2005) and the European Directive 2010/63/EU for the protection of animals used for experimentation and other scientific purposes. The personnel participating in the project are suitably trained and experienced for the design and performance of experimental studies with aquatic animals and to avoid or minimize animal pain and suffering.

At **FUNDP**, all experimental protocols involving vertebrate animals including fish (except fish embryos) must be submitted to – and accepted by – the Ethical Committee for Animal Experimentation and Welfare prior to conducting the experiments. Moreover, regular control of the facilities and animals are done by the Federal Public Health service of Belgium. The Research Unit of Environmental and Evolutionary Biology (URBE) of FUNDP has the agreements for all their animal research facilities (LA 1900048 and LA 2900365). All experiments are under the responsibility and supervision of a “master of animal experimentation” (Prof. P. Kestemont and Dr R. Mandiki) and all scientists and technicians involved in the experimentations are graduated as “animal biotechnicians”. This certificate is obtained after a successful examination related to 40h-lectures on animal experimentation procedures.

The work of **CTAQUA** in DIVERSIFY implies the use of fish as experimental animals in nutritional evaluations. CTAQUA working protocols implement the 3R principle for the minimization of experimental



individuals to obtain results of statistical value. CTAQUA carries out animal experiments according to the Spanish legislation (RD1201/2005) and the European Directive 2010/63/EU for the protection of animals used for experimentation and other scientific purposes. The research staff directly related with animal testing is authorized and has the legal expertise requested for this type of animal testing.

In all the experiments involving animals held at the **CMRM** the experimentation will be performed according to the Spanish legislation (RD1201/2005) and the European Directive 2010/63/EU for the protection of animals used for experimentation and other scientific purposes. The personnel participating in the project are suitably trained and experienced for the design and performance of experimental studies with aquatic animals and to avoid or minimize animal pain and suffering.

All nutritional, larval and husbandry experiments undertaken at **DTU** will respect strictly the indications of the European Directives and recommendations. The experiments will be performed under the responsibility of scientists having high skill for the design and performance of experiments on animals, which includes acquired ethic background and animal welfare practices. All technicians in the experimental facilities hold basic background on how to minimize animal pain and suffering and the facilities themselves are approved for animal experiments by the local health services, as competent authorities. When stress challenge testing experiments are forecast, which could imply discomfort for some of the fish used, experimental protocols will be submitted for approval to the local Ethical Committees. As all Partners of DIVERSIFY, DTU strive to comply with the 3Rs principles: i) For each experiment to reduce the number of involved animals to minimum necessary; ii) Refine experimental protocols in order to diminish to an absolute minimum the amount of stress imposed on those animals that will be used. iii) Replace animal experiments by *in vitro* investigations or *in silico* simulations whenever possible.

The contribution of **SWH** to the project is mostly developing protocols for larval rearing in recirculatory systems (RAS) and on-growing Artemia, and to supply mature females and holding facilities for hormone injection trials. All fish will be maintained under standard aquaculture conditions. Hormone injection trials on broodstock will be performed according to Norwegian legislation and the European Directive 2010/63/EU, for the protection of animals used for experimentation and other scientific purposes.

The experiments involving animals at the SMEs **ARGO** and **GEI** (Greece) will be done in the context of production processes. The experimentation will be performed according to the Greek legislation and the European Directive 2010/63/EU for the protection of animals used for experimentation and other scientific purposes. The personnel participating in the project are suitably trained and experienced in handling cultured fishes and every possible effort is made to avoid or minimize animal pain and suffering, as this affects the quality of the final product.

Although there are no fish welfare guidelines for the rearing of fish larvae prior to the juvenile stage in Israel, in **DOR** all brood stock and grow-out grey mullet are maintained in a minimal stress environment where the fish are regularly fed, stocked at low densities while oxygen and other water parameters are constantly monitored and maintained at optimal levels. All personnel involved in maintaining fish are well trained to minimize stress to the fish during handling, treatment and harvesting. Moreover, fish are quickly removed if there are any signs of disease or infection and taken to the regional veterinary diagnostic center for diagnosis and advice on treatment.

The experiments involving animals at the SME **FORKYS** will be done in the context of production processes. Husbandry and management are crucial factors to ensure the health, welfare, high performance and premium quality of farmed European seabass and gilthead seabream. So, every possible measure will be taken to assure animal welfare. This concerns also the larval and the grow-out section of the experimental process. The experimentation will be performed according to the Greek legislation and the European Directive 2010/63/EU for the protection of animals used for experimentation and other scientific purposes. The personnel participating in the project are suitably trained and experienced, dealing with marine fish and to avoid or minimize animal pain and suffering.

Trials with greater amberjack held in the SME **CANEXMAR** will be developed according to the Spanish legislation (32/2007), which regulates the transport, rearing conditions and sacrificing method of animals.





As with other SMEs, the experiments at **ASIALOR** will be done in the context of production processes. Husbandry and management are crucial factors to ensure the health, welfare, high performance and premium quality of farmed European seabass and gilthead seabream. So, every possible measure will be taken to assure animal welfare. This concerns also the larval and the grow-out section of the experimental process. The experimentation will be performed according to the French legislation and the European Directive 2010/63/EU for the protection of animals used for experimentation and other scientific purposes. The personnel participating in the project are suitably trained and experienced, dealing with marine fish and to avoid or minimize animal pain and suffering.

## References

- EEC. 1986. Council Directive 86/609 EEC for the protection of animals used for experimental and other scientific purposes. Official Journal, L358: 1–28.
- Anonymous. 2006. Ethical justification for the use and treatment of fishes in research. J Fish Bio, 68:1–2.

*Include the Ethics issues table below. If you indicate YES to any issue, please identify the pages in the proposal where this ethics issue is described. Answering 'YES' to some of these boxes does not automatically lead to an Ethics Review. It basically enables the independent experts to decide if an Ethics Review is required. If you are sure that none of the issues apply to your proposal, simply tick the YES box in the last row.*

*(No maximum length for Section 4: Depends on the number of such issues involved)*

### Note:

*Only in exceptional cases will additional information be sought for clarification, which means that any Ethics Review will be performed solely on the basis of the information available in the proposal.*



## ETHICS ISSUES TABLE

All FP7 funded research shall comply with the relevant national, EU and international ethics-related rules and professional codes of conduct. Where necessary, the beneficiary(ies) shall provide the responsible Commission services with a written confirmation that it has received (a) favourable opinion(s) of the relevant ethics committee(s) and, if applicable, the regulatory approval(s) of the competent national or local authority(ies) in the country in which the research is to be carried out, before beginning any Commission approved research requiring such opinions or approvals. The copy of the official approval from the relevant national or local ethics committees must also be provided to the responsible Commission services.

**Guidance notes on informed consent, dual use, animal welfare, data protection and cooperation with non-EU countries are available at : [http://cordis.europa.eu/fp7/ethics\\_en.html#ethics\\_sd](http://cordis.europa.eu/fp7/ethics_en.html#ethics_sd)**

**For real time updated information on Animal welfare also see: [http://ec.europa.eu/environment/chemicals/lab\\_animals/home\\_en.htm](http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm)**

**For real time updated information on Data Protection also see: [http://ec.europa.eu/justice/data-protection/index\\_en.htm](http://ec.europa.eu/justice/data-protection/index_en.htm)**

<b>Research on Human Embryo/ Foetus</b>		<b>YES</b>	<b>Page</b>
	Does the proposed research involve human Embryos?		
	Does the proposed research involve human Foetal Tissues/ Cells?		
	Does the proposed research involve human Embryonic Stem Cells (hESCs)?		
	Does the proposed research on human Embryonic Stem Cells involve cells in culture?		
	Does the proposed research on Human Embryonic Stem Cells involve the derivation of cells from Embryos?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	YES	

<b>Research on Humans</b>		<b>YES</b>	<b>Page</b>
	Does the proposed research involve children?		
	Does the proposed research involve patients?		
	Does the proposed research involve persons not able to give consent?		
	Does the proposed research involve adult healthy volunteers?		
	Does the proposed research involve Human genetic material?		
	Does the proposed research involve Human biological samples?		
	Does the proposed research involve Human data collection?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	YES	

<b>Privacy</b>		<b>YES</b>	<b>Page</b>
	Does the proposed research involve processing of genetic information or personal data (e.g. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?		
	Does the proposed research involve tracking the location or observation of people?		



	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	YES	
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<b>Research on Animals</b>		<b>YES</b>	<b>Page</b>
	Does the proposed research involve research on animals?	YES	DOW
	Are those animals transgenic small laboratory animals?	NO	
	Are those animals transgenic farm animals?	NO	
	Are those animals non-human primates?	NO	
	Are those animals cloned farm animals?	NO	
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		

<b>Research Involving non-EU Countries (ICPC Countries<sup>2</sup>)</b>		<b>YES</b>	<b>Page</b>
	Is the proposed research (or parts of it) going to take place in one or more of the ICPC Countries?		
	Is any material used in the research (e.g. personal data, animal and/or human tissue samples, genetic material, live animals, etc) :		
	a) Collected and processed in any of the ICPC countries?		
	b) Exported to any other country (including ICPC and EU Member States)?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	YES	

<b>Dual Use</b>		<b>YES</b>	<b>Page</b>
	Research having direct military use		
	Research having the potential for terrorist abuse		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	YES	

<sup>2</sup> In accordance with Article 12(1) of the Rules for Participation in FP7, 'International Cooperation Partner Country (ICPC) means a third country which the Commission classifies as a low-income (L), lower-middle-income (LM) or upper-middle-income (UM) country. Countries associated to the Seventh EC Framework Programme do not qualify as ICP Countries and therefore do not appear in this list.



## B5. Gender aspects (optional)

*The consortium or individual beneficiaries have the option to give an indication of the kind of actions that would be undertaken during the course of the project to promote gender equality in your project, or in your field of research.*

*Relevant activities could include actions related to the project consortium (e.g. improving the gender balance in the project consortium, measures to help reconcile work and private life, awareness raising within the consortium) or, where appropriate, actions aimed at a wider public (e.g. events organised in schools or universities).*

*The gender dimension of the research content should also be considered.*

*Gender Aspects should be addressed in a work package or task within a work package. See appendix 8 of Negotiation Guidance Notes*

Although gender equality in the European work force has been advocated as a core policy since 1957 (Rome Treaty), the reality is different. During FP5 and FP6 women were systematically under-represented in research projects. For FP7, the EU has set a target of at least 40% women participation at all levels of research in order to encourage equal opportunities under gender sensitive working conditions. It is worth mentioning that Aquaculture (especially land-based hatcheries) is among the industrial and scientific activities with a greater presence of women.

In DIVERSIFY, **several issues will be addressed regarding equal participation of women as RTD researchers and SME staff**, and a commitment to use gender-impartial language will be made. Particular attention will also be given to gender sensitive issues when organising the project, such as scheduling annual meetings that require mobility. Work from men and women will be valued equally.

In the current project women participate as **Species Leaders** (3 of 6; IRTA, IEO and IMR), Scientific Discipline Leaders (or GWP leaders) (2 of 8; SWR and CTAQUA), **Work package Leaders** (14 of 31; **IMR, IOLR, FCPCT, IEO, NIFES, CMRM, SWR, CTAQUA**) and the **Scientists in charge** for the Partners (14 of 38; FCPCT, IRTA, IMR, IEO, SWR, IMR, ULL, NIFES, CTAQUA, CMRM, SWH, ANFACO, EUFIC, HRH). In terms of Researchers and Technicians, the overall participation of women in all the work packages is 42.5%. These women researchers are working as fellow researchers in all the participating countries. Pay scales in the participating institutions are independent of gender for the same position. Several of the participating institutions (HCMR, FCPCT, IRTA, IOLR, UNIABDN, IMR, UNIBA, IFREMER, ULL, SARC, DTU, SWH and DOR) have policies targeting gender equity at work. The hiring process for new personnel (technicians, graduate students, post-doctoral fellows) will be based on equal gender opportunity in order to contribute to the advancement of women in post-doctoral and top decision-making positions. The SMEs participating in this proposal employ women, mainly in land-based facilities in activities that include broodstock management, live food production, hatchery, nursery and pathology. Finally, when performing the consumer demand studies, results will be presented by gender and when required they will be analysed separately.

In an attempt to perform gender-sensitive research, the scheduling and organising of meetings and other activities requiring mobility will take into account gender issues and alternatives such as virtual meeting will be considered. Places for such activities will be chosen taking into account not only practical logistics, but also requests from parents with limited mobility. Institutes will consider the use of flexible working hours for this project. A questionnaire will be sent to participating institutions to find out whether participating researchers, especially women have limited availability to travel and which time of the year and for how long is not convenient to travel. The questionnaire will serve to verify the gender ratio of participation in this project. At the end of the project, participants will answer a small questionnaire on this issue to understand whether they felt under different levels of pressure when participating in tasks that required mobility or whether a balance situation was achieved. This questionnaire will ask which meetings they attended and how easy/difficult was for them to integrate the travel in their work and private life calendar.

Finally a **person to deal with gender issues**, should they arise, has been appointed from one of the researchers of the participants (Dr Ana Roque, IRTA).



## Bibliography

- Abdel-Hameid, N.A.H., Abidi, S.F., Khan, M.A., 2012. Dietary vitamin E requirement for maximizing the growth, conversion efficiency, biochemical composition and haematological status of fingerling *Channa punctatus*. *Aquaculture Research* 43: 226–238.
- Abreu, N., Socorro, J., Betancor, M., Caballero, M.J., Fernández-Palacios, H., Hernández-Cruz, C.M., Roo, J., Schuchart, D., 2009. New findings in organogenesis in meagre larvae (*Argyrosomus regius* Asso, 1801). XII Congreso Nacional de Acuicultura, Madrid, Spain.
- AIPCE-CEP, 2012. Finfish study 2012, Brussels.
- Aizen, J., Meiri, I., Tzchori, Levavi-Sivan, B., Rosenfeld, H., 2005. Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. *Gen. Comp. Endocrinol.* 142: 212-221.
- Aly, T.S., Garcia, A., Izquierdo, M.S., Jover, M., 1999. Utilization of different sources of lipids in extruded diets for *Seriola dumerilii* (Risso). VII Congreso Nacional de Acuicultura, Las Palmas, Spain.
- Andaloro, F., Pipitone, C., 1997. Food and feeding habits of the amberjack, *Seriola dumerili*, in the Central Mediterranean Sea during the spawning season. *Cah. Biol. Mar.* 38: 91-96.
- Anderson, S.A., Salinas, I., Walker, S.P., Gublin, Y., Pether, S., Kohn, Y.Y., Symonds, J.E., 2012. Early development of New Zealand hapuku *Polyprion oxygeneios* eggs and larvae. *J. Fish Biol.* 80: 555-571.
- Andree, K., Axtner, J., Bagley, M.J., Barlow, E.J., Beebe, T.J.C., et al., 2010. Permanent Genetic Resources Note. Permanent Genetic Resources added to Molecular Ecology. *Resources Database* 1 April 2010–31 May 2010. *Molecular Ecology Resources* 10: 1098–1105.
- Anonymous, 2008. Innovative Methodologies for the reproduction and larval rearing of fast growers. Final Report. OPF 2000-2006 Measure 4.6, 66 pp.
- Atalah, E., Hernández-Cruz, C.M., Benitez, T., Ganga, R., Roo, J., Izquierdo, M.S., 2011. Importance of relative levels of dietary ARA and EPA for culture performance of gilthead seabream (*Sparus aurata*). *Aquaculture Research* 42: 1279-1288.
- Attramadal, K.J.K., 2011. Water treatment as an approach to increase microbial control in the culture of cold water marine larvae. Doctoral thesis at NTNU, Norwegian University of Science and Technology, NTNU, 2011:231, ISBN 978-82-471-3021-6 (printed ver.).
- Attramadal, K.J.K., Tøndel, B., Salvesen, I., Øie, G., Vadstein, O., Olsen, Y., 2012. Ceramic clay reduces the load of organic matter and bacteria in marine fish larval culture tanks. *Aquaculture Engineering* 49: 23-34.
- Awad, E., Austin, D., Lyndon, A.R., 2013. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture* (388-391): 193-197.
- Babiak, J., Babiak, I., Harboe, T., Haugen, T., van Nes, S. and Norberg, B., 2012. Induced sex reversal using an aromatase inhibitor, Fadrozole, in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* 324-325: 276-280.
- Babiak, I., Mandiki, R., Ratsinjomanana, R., Kestemont, P., 2004. Initial weight and its variation in post-larval Eurasian perch affect quantitative characteristics of juvenile cohorts under controlled conditions. *Aquaculture* 234: 263-276.
- Bakeer, M.N., 2006. Performance of grey mullet (*Mugil cephalus*) reared in monoculture in the New Desert Area. *Journal of the Arabian Aquaculture Society* 1: 44-56.
- Baily, J.E., Bretherton, M.J., Gavine, F.M., Ferguson, H.W., Turnbull, J.F., 2005. The pathology of chronic erosive dermatopathy in Murray cod, *Maccullochella peelii peelii* (Mitchell). *J. Fish Diseases* 28: 3-12.
- Ball, A.O., Sedberry, G.R., Zatzoff, M.S., Chapman, R.W., Carlin, J.L., 2000. Population structure of wreckfish *Polyprion americanus* determined with microsatellite genetic markers. *Mar. Biol.* 137: 1077-1090.
- Baras, E., Hafsaridewi, R., Slembrouck, J., Priyadi, A., Moreau, Y., Pouyaud, L., Legendre, M., 2010. Why is cannibalism so rare among cultured larvae and juveniles of *Pangasius djambal*? Morphological, behavioural and energetic answers. *Aquaculture* 305: 42-51.
- Baras, E., Kestemont, P., Mélard, C., 2003. Effect of stocking density on the dynamics of cannibalism in sibling larvae of *Perca fluviatilis* under controlled conditions. *Aquaculture* 219: 241-255.
- Baras, E., Raynaud, T., Slembrouck, J., Cochet, C., Caruso, D., Legendre, M., 2011. Interactions between temperature and size on growth, size heterogeneity, mortality and cannibalism in cultured larvae and



- juveniles of the Asian catfish, *Pangasianodon hypophthalmus* (Sauvage). *Aquac. Res.* 42: 260-276.
- Barczak, G., Griffin, A., Kahn, K. B., 2009. Perspective: Trends and Drivers of Success in Npd Practices: Results of the 2003 Pdma Best Practices Study, *The Journal of Product Innovation Management* 26 (1): 3.
- Barman, U.k., Jana, S.N., Garg, S.K., Bhatnagar, A., Arasu, A.R.T., 2005. Effect of inland water salinity on growth, feed conversion efficiency and intestinal enzyme activity in growing grey mullet, *Mugil cephalus* (Linn.): Field and laboratory studies. *Aquaculture International* 13 (3): 241-256.
- Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* 42: 517-525.
- Bastardo, A., Ravelo, C., Castro, N., Calheiros, J., and Romalde, J.L., 2012. Effectiveness of bivalent vaccines against *Aeromonas hydrophila* and *Lactococcus garvieae* infections in rainbow trout *Oncorhynchus mykiss* (Walbaum). *Fish and Shellfish Immunology* 32 (5): 756-761.
- Bellentani, S., Pecorari M., Cordoma, P., Marchegiano, P., Manenti, F., Bosisio, E., De Fabiani, E., Galli, G., 1987. Taurine increases bile acid pool size and reduces bile saturation index in the hamster. *J. Lipid Res.* 28: 1021-1027.
- Bergh, Ø., Naas, K.E., Harboe, T., 1994. Shift in the intestinal microflora of Atlantic halibut (*Hippoglossus hippoglossus*) larvae during first feeding. *Can. J. Fish. Aquat. Sci.* 51: 1899-1903.
- Betancor, M.B., Atalah, E., Caballero, M.J., Benítez-Santana, T., Roo, J., Montero, D., Izquierdo, M.S., 2011.  $\alpha$ -Tocopherol in weaning diets for European sea bass (*Dicentrarchus labrax*) improves survival and reduces tissue damage caused by excess dietary DHA contents. *Aquaculture Nutrition* 17(2): 112-122.
- Betancor, M., Caballero, M.J., Terova, G., Saleh, R., Atalah, E., Benítez-Santana, T., Bell, J. G., Izquierdo, M.S., 2012a. Selenium inclusion decreases oxidative stress indicators and muscle injuries in sea bass larvae fed high-DHA microdiets. *British Journal of Nutrition* 8 (12): 2115-2128.
- Betancor, M., Caballero, M.J., Terova, G., Saleh, R., Benítez-Santana, T., Bell, J. G., Hernandez Cruz, C.M., Izquierdo, M.S., 2012b. Vitamin C enhances vitamin E status and reduces oxidative stress indicators in sea bass larvae fed high DHA microdiets. *Lipids* 47: 1193-1207.
- Bianci, E., 2012. First ideas on strategic guidelines for sustainable aquaculture, DG MARE. European Aquaculture: the Path for Growth, 23 November 2012, A Coruna, Spain.
- Björklund, M., Aho, T., Arson, L.C., 2007. Genetic differentiation in pikeperch (*Sander lucioperca*): the relative importance of gene flow, drift and common history. *Journal of Fish Biology* 71: 264-278.
- Björndahl, L., Söderlund I. and Kvist U., 2003. Evaluation of the one step eosin nigrosin staining technique for human sperm vitality assessment. *Hum. Reprod.* 18 (4): 813-816.
- Borer, S.O., Miller, L.M., Kapuscinski, A.R., 1999. Microsatellites in walleye *Stizostedion vitreum*. *Molecular Ecology* 8: 335-346.
- Boryshpolets, S. (2009). Dynamics of ATP and movement in Eurasian perch (*Perca fluviatilis* L.) sperm in conditions of decreasing osmolality. *Theriogenology* 72:851-859.
- Bostock, J., Murray, F., Muir, J., Telfer, T., Lane, A., Anagnopoulos, N., Papageorgiou, P., Alday-Sanz, V., 2009. European aquaculture competitiveness: Limitations and possible strategies. European Parliament. European Parliament, Brussels.
- Brandsen, M.P., Carter, C.G., Nichols, P.D., 2003. Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth performance, tissue fatty acid composition and disease resistance. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 135: 611-625.
- Brick, M., Klippel, S., 2003. Reproductive biology of southwestern Atlantic wreckfish, *Polyprion americanus* (Teleostei: Polyprionidae). *Environmental Biology of Fishes* 68: 163-173.
- Brick Peres, M., Haimovi, M. 2003. Alimantação do cherne-poveiro *Polyprion americanus* (Poliprionidae, Teleostei) no sul de Brasil. *Atlantica, Rio Grande* 25 (2): 201-208.
- Bruhn, C.M., 2007. Enhancing consumer acceptance of new processing technologies. *Innovative Food Science and Emerging Technologies. Agricultural Economics* 60, nr 2, 8: 555-558.
- Bruzón, M.A., 2007. Reproduction and culture of the dusky grouper *Epinephelus marginatus* (Lowe, 1834) in the South of Spain. 2nd International Symposium on Mediterranean Sea, 10-13 May, Nice, France.
- Buchet, V., Zambonino Infante, J.L., Cahu, C.L., 2000. Effect of lipid level in a compound diet on the development of red drum (*Sciaenops ocellatus*) larvae. *Aquaculture* 184: 339-347.
- Bunte, F.H.J, van Galen, M.A., Kuiper, W.E., Tacke, G.M.L., 2010. Limits to the growths of organic sales. *De Economist* 158 (4): 387-410.
- Cacot, P., Legendre, M., Dan, T.Q., Tung, L.T., Liem, P.T., Mariojous, C., Lazard, J., 2002. Induced ovulation of *Pangasius bocourti* (Sauvage, 1880) with a progressive hCG treatment. *Aquaculture* 213:





- 199-206.
- Cardello, A.V., Schutz, H.G., Lesher, L.L., 2007. Consumer perceptions of foods processed by innovative and emerging technologies: A conjoint analytic study. *Innovative Food Science and Emerging Technologies* 8: 73–83.
- Carton, A.G., 2005. The impact of light intensity and algal-induced turbidity on first-feeding *Seriola lalandi* larvae. *Aquaculture Research* 36: 1588-1594.
- Cergole, M.C., Olinto, A., Del Bianco, C.L., 2005. Análise das principais piscarias comerciais da região sudeste-sul do Brasil: dinâmica populacional das espécies em exploração. *Serie documentos Revizee: Score Sul*. ISBN 85-98729-05-1.
- Chatzifotis, S., Panagiotidou, M., Divanach, P., 2012. Effect of protein and lipid dietary levels on the growth of juvenile meagre (*Argyrosomus regius*). *Aquacult. Int.* 20: 91-98.
- Chatzifotis, S., Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, I., Mylonas, C.C., 2010. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. *Aquaculture* 307: 65-70.
- Chatzifotis, S., Polemitou, I. Divanach, P., Antonopoulou, E., 2008. Effect of dietary taurine supplementation on growth performance and bile salt activated lipase activity of common dentex, *Dentex dentex*, fed a fish meal/soy protein concentrate-based diet. *Aquaculture* 275: 201-208.
- Chatzifotis, S., Villamor Martin-Prat, A., Limberis, N., Papandroulakis, N., Divanach, P., 2006. First data on growth of cultured brown meagre *Sciaena umbra* using diets with different protein and fat contents. *Fisheries Science* 72: 83–88.
- Chauvigné, F., Verdura, S., Mazón, M.J., Duncan, N., Zanuy, S., Gómez, A., Cerda, J., 2012. Follicle-stimulating hormone and luteinizing hormone mediate the androgenic pathway in Leydig cells of an evolutionary advanced teleost. *Biology of Reproduction* 87(2): 35.
- Chen, J.N., Takeuchi, T., Takahashi, T., Tomoda, T., Koiso, M., Kuwada, H., 2004. Effect of rotifers enriched with taurine on growth and survival activity of red sea bream *Pagrus major* larvae. *Nippon Suisan Gakkaishi* 70: 542–547.
- Chen, J.N., Takeuchi, T., Takahashi, T., Tomoda, T., Koiso, M., Kuwada, H., 2005. Effect of rotifers enriched with taurine on growth in larvae of Japanese flounder *Paralichthys olivaceus*. *Nippon Suisan Gakkaishi* 71: 342–347.
- Civitelli, R., Villareal, D.T., Agnusdei, D., Nardi, P., Avioli, L.V., Gennari, C., 1992. Dietary L-lysine and calcium metabolism in humans. *Nutrition* 8: 400-405.
- Conceição, L.E.C., van der Meeren, T., Verreth, J.A.J., Evjen, M.S., Houlihan, D.F., Fyhn, H.J., 1997. Amino acid metabolism and protein turnover in larval turbot (*Scophthalmus maximus*) fed natural zooplankton or *Artemia*. *Mar. Biol.* 129: 255–265.
- Conte, F.S., 2004. Stress and the welfare of cultured fish. *Applied Animal Behaviour Science* 86 (3-4): 205–223.
- Cooke, S.J., Sneddon, L.U., 2007. Animal welfare perspectives on catch-and-release recreational angling. *Applied Animal Behaviour Science* 104: 176–198.
- Crespo, S., Grau, A., Padrós, F., 1990. Epitheliocystis disease in the cultured amberjack, *Seriola dumerili* Risso (Carangidae). *Aquaculture* 90: 197-207.
- Crespo, S., Grau, A., Padrós, F., 1994. The intensive culture of 0+ amberjack in the western Mediterranean is compromised by disease problems. *Aquaculture International* 2: 1–4.
- Cummings, N.J., Turner, S.C., McClellan, D.B., Legault, C.M., 1999. Atlantic greater amberjack abundance indices from commercial handline and recreational charter, private, and headboat fisheries through fishing year 1997. *National Oceanic and Atmospheric Sciences*, 77 pp.
- D’Alvise, P.W., Lillebø, S., Prol-García, M.J., Wergeland, H.I., Nielsen, K.F., et al., 2012. *Phaeobacter gallaeciensis* reduces *Vibrio anguillarum* in cultures of microalgae and rotifers, and prevents vibriosis in cod larvae. *PLoS ONE* 7(8): e43996. doi:10.1371/journal.pone.0043996.
- D’Alvise, P.W., Lillebø, S., Wergeland, H.I., Nielsen, K.F., Gram, L.K., Bergh, Ø., 2013 (in press). Protection of doc larvae from vibriosis by *Phaeobacter* spp. A comparison of strains and introduction times. *Aquaculture* (in press).
- Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Pedersen, P.B., 2013 (in press) Farming different species in RAS in Nordic countries: Current status and further perspectives. *Aquacultural Engineering*. doi:10.1016/j.aquaeng.2012.11.008.



- Daniels, W.H., Robinson E.H., 1986. Protein and energy requirements of juvenile red drum (*Sciaenops ocellatus*). *Aquaculture* 53: 243-252.
- Dantagnan, P., Bórquez, A., Hernández, A., Izquierdo, M.S., 2010. Effect of EPA/DHA ratios on the growth and survival of *Galaxias maculatus* (Jenys, 1842) larvae reared under different salinity regimes. *Aquaculture Research* 41: 239-244. doi: 10.1111/j.1365-2109.2010.02512.x.
- Darawiroj, D., Kondo, H., Hirono, I., Aoki, T., 2008. Immune-related gene expression profiling of yellowtail (*Seriola quinqueradiata*) kidney cells stimulated with ConA and LPS using microarray analysis. *Fish and Shellfish Immunol.* 24: 260-266.
- Deliza, R., Rosenthal, A., Abadio, F.B.D., Carlos, Silva, H.O., Castillo, C., 2005. Application of high pressure technology in the fruit juice processing: benefits perceived by consumers. *Journal of Food Engineering* 67: 241-246.
- De Monbrison, D., Tzchori, I., Holland, M.C., Zohar, Y., Yaron, Z., Elizur, A., 1997. Acceleration of gonadal development and spawning induction in the Mediterranean grey mullet, *Mugil cephalus*: Preliminary studies. *Isr. J. Aquacult. Bamid.* 49: 214-221.
- Deudero, S., Morales-Nin, B., 2000. Occurrence of *Polyprion americanus* under floating objects in western Mediterranean oceanic waters, inference from stomach contents analysis. *J. Mar. Biol. Ass. UK.* 80: 751-752.
- Díaz-López, M., Pérez, M.J., Acosta, N.G., Jerez, S., Dorta-Guerra, R., Tocher, D.R., Lorenzo, A., Rodríguez, C., 2010. Effects of dietary fish oil substitution by *Echium* oil on enterocyte and hepatocyte lipid metabolism of gilthead seabream (*Sparus aurata* L.). *Comparative Biochemistry and Physiology (B)* 155: 371-379.
- Díaz-López, M., Pérez M.J., Acosta, N.G., Tocher, D.R., Jerez, S., Lorenzo, A., Rodríguez, C., 2009. Effect of dietary substitution of fish oil by *Echium* oil on growth, plasma parameters and body lipid composition in gilthead seabream (*Sparus aurata*). *Aquaculture Nutrition* 15: 500-512.
- Díaz-Rosales, P., Arijo, S., Chabrilón, M., Alarcón, F.J., Tapia-Paniagua, S.T., Martínez-Manzanares, E., Balebona, M.C., Moriñigo, M.A., 2009. Effects of two closely related probiotics on respiratory burst activity of Senegalese sole (*Solea senegalensis*, Kaup) phagocytes, and 1 protection against *Photobacterium damsela* subsp. piscicida. *Aquaculture* 293: 16-21.
- Dierckens, K., Rececki, A., Laureau, S., Sorgeloos, P., Boon, N., van den Broeck, W., Bossier, P., 2009. Development of a bacterial challenge test for gnotobiotic sea bass (*Dicentrarchus labrax*) larvae. *Environmental Microbiology* 11(2): 526-533.
- Divakaran, S., 2006. Taurine: An amino acid rich in fish meal. In: L. Elizabeth Cruz Suárez, Denis Rique Marie, Mireya Tapia Salazar, Martha G. Nieto López, David A. Villarreal Cavazos, Ana C. Puello Cruz y Armando García Ortega. *Avances en Nutrición Acuicola VIII. VIII Symposium Internacional de Nutrición Acuicola.* Nov. 15-17. Universidad Autónoma de Nuevo León, Monterrey. Nuevo León, México, pp. 310-317.
- Douxflis, J., Mandiki, S.N.M., Marotte, G., Wang, N., Silvestre, F., Milla, S., Henrotte, E., Vandecan, M., Rougeot, C., Mélard, C., Kestemont, P., 2011. Does domestication process affect stress response in juvenile Eurasian perch *Perca fluviatilis*? *Comparative Biochemistry and Physiology Part A* 159: 92-99.
- Douxflis, J., Deprez, M., Mandiki, S.N.M., Milla, S., Henrotte, E., Mathieu, C., Silvestre, F., Vandecan, M., Rougeot, C., Mélard, C., Dieu, M., Raes, M., Kestemont, P., 2012. Physiological and proteomic responses to single and repeated hypoxia in juvenile Eurasian perch under domestication - Clues to physiological acclimation and humoral immune modulations. *Fish and Shellfish Immunology* 33: 1112-1122.
- Dubut, V., Grenier, R., Meglécz, E., Chappaz, R., Costedoat, C., Danancher, D., Descloux, S., Malausa, T., Martin, J.F., Pech, N., Gilles, A., 2010. Development of 55 novel polymorphic microsatellite loci for the critically endangered *Zingel asper* L. (Actinopterygii: Perciformes: Percidae) and cross-species amplification in five other percids. *Eur J Wildl Res.*
- Dunbar, C.E., Herman, R.L., 1971. Visceral granuloma in brook trout (*Salvelinus fontinalis*). *J. Nutrition* 101: 1445-1452.
- Duncan, N.J., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C.M., Roo, J., Schuchardt, D., Vallés, R., 2013a. Aquaculture production of meagre (*Argyrosomus regius*): hatchery techniques, ongrowing and market. In: Allan, G., Burnell, G. (Eds.), *Advances in aquaculture hatchery technology.* Woodhead Publishing Limited, Cambridge, UK.





- Duncan, N., Estévez, A., Porta, J., Carazo, I., Norambuena, F., Aguilera, C., Gairin, I., Bucci, F., Valles, R., Mylonas, C.C., 2012. Reproductive development, GnRHa-induced spawning and egg quality of wild meagre (*Argyrosomus regius*) acclimatized to captivity. *Fish Physiology and Biochemistry* 38: 1273-1286.
- Duncan, N.J., Sonesson, A.K., Chavanne, H., 2013b. Principles of finfish broodstock management in aquaculture: control of reproduction and genetic improvement. In: Allan, G., Burnell, G. (Eds.), *Advances in aquaculture hatchery technology*, Woodhead Publishing Limited, Cambridge, UK.
- EATIP (European Aquaculture Technology and Innovation Platform) 2012. The future of European Aquaculture: a strategic agenda for research and innovation. [www.eatip.eu](http://www.eatip.eu)
- Eda, H., Murashige, R., Oozeki, Y., Hagiwara, A., Eastham, B., Bass, P., Tamaru, C.S., Sheng Lee, C., 1990. Factors affecting intensive larval rearing of striped mullet *Mugil cephalus*. *Aquaculture* 91: 281-294.
- Elkesh, A., Kantham, K.P.L., Shinn, A.P., Crumlish, M., Richards, R.H., 2012. Systemic nocardiosis in a Mediterranean population of cultured meagre, *Argyrosomus regius* Asso (Perciformes: Sciaenidae). *Journal of Fish Diseases*. doi:10.1111/jfd.12015.
- Ernst and Young, Cogea and Eurofish, 2008, Evaluation of the Common Organisation of the Markets in Fishery and Aquaculture Products. Directorate-General for Maritime Affairs and Fisheries (DG MARE), Brussels.
- Estevez, A., Treviño, L., Kotzamanis, Y., Karacostas, I. Tort, L., Gisbert, E., 2011. Effects of different levels of plant proteins on the on-growing of meagre (*Argyrosomus regius*) juveniles at low temperatures. *Aquacult. Nutr.* 17: 572-582. doi: 10.1111/j.1365-2095.2010.00798.x.
- Failler, P., 2007. Future prospects for fish and fishery products. 4. Fish consumption in the European Union in 2015 and 2030. Part 1. European overview. FAO Fisheries Circular. No. 972/4, Part 1. Rome, FAO, 2007, 204p.
- FAO 2003-2012. Fisheries and Aquaculture topics. Fisheries technology, Topics Fact Sheets. In: FAO Fisheries and Aquaculture Department [online], Rome, Updated 31 October 2001. [Cited 14 December 2012]. <http://www.fao.org/fishery/topic/2800/en>.
- FAO 2005-2011. Cultured Aquatic Species Information Programme. *Argyrosomus regius*. Cultured Aquatic Species Information Programme. Text by Stipa, P., Angelini, M. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 10 February 2005. [Cited 24 September 2011]. [http://www.fao.org/fishery/culturedspecies/Argyrosomus\\_regius/en](http://www.fao.org/fishery/culturedspecies/Argyrosomus_regius/en).
- FAO, 2009. The state of the world fisheries and aquaculture 2008. FAO, OUN. Rome, 216 pp.
- FAO, 2010. [http://www.fao.org/fishery/culturedspecies/Mugil\\_cephalus/en](http://www.fao.org/fishery/culturedspecies/Mugil_cephalus/en).
- FAO, 2012. The State of World Fisheries and Aquaculture: 2012. Rome: Food and Agriculture Organization of the United Nations. 209 pp.
- FAO, 2012. FAO Fisheries Department, Fishery Information, Data and Statistics Unit. FISHSTAT Plus: Universal software for fishery statistical time series. Version 2.3. 2000. Data sets: Aquaculture production: quantities and values 1950-2010.
- FAO, 2012 [http://www.fao.org/fishery/culturedspecies/Seriola\\_quinqueradiata/en](http://www.fao.org/fishery/culturedspecies/Seriola_quinqueradiata/en)
- Fauvel, C.H., Boryshpolets, S., Cosson, J., Wilson Leedy, J.G., Labbé, C., Haffray, P., Suquet, M., 2012. Improvement of chilled seabass sperm conservation using a cell culture medium. *Journal of Applied Ichthyology* 28 (6): 961-966.
- Fauvel, C.H., Suquet, M., Sévère, A., Mylonas, C., Papandroulakis, N., 2008. Slow-release GnRHa treatment prevented atresia during vitellogenesis and induced ovulation of captive wreckfish (*Polyprion americanus*). *Cybio* 32 (2) suppl.: 191.
- Fauvel, C., Suquet, M., Cosson, J., 2010. Evaluation of fish sperm quality. *J. Appl. Ichthyol.* 26 (5): 636-643.
- Fernández-Palacios, H., Hernández-Cruz, C.M., Schuchardt, D., Izquierdo, M.S., Roo, F.J., 2009a. Effect of co-feeding regimes on biological performance and biochemical composition of meagre (*Argyrosomus regius* Asso, 1801) larvae. *Europ. Aquac. Soc. Spec. Publ.* 38: 108-111.
- Fernández-Palacios, H., Izquierdo, M.S., Norberg, B., Hamre, K., 2011a. Effect of broodstock diet on eggs and larvae. In: Holt, J. (ed.), *Larval Fish Nutrition*, Wiley – Blackwell, John Wiley and Sons, ISBN: 978-0-8138-1792-7, pp. 153-183.
- Fernández-Palacios, H., Schuchardt, D., Roo, J., Borrero, C., Hernández-Cruz, C.M., Socorro, J. 2007. Morphometry of meagre (*Argyrosomus regius*, Asso, 1801) during the first month of rearing. XI Congreso Nacional de Acuicultura, Vigo, Spain.



- Fernández-Palacios, H., Schuchardt, D., Roo, J., Hernández-Cruz, C.M., Duncan, N., 2009b. Efecto de distintas dosis de GnRHa sobre la calidad de la puesta de corvina (*Argyrosomus regius*). In Libro de Actas, XII Congreso Nacional de Acuicultura, Madrid, España, 24-26 Noviembre 2009, pp. 554-555 (in Spanish, abstract in English).
- Fernández-Palacios, H., Schuchardt, D., Roo, J., Hernández-Cruz, C.M., Sabater, C., Duncan, N., 2011b. Efecto de diferentes intervalos de tiempo entre inyecciones con GnRHa, sobre las puestas de reproductores de corvina (*Argyrosomus regius*). XIII Congreso Nacional Acuicultura, Book of Abstracts, Universitat Politècnica de Catalunya ESAB-Castelldefels, 21-24 November 2012, Barcelona, Spain (in Spanish, abstract in English).
- Folkvord, A., Otterå, H., 1993. Effects of initial size distribution, day length, and feeding frequency on growth, survival and cannibalism in juvenile Atlantic cod (*Gadus morhua* L.). *Aquaculture* 114: 243-260.
- Fonseca-Madrugal, J., Pineda-Delgado, D., Martínez-Palacios, C., Rodríguez, C. and Tocher D.R., 2012. Effect of salinity on the biosynthesis of n-3 long-chain polyunsaturated fatty acids in silverside *Chirostoma estor*. *Fish Physiology and Biochemistry* 38 (4): 1047-1057.
- Fontaine, P., Wang, N., Teletchea, F., 2012. Domestication of new species and diversification in inland aquaculture, the example of Percid fish. Third workshop on fish culture, 3-4th July, Paris, France (in French).
- Furutani, T., Masumoto, T., Fukada, H., 2012. Response of cholecystokinin and digestive enzyme mRNA levels to various feed ingredients in yellowtail *Seriola quinqueradiata*. *Fish. Sci.* 78: 1075–1082.
- Ganga, R., Tort, L., Acerete, L., Montero, D., Izquierdo, M.S., 2006. Modulation of ACTH-induced cortisol release by polyunsaturated fatty acids in interregional cells from gilthead seabream, *Sparus aurata*. *Journal of Endocrinology*: 190, 39-45.
- García de la Banda, I., Lobo, C., Chabrilón, M., León-Rubio, J.M., Arijo, S., Pazos, G., Lucas, L.M., Moriñigo, M.A., 2012. Influence of dietary administration of a probiotic on Senegalese sole (*Solea senegalensis*, Kaup, 1858) growth, body composition and resistance to *Photobacterium damsela* subsp. *piscicida*. *Aquacult. Res.* 43(5): 662-669.
- Gatlin, D.M. III, 1995. Review of red drum nutrition. In: Limm, C.E., Sessa, D.J. (Eds.), *Nutrition and Utilization Technology in Aquaculture*, AOCS Press, Champaign, pp. 41-49.
- Gatlin, D.M. III, 2002. Nutrition and fish health. In: Halver, J.E., Hardy, R.W. (Eds.), *Fish Nutrition*, 3rd edition, Academic Press, San Diego, CA, USA, pp. 671–702.
- Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R., Herman, E., Hu, G., Kroghdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research* 38: 551- 579.
- García de la Banda, I., Lobo, C., Leon-Rubio, J.M., Tapia-Paniagua, S., Balebona, M.C., Moriñigo, M.A., Moreno-Ventas, X., Lucas, L.M., Linares, F., Arce, F., Arijo, S., 2010. Influence of two closely related probiotics on juvenile Senegalese sole (*Solea senegalensis*, Kaup 1858) performance and protection against *Photobacterium damsela* subsp. *piscicida*. *Aquaculture* 306: 281-288.
- Georgakopoulou, E., Angelopoulou, A., Kaspiris, P., Divanach, P., Koumoundouros, G., 2007. Temperature effects on cranial deformities in European sea bass, *Dicentrarchus labrax* (L.) *J. Appl. Ichthyol.* 23: 99–103.
- Gerlach, G., Schardt, U., Eckmann, R., Meyer, A., 2001. Kin-structured subpopulations in Eurasian perch (*Perca fluviatilis* L.). *Heredity* 86: 213-221.
- Ghittino, C., Manuali, E., Latini, M., Agnetti, F., Rogaro, F., Agonigi Colussi, S., Prearo, M., 2004. Caso di granulomatosi sistemica in ombrina boccardoro (*Argyrosomus regius*) e raffronto con le lesioni istologiche presenti nell'orata. *Ittipatologia* 1: 59-67.
- Gillespie, A., 2011. *Foundations of economics*, Oxford Press, New York, additional chapter Business strategy.
- Ginés, R., Robaina, L., Rodríguez-Lozano, A., Domínguez-Montesdeoca, D., Hernández-Cruz, C.M., Romero, J., 2013 (in press). Effect of the different dietary levels of vitamin E on fillet quality of meagre (*Argyrosomus regius*). *Aquaculture* (in press).
- Gjedrem, T., 2012. Genetic improvement for the development of efficient global aquaculture: A personal opinion review. *Aquaculture* 344-349: 12–22.
- Goto, T., Matsumoto, T., Murakami, S., Takagi, S., Hasumi, F., 2003. Conversion of cysteate into taurine in liver of fish. *Fish. Sci.* 69: 216–218.



- Grossi, E., Fernández-Palacios, H., Abreu, N., Socorro, J., Roo, J., Hernández-Cruz, C. M., Schuchardt, D. 2009. Organogenesis and morfometry in the lecithotrophic phase in *Seriola rivoliana* (Valenciennes, 1883) larvae. XII Congreso Nacional de Acuicultura, Madrid, Spain.
- Grau, A., Crespo, S., 1991. Epitheliocystis in the wild and cultured amberjack, *Seriola dumerili* Risso: Ultrastructural observations. *Aquaculture* 95:1-6.
- Grau, A., Crespo, S., Pastor, E., Gonzalez, P., Carbonell, E., 2003. High infection by *Zeuxapta seriolae* (Monogenea: Heteraxinidae) associated with mass mortalities of amberjack *Seriola dumerili* Risso reared in sea cages in the Balearic Islands (western Mediterranean). *Bull. Eur. Ass. Fish Pathol.* 23: 139–142.
- Guiltinan, J. P., 1999. Launch strategy, launch tactics, and demand outcomes, *Journal of Product Innovation Management* 16: 509-529.
- Haffray, P., Malha, R., Sidi, M.O.T., Prista, N., Hassan, M., Castelnaud, G., Karahan-Nomm, B., Gamsiz, K., Sadek, S., Bruant, J.S., Balma, P., Bonhomme, F., 2012. Very high genetic fragmentation in a large marine fish, the meagre *Argyrosomus regius* (Sciaenidae, Perciformes): impact of reproductive migration, oceanographic barriers and ecological factors. *Aquatic Living Resources* 25: 173-183.
- Hagey, L.R., Møller, P.R., Hofmann, A.F., Krasowski, M.D., 2010. Diversity of bile salts in fish and amphibians: Evolution of a complex biochemical pathway. *Physiol. Biochem. Zool.* 83: 308-321.
- Hamasaki, K., Tsuruoka, K., Teruya, K., Hashimoto, H., Hamada, K., Hotta, T., Mushiake, K., 2009. Feeding habits of hatchery-reared larvae of greater amberjack *Seriola dumerili*. *Aquaculture* 288: 216-225.
- Hamre, K., Harboe, T., 2008a. Critical levels of essential fatty acids for normal pigmentation in atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Aquaculture* 277: 101-108.
- Hamre, K., Harboe, T., 2008b. Artemia enriched with high n-3 HUFA may give a large improvement in performance of Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Aquaculture* 277: 239-243.
- Hamre, K., Lie, Ø., 1995. Minimum requirement of vitamin E for Atlantic salmon, *Salmo salar* L., at first feeding. *Aquaculture Research* 26: 175–184.
- Hamre, K., Yufera, M., Ronnestad, I., Boglione, C., Conceição, L.E.C., Izquierdo, M., 2013 (in press). Fish larval nutrition and feed formulation – knowledge gaps and bottlenecks for advances in larval rearing. *Reviews in aquaculture* (in press).
- Hamza, N., Mhetli, M., Khemis, I.B., Cahu, C., Kestemont, P., 2008. Effect of dietary phospholipid levels on performance, enzyme activities and fatty acid composition of pikeperch (*Sander lucioperca*) larvae. *Aquaculture* 275 (1-4): 274-282.
- Hansen, S.H., Mortensen, O.H., 2012. Taurine and metabolic disease. In: El Idrissi, A. and L'Amoreaux, W. (Eds.), *Taurine in health and disease*, Transworld Research Network, pp. 167-190.
- Harboe, T., Reitan, K.I., 2005. Halibut fry production. Larvi '05 – Fish and shellfish larviculture symposium, Hendry, C.I., Van Stappen, G., Wille, M. and Sorgeloos, P. (Eds.), European Aquaculture Society, Special publication No. 36, Oostende, Belgium.
- Harboe, T., Mangor-Jensen, A., Moren, M., Hamre, K., Rønnestad, I., 2009. Control of light condition affects the feeding regime and enables successful eye migration in Atlantic halibut juveniles. *Aquaculture* 290: 250-255.
- Harel, M., Ben-Atya, S., Zlotkin, V., Tandler, A., 1998. Mass production of grey mullet, *Mugil cephalus*: Effect of environmental and nutritional factors on larval performances. *Israeli Journal of Aquaculture* 50: 91-98.
- Hendry, C.I., Martin-Robichaud, D.J., Benfey, T.J., 2003. Hormonal sex reversal of Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture* 219: 769-781.
- Henrotte, E., Mandiki, R.S.N.M., Prudencio, A.T., Vandecan, M., Mélard, C., Kestemont, P., 2010. Egg and larval quality, and egg fatty acid composition of Eurasian perch breeders (*Perca fluviatilis*) fed different dietary DHA/EPA/AA ratios. *Aquaculture Research* 41: 53–61.
- Hernández-Cruz, C.M., Schuchardt, D., Roo, J., Borrero, C., Fernández-Palacios, H. 2007. Optimization of weaning protocol in meagre (*Argyrosomus regius*, Asso, 1801). XI Congreso Nacional de Acuicultura, Vigo, Spain.
- Huxtable, R.J. 1992. Physiological actions of taurine. *Physiol. Rev.* 72: 101–163.
- Izquierdo, M.S., 1996. Essential fatty acid requirements of cultured marine fish larvae. *Aquaculture Nutrition* 2(4): 183-191.
- Izquierdo, M.S., Fernandez-Palacios, H., Tacon, A.G.J., 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* 197: 25-42.



- Izquierdo, M.S., Koven, W., 2011. Lipids: In: Holt, J. (ed.), Larval Fish Nutrition, Wiley-Blackwell, John Wiley and Sons, ISBN: 978-0-8138-1792-7, pp. 47-82.
- Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Rosenlund, G., Gines, R., 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture* 250: 431-444.
- Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., Rosenlund, G., 2003. Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. *Aquaculture Nutrition* 9: 397-407.
- Izquierdo M.S., Scolamacchia, M., Betancor, M., Roo, J., Caballero, M.J., Terova, G., Witten, P.E., 2012. Effects of dietary DHA and *a*-tocopherol on bone development, early mineralisation and oxidative stress in *Sparus aurata* (Linnaeus, 1758) larvae. *British Journal of Nutrition*. doi:10.1017/S0007114512003935.
- Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernandez-Cruz, C.M., 2000. Recent advances in lipid nutrition in fish larvae. *Fish Physiology and Biochemistry* 22: 97-107.
- Izquierdo, M.S., Socorro, J., Roo, J., 2010. Review: Studies on the appearance of skeletal anomalies in red porgy: effect of culture intensiveness, feeding habits and nutritional quality of live preys. *J. Appl. Ichthyol.* 26: 320-326.
- Jentoft, S., Aastveit, A.H., Torjesen, P.A., Andersen, O., 2005. Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part A* 141: 353-358.
- Jerez, S., Cejas, J.R., Martín, V., Bolaños, A., Rodríguez, D., Lorenzo, A., 2011. Efecto del tipo de alimento y densidad de cultivo en el engorde de *Seriola dumerili*. *Actas XIII Congr. Nac. Acuicult.*, Castelfelers, Barcelona (España), 21-24 November, 2011.
- Jerez, S., Cejas, J.R., Samper, M., Felipe, B.C., Santamaría, F.J. and Villamandos, J.E. 2007. Crecimiento y maduración sexual en ejemplares de medregal *Seriola dumerili* nacidos en cautividad en Canarias. XI Congreso Nacional de Acuicultura, Vigo (España), 24/09/2007 a 28/09/2007.
- Jerez, S., Hernández, I., Cejas, J.R., Almansa, E., Samper, M., Villamandos, J.E., Felipe, B.C., 2009a. Efectos de la estrategia de alimentación en la hematología y bioquímica sanguínea del medregal (*Seriola dumerili*) en condiciones de cultivo. XII Congreso Nacional de Acuicultura. Madrid, 24-26 November 2009.
- Jerez, S., Hernández, I., Cejas, J.R., Almansa, E., Samper, M., Santamaría, F.J., 2009b. Efecto de la estrategia de alimentación en el crecimiento del medregal (*Seriola dumerili*) en condiciones de cultivo. XII Congreso Nacional de Acuicultura, Madrid, 24-26 November 2009.
- Jerez, S., Samper, M., Santamaría, F.J., Villamandos, J.E., Cejas, J.R., Felipe, B.C., 2006. Natural spawning of greater amberjack (*Seriola dumerili*) kept in captivity in the Canary Islands. *Aquaculture* 252: 199-207.
- Jover, M., Garcia-Gomez, A., Tomas, A., De la Gandara, F., Pérez, L., 1999. Growth of Mediterranean yellowtail (*Seriola dumerilii*) fed extruded diets containing different levels of protein and lipid. *Aquaculture* 179: 25-33.
- Kanazawa, A., Teshima, S., Koshio, S., Higashi, M., Itoh, S., 1992. Effect of l-ascorbyl-2-phosphate-Mg on the yellowtail *Seriola quinqueradiata* as a vitamin C source. *Nippon Suisan Gakkaishi* 58: 337-341.
- Katharios, P., Kokkari, K., Papadaki, M., Papandroulakis, N., 2011a. Systemic granulomas in cultured meagre, *Argyrosomus regius*. In: *Aquaculture Europe* 11, Rhodes, pp. 537-538.
- Katharios, P., Papadaki, M., Papandroulakis, N., Divanach, P., 2008. Severe mortality in mesocosm-reared sharpsnout sea bream *Diplodus puntazzo* larvae due to epitheliocystis infection. *Diseases of Aquatic Organisms* 82: 55-60.
- Katharios, P., Papadaki, M., Ternengo, S., Kantham, P.K., Zeri, C., Petraki, P.E., Divanach, P., 2011b. Chronic ulcerative dermatopathy in cultured marine fishes. Comparative study in sharpsnout sea bream, *Diplodus puntazzo* (Walbaum). *J. Fish Diseases* 34: 459-74.
- Katz, T., Herut, B., Genin, A., Angel, D.L., 2002. Gray mullets ameliorate organically enriched sediments below a fish farm in the oligotrophic Gulf of Aqaba (Red Sea). *Marine Ecol. Prog. Series* 234: 205-214.
- Kaushik, S.J., 1998. Nutritional bioenergetics and estimation of waste production in non-salmonids. *Aquat. Living Resour.* 11: 211-217.
- Kaushik, S.J., Coves, D., Dutto, G., Blanc, D. 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture* 230: 391-404.





- Kawanishi, M., Kojima, A., Ishihara, K., Esaki, H., Kijima, M., Takahashi, T., Suzuki, S., Tamura, Y., 2005. Drug resistance and pulsed-field gel electrophoresis patterns of *Lactococcus garvieae* isolates from cultured *Seriola* (yellowtail, amberjack and kingfish) in Japan. *Lett. Appl. Microbiol.* 40: 322-8.
- Kestemont, P., Jourdan, S., Houbart, M., Mélard, C., Paspatis, M., Fontaine, P., Cuvier, A., Kentouri, M., Baras, E., 2003. Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. *Aquaculture* 227: 333-356.
- Kestemont, P., Melard, C., 2000. Chapter 11. Aquaculture. In: Craig, J.F. (ed.), *Percid Fish Systematics, Ecology and Exploitation*, Blackwell Science, Oxford, UK, pp. 191–224.
- Kestemont, P., Mélard, C., Fiogbe, E., Vlavonou, R., Masson, G., 1996. Nutritional and animal husbandry aspects of rearing early life stages of Eurasian perch *Perca fluviatilis*. *Journal of Applied Ichthyology* 12: 157–165.
- Kestemont, P., Xu, X., Hamza, N., Maboudou, J., Imorou, Toko, I., 2007. Effect of weaning age and diet on pike perch larviculture. *Aquaculture* 264: 197-204.
- Kibe, A., Wake, C., Kuramoto, T., Hoshita, T., 1980. Effect of dietary taurine on bile acid metabolism in guinea pigs. *Lipids* 15: 224–229.
- Kiron, V., Thawonsuwan, J., Panigrahi, A., Scharsack, J.P., Satoh, S., 2010. Antioxidant and immune defences of rainbow trout (*Oncorhynchus mykiss*) offered plant oils differing in fatty acid profiles from early stages. *Aquacult Nutr.* doi:10.1111/J.1365-2095.2009.00715.x.
- Kobayashi, T., Pakarinen, P., Torgersen, J., Huhtanemi, I., Andersen, Ø., 2008. The gonadotropin receptors FSH-R and LH-R of Atlantic halibut (*Hippoglossus hippoglossus*)-2. Differential follicle expression and asynchronous oogenesis. *Gen. Comp. Endocrinol.* 156: 595-602.
- Kocabas, Gatlin III D., 1999. Dietary vitamin E requirement of hybrid striped bass (*Morone chrysops* female × *M. saxatilis* male). *Aquaculture Nutrition* 5: 3–7.
- Kohlmann, K., Kersten, P., 2008. Isolation and characterization of nine microsatellite loci from the pike-perch, *Sander lucioperca* (Linnaeus, 1758). *Molecular Ecology Resources* 8: 1085–1087.
- Kolkovski, S., Lazzo, J.P., Leclercq, D., Izquierdo, M., 2009. Larval nutrition and diets: new developments. In: Burnell, G., Allan, G. (Eds.), *New Technologies in Aquaculture: improving production efficiency, quality and environmental management* 78, 315-369. Woodhead Publishing Limited, Cambridge, UK.
- Kolkovski, S., Curnow, J., King, J., 2010. Development towards commercialization of marine fish larvae feeds – Microdiets. Project No. 2004/258. Fisheries Research Report No. 198. Department of Fisheries, Western Australia, 180p.
- Koven, W.M., 2003. Key factors influencing juvenile quality in mariculture: A Review. *Israeli Journal of Aquaculture/Bamidgeh* 55 (4): 283-297.
- Koven, B., Nixon, O., Eliezer, D., Tandler, A., 2012. Larval Feeds: Ciliates improve survival, maintain growth and are preferred over rotifers in first-feeding fish larvae according to Israeli researchers. *Hatchery International*, volume 13 (5), September/October.
- Koven, W., Nixon-Shtupler, O., Avraham, S., Falcon, J., Besseau, L., Escande, M., El Sadin, S., Levitan, A., Tandler, A., 2012. The combined effect of DHA and taurine improves retinal development, prey ingestion and growth in first feeding larvae of Atlantic blue fin tuna *Thunnus thynnus* (abstract). *Aquaculture* 2013. February 21-25 2013, Nashville, Tennessee, USA.
- Koyuncu, C. E., Castro Romero, R., Karaytug, S., 2012. *Lernanthropus indefinitus* N. Sp (Copepoda, Siphonostomatoida, Lernanthropidae) parasitic on *Argyrosomus Regius* (Asso, 1801) (Pisces, Sciaenidae). *Crustaceana* 85: 1409-1420.
- Kozul, V., Skaramuca, B., Glamuzina, B., Glavic, N., Tutman, P., 2001. Comparative gonadogenesis and hormonal induction of spawning of cultured and wild Mediterranean amberjack (*Seriola dumerili*, Risso 1810). *Sci. Mar.* 65: 215-220.
- Kucharczyk, D., Kestemont, P., Mamcarz, A., 2007. Artificial reproduction of pikeperch. Practical manual, Polish Ministry of Science, 80 pp.
- Kvåle, A., Mangor-Jensen, A., Moren, M., Espe, M., Hamre, K., 2007. Development and characterisation of some intestinal enzymes in Atlantic cod (*Gadus morhua* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Aquaculture* 264: 457–468.
- Lall, S.P., 2000. Nutrition and health of fish. In: Cruz -Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A., Civera-Cerecedo, R., (eds.), *Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola*, Mérida, Yucatán, Mexico.



- Lazo, J.P., Mendoza, R., Holt, G.J., Aguilera, C., Arnold, C.R., 2007. Characterization of digestive enzymes during larval development of red drum (*Sciaenops ocellatus*). *Aquaculture* 265: 194–205.
- Leclerc, D., Wirth, T., Bernatchez, L., 2000. Isolation and characterization of microsatellite loci in the yellow perch (*Perca flavescens*), and cross species amplification within the family Percidae. *Molecular Ecology* 9: 993–1011.
- Ledoré, Y., Gardeur, J.-N., Rérat, R., Atmane, D., Fontaine, P., 2010. Développement et optimisation d'une production d'alevins sevrés de perche commune en circuit fermé. Rapport final, contrat de recherche OSEO-Innovation – Lucas Perches, 69 pp. (in French).
- Lee, Y., O'Connor, G.C., 2003. The Impact of Communication Strategy on Launching New Products: The Moderating Role of Product Innovativeness. *Journal of Product Innovation Management* 20: 4–21.
- Li, L., Wang, H.P., Givens, C., Czesny, S., Brown, B., 2007. Isolation and characterization of microsatellites in yellow perch (*Perca flavescens*). *Molecular Ecology* 7: 600–603.
- Li, P., Mai, K., Trushenski, J., Wu, G., 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids* 37 (1): 43–53.
- Liao, I.C. and Leano, E.M., 2008. The aquaculture of Groupers. The Fisheries Society of Taiwan, National Taiwan Ocean University, Keelung, Taiwan.
- Lin, Y.H., Shiau, S.Y., 2005. Dietary vitamin E requirement of grouper *Epinephelus malabaricus* at two lipid levels and their effects on immune responses. *Aquaculture* 248: 235–244.
- Liu, C.H., 2001. Early osteological development of the yellowtail *Seriola dumerili* (Pisces: Carangidae). *Zool. Stud.* 40: 289–298.
- Lobo, C., Moreno-Ventas, X., Tapia-Paniagua, S., Rodriguez, C., Moriñigo, M.A., García de la Banda, I., 2013. Enhancement of Senegalese sole (*Solea senegalensis*) larval and fry culture by Pdp11 *Shewanella putrefaciens* probiotic addition. *Fish Physiology and Biochemistry* (in press).
- López-Escalera, R., Morán, J., Pasantes-Morales, H., 1988. Taurine and nifedipine protect retinal rod outer segment structure altered by removal of divalent cations. *Journal of Neuroscience Research* 19: 491–496.
- Lovatelli, A., Holthus, P.F., 2008. Capture-based aquaculture; Global overview. Food and Agriculture Organization of the United Nations, Rome, 298 pp.
- Lubzens, E., Gibson, O., Zmora, O., Sukenik, A., 1995. Potential advantages of frozen algae (*Nannochloropsis* sp.) for rotifer (*Brachionus plicatilis*) culture. *Aquaculture* 133: 295–309.
- Lund, I., Skov, P.V., Hansen, B.W., 2012. Dietary supplementation of essential fatty acids in larval pikeperch (*Sander lucioperca*); short and long term effects on stress tolerance and metabolic physiology. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 162 (4): 340.
- Lund, I., Steinfeldt, S.J., 2011. The effects of dietary long-chain essential fatty acids on growth and stress tolerance in pikeperch larvae (*Sander lucioperca* L.). *Aquaculture Nutrition* 17: 191–199.
- Lunger, A.N., McLean, E., Gaylord, T.G., Kuhn, D., Craig, S.R., 2007. Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). *Aquaculture* 271: 401–410.
- Lupatsch, I., Katz, T., Angel, D., 2003. Assessment of the removal efficiency of fish farm effluents by grey mullets: a nutritional approach. *Aquaculture Research* 34: 1367–377.
- Ma, Z., Qin, J.G., Hutchinson, W., Chen, B.N., 2012. Food consumption and selectivity by larval yellowtail kingfish *Seriola lalandi* cultured at different live feed densities. *Aquaculture Nutrition*. doi: 10.1111/anu.12004.
- Magretta, J., 2002. Why business models matter. *Harvard business review*, 80 (5): 86–93.
- Mandiki, S.N.M., Blanchard, G., Mélard, C., Koskela, J., Kucharczyk, D., Fontaine, P., Kestemont, P., 2004. Effects of geographic origin on growth and food intake in Eurasian perch (*Perca fluviatilis* L.) juveniles under intensive culture conditions. *Aquaculture* 229: 117–128.
- Mandiki, S.N.M., Babiak, I., Krol, J., Rasolo, J.F.R., Kestemont, P., 2007. How initial predator-prey ratio affects intra-cohort cannibalism and growth in Eurasian perch *Perca fluviatilis* L larvae and juveniles under controlled conditions? *Aquaculture* 268: 149–155.
- Marino, G., Panini, E., Longobardi, A., Mandich, A., Finoia, M.G., Zohar, Y., Mylonas, C.C., 2003. Induction of ovulation in captive-reared dusky grouper, *Epinephelus marginatus* (Lowe, 1834) with a sustained-release GnRH<sub>a</sub> implant. *Aquaculture* 219: 841–858.



- Martínez-Llorens, S., Espert, J., Moya, J., Jover Cerdá, M., Tomás Vidal, A., 2011. Growth and nutrient efficiency of meagre (*Argyrosomus regius*, Asso 1801) fed extruded diets with different protein and lipid levels. *Int. J. Fish. Aquac.* 3(10): 195-203.
- Masuda, R., Takeuchi, T., Tsukamoto, K., Sato, H., Shimizu, K., Imaizumi, K., 1999. Incorporation of Dietary Docosahexaenoic Acid into the Central Nervous System of the Yellowtail *Seriola quinqueradiata*. *Brain Behav. Evol.* 53: 173–179.
- Matsunari, H., Arai, D., Koiso, M., Kuwada, H., Takahashi, T., Takeuchi, T., 2005. Effect of feeding rotifers enriched with taurine on growth performance and body composition of pacific cod larvae *Gadus macrocephalus*. *Aquac. Sci.* 53: 297–304.
- Matsunari, H., Hamada, K., Mushiake, K., Takeuchi, T., 2006. Effects of taurine levels in broodstock diet on reproductive performance of yellowtail *Seriola quinqueradiata*. *Fisheries Science* 72: 955–960.
- Matsunari, H., Hashimoto, H., Oda, K., Masuda, Y., Imaizumi, H., Teruya, K., Furuita, H., Yamamoto, T., Hamada, K., Mushiake, K. 2012. Effect of different algae used for enrichment of rotifers on growth, survival, and swim bladder inflation of larval amberjack *Seriola dumerili*. *Aquaculture International* 20 (5): 981-992.
- Matsunari, H., Hashimoto, H., Oda, K., Masuda, Y., Imaizumi, H., Teruya, K., Furuita, H., Yamamoto, T., Hamada, K., Mushiake, K., 2012. Effects of docosahexaenoic acid on growth, survival and swim bladder inflation of larval amberjack (*Seriola dumerili*, Risso). *Aqua. Res.* doi: 10.1111/j.1365-2109.2012.03174.x.
- Mazzola, A., Favalaro, E., Sara, G., 2000. Cultivation of the Mediterranean amberjack, *Seriola dumerili* (Risso, 1810), in submerged cages in the Western Mediterranean Sea. *Aquaculture* 181: 257-268.
- McGoogan, B.B., Gatlin, D.M. III, 1999. Dietary manipulations affecting growth and nitrogenous waste production of red drum, *Sciaenops ocellatus*. I. Effects of dietary protein and energy levels. *Aquaculture* 178 (3/4): 333-348.
- Merella, P., Cherchi, S., Garippa, G., Fioravanti, M.L., Gustinelli, A., Salati, F., 2009. Outbreak of *Sciaenacotyle panceri* (Monogenean) on cage-reared meagre *Argyrosomus regius* (Osteichthyes) from the western Mediterranean Sea. *Dis. Aquatic Organisms* 86: 169-73.
- Messenger, J.L., Ansquer, D., Metailler, R., J., Person-Le-Ruvet, J., 1986. Experimental induction of the "granulomatous hypertyrosinemia" of reared turbot (*Scophthalmus maximus*) with an ascorbic acid deficient diet. *Ichthyophysiological Acta* 10: 201-214.
- Micale, V., Maricchiolo, G., Genovese, L., 1999. The reproductive biology of the amberjack, *Seriola dumerilii* (Risso 1810). I. Oocyte development in captivity. *Aqua. Res.* 30: 349-355.
- Miki, T., Nakatsukasa, H., Takahashi, N., Murata, O., Ishibashi, Y., 2011. Aggressive behaviour and cannibalism in greater amberjack, *Seriola dumerili*: effects of stocking density, feeding conditions and size differences. *Aquaculture Research* 42: 1339–1349.
- Milstein, A., Alkon, A., Avnimelech, Y., Kochba, M., Hulata, G., Schroeder, G., 1991. Effects of manuring rate on ecology and fish performance in polyculture ponds. *Aquaculture* 96 (2): 119-138.
- Mittelholzer, C., Andersson, E., Taranger, G. L., Consten, D., Hirai, T., Senthilkumaran, B., Nagahama, Y., Norberg, B., 2009. Molecular characterization and quantification of the gonadotropin receptors FSH-R and LH-R form Atlantic cod (*Gadus morhua*). *Gen. Comp. Endocrinol.* 160 (1): 47-58.
- Monfort, M.C., 2010. Present market situation and prospects of meagre (*Argyrosomus regius*), as an emerging species in Mediterranean aquaculture, *Studies and Reviews, General Fisheries Commission for the Mediterranean* No. 89, FAO, Roma, pp. 28.
- Montero, D., Izquierdo, M.S., 2010. Welfare and health of fish fed vegetable oils as alternative lipid sources to fish oil. In: Turchini, G., Ng, W. and Tocher, D. (Eds.), *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*, CRC Press, Cambridge, UK, ISBN: 978-1-4398-0862-7, pp. 439-485.
- Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M.J., Izquierdo, M.S., 2003. Vegetable lipid sources for gilthead seabream (*Sparus aurata*): Effects on fish health. *Aquaculture* 225: 353-370.
- Montero, D., Marrero, M., Izquierdo, M.S., Robaina, L., Vergara, J.M., Tort, L., 1999. Effect of vitamin E and C dietary supplementation on some immune parameters of gilthead seabream (*Sparus aurata*) juveniles subjected to crowding stress. *Aquaculture* 171: 269-278.
- Montero, D., Tort, L., Izquierdo, M.S., Robaina, L., Vergara, J.M., 1998. Depletion of serum alternative complement pathway activity in gilthead seabream caused by alpha-tocopherol and n-3 HUFA dietary deficiencies. *Fish Physiology and Biochemistry* 18(4): 399-407.



- Montero, F.E., Crespo, S., Padrós, F., De la Gándara, F., García, A., Raga, J.A., 2004. Effects of the gill parasite *Zeuxapta seriolae* (Monogenea: Heteraxinidae) on the amberjack *Seriola dumerili* Risso (Teleostei: Carangidae). *Aquaculture* 232: 153–163.
- Morris, A.L., Hamlin, H.J., Francis-Floyd, R., Sheppard, B.J., Guillette, L.J. 2011. Nitrate-induced goiter in captive whitespotted bamboo sharks *Chiloscyllium plagiosum*. *Journal of Aquatic Animal Health* 23: 92–99.
- Mozes, N., Papandroulakis, N., Vergara, J.M, Biswas, A.K., Takii, K., Ntatsopoulos, A., 2011. Production systems. In Pavlides, M., Mylonas, C.C. (eds.), *Sparidae: Biology and Aquaculture*, Wiley-Blackwell, Oxford, 390 pp.
- Murashige, R., Bass, P., Wallace, L., Molnar, A., Eastham, B., Sato, V., Tamaru, C.S., Lee, C.-S., 1991. The effect of salinity on the survival and growth of striped mullet, *Mugil cephalus*, larvae in the hatchery. *Aquaculture* 96: 249-254.
- Mylonas, C.C., Bridges, C.R., Gordin, H., Belmonte Ríos, A., García, A., De la Gándara, F., Fauvel, C., Suquet, M., Medina, A., Papadaki, M., Heinisch, G., De Metrio, G., Corriero, A., Vassallo-Agius, R., Guzmán, J.M., Mañanos, E., Zohar, Y., 2007. Preparation and administration of gonadotropin-releasing hormone agonist (GnRHa) implants for the artificial control of reproductive maturation in captive-reared Atlantic bluefin tuna (*Thunnus thynnus thynnus*). *Rev. Fish. Sci.* 15: 183-210.
- Mylonas, C.C., Corriero, A., De la Gándara, F., Belmonte Ríos, A., 2010b. Atlantic bluefin tuna (*Thunnus thynnus*) farming and fattening in the Mediterranean Sea. *Rev. Fish. Sci.* 18: 266-280.
- Mylonas, C.C., Fostier, A., Zanuy, S., 2010a. Broodstock management and hormonal manipulations of reproduction. *Gen. Comp. Endocrinol.* 165: 516-534.
- Mylonas, C.C., Mitrizakis, N., Sigelaki, I., Papadaki, M., 2011. Spawning kinetics of individual female meagre (*Argyrosomus regius*) after treatment with GnRHa implants. *Indian Journal of Science and Technology*, 9th ISRPF Issue Vol. 4 No. 5, pp. 232-233.
- Mylonas, C.C., Papandroulakis, N., Smboukis, A., Papadaki, M., Divanach, P., 2004. Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. *Aquaculture* 237: 141-154.
- Mylonas, C.C., Sigelaki, I., Divanach, P., Mañanos, E., Carillo, M., Afonso-Polyviou, A., 2003. Multiple spawning and egg quality of individual European sea bass (*Dicentrarchus labrax*) females after repeated injections of GnRHa. *Aquaculture* 221: 605-620.
- Mylonas, C.C., Zohar, Y., 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. *Rev. Fish Biol. Fish.* 10: 463-491.
- Nakada, M., 2000. Yellowtail and related species culture. In: Stickney, R. (Ed.), *Encyclopedia of Aquaculture*, Wiley, pp. 1007–1036.
- Nash, C.E., Koningsberg, R.M., 1981. Artificial propagation. In: Oren, O.H. (ed.), *Aquaculture of Grey Mulletts*, Cambridge University Press, pp. 265-312.
- Nayak, S.K., 2010. Role of gastrointestinal microbiota in fish. *Aquacult. Res.* 41: 1553–1573.
- Nishiki, I., Noda, M., Itami, T., Yoshida, T. 2010. Homogeneity of *Streptococcus dysgalactiae* from farmed amberjack *Seriola dumerili* in Japan. *Fisheries Sci.* 76: 661.
- Norambuena, F., Mackenzie, S., Bell, J.G., Callol, A., Estévez, A., Duncan, N., 2012. Prostaglandin (F and E, 2- and 3-series) production and cyclooxygenase (COX-2) gene expression of wild and cultured broodstock of senegalese sole (*Solea senegalensis*). *General and Comparative Endocrinology* 177: 256–262.
- Norambuena, F., Morais, S., Estévez, A., Bell, J.G., Tocher, D.R., Navarro, J.C., Cerdà, J., Duncan, N., 2013. Dietary modulation of arachidonic acid metabolism in senegalese sole (*Solea Senegalensis*) broodstock reared in captivity. *Aquaculture* 372–375: 80–88.
- Norberg, B., 1995. Atlantic halibut (*Hippoglossus hippoglossus*) vitellogenin: induction, isolation and partial characterization. *Fish Physiology and Biochemistry* 14 (1): 1-13.
- Norberg, B., Valkner, V., Huse, J., Karlsen, I., Lerøy Grung, G., 1991. Ovulatory rhythms and egg viability in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* 97 (4): 365-371.
- Norberg, B., Weltzien, F.-A., Karlsen, Ø., Holm, J.C., 2001. Effects of photoperiod on sexual maturation and somatic growth in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Comp. Biochem. Physiol.* 129B (2-3): 357-366.
- Ohno, Y., Kawano, F., Hirazawa, N., 2008. Susceptibility by amberjack (*Seriola dumerili*), yellowtail (*S. quinqueradiata*) and Japanese flounder (*Paralichthys olivaceus*) to *Neobenedenia girellae* (Monogenea) infection and their acquired protection. *Aquaculture* 274: 30–35.





- Olsen, A.I., Attramadal, I., Jensen, A., Olsen, Y., 1999. Influence of size and nutritional value of *Artemia franciscana* on growth and quality of halibut larvae (*Hippoglossus hippoglossus*) during the live feed period. *Aquaculture* 179 (1-4): 475-487.
- Oren, O.H., 1981. *Aquaculture of Grey Mulletts*, Cambridge University Press, 506 pp.
- Ostaszewska, T., Dabrowski, K., Katarzyna, C., Wanda, O., Olejniczak, M., 2005. Rearing of pike-perch larvae using formulated diets – first success with starter feeds. *Aquaculture Res.* 36: 1167-1176.
- Osterwalder, A., Pigneur, Y., 2010. *Business model generations; a handbook for visionaries, game changers and challengers*, Lavoisier, Cachan, France ch 1.
- Panagiotidou, M., Chatzifotis, S., Papaioannou, N., Papisolomontos, S., Pavlidis, M., Nengas, I., Mylonas, C.C., 2007. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*). *Aquaculture Europe 2007*, Estambul, Turkey, 24-27 October.
- Panini, E., Mylonas, C.C., Zanuy, S., Carrillo, M., Ramos, J., Bruce, M., 2001. Incubation of embryos and larvae of marine fish using microtiter plates. *Aquacult. Int.* 9: 189-196.
- Papadakis, I., Kentouri, M., Divanach, P., Mylonas, C.C., 2013. Ontogeny of the digestive system of meagre *Argyrosomus regius* reared in a mesocosm, and quantitative changes of lipids in the liver from hatching to juvenile. *Aquaculture* (in press).
- Papadakis, I.E., Zaiss, M.M., Kyriakou, Y., Georgiou, G., Divanach, P., Mylonas, C.C., 2009. Histological evaluation of the elimination of *Artemia* nauplii from larval rearing protocols on the digestive system ontogeny of shi drum (*Umbrina cirrosa* L.). *Aquaculture* 286: 45-52.
- Papandroulakis, N., Divanach, P., Anastasiadis, P., Kentouri, M., 2002. The pseudo-green water technique for intensive rearing of sea bream (*Sparus aurata*) larvae. *Aquaculture International* 9 (3): 205-216.
- Papandroulakis, N., Kentouri, M., Maingot, E., Divanach, P., 2004. Mesocosm: a reliable technology for larval rearing of *Diplodus puntazzo* and *Diplodus sargus sargus*. *Aquacult. Int.* 12: 345-355.
- Papandroulakis, N., Mylonas, C.C., Maingot, E., Divanach, P., 2005. First results of greater amberjack (*Seriola dumerili*) larval rearing in mesocosm. *Aquaculture* 250: 155–161.
- Papandroulakis, N., Mylonas, C.C., Syggelaki, E., Katharios, P., Divanach, P., 2008. First reproduction of captive-reared wreckfish (*Polyprion americanus*) using GnRH $\alpha$  implants. *Aquaculture Europe 08*, September 15-18, Krakow, Poland, European Aquaculture Society Special Publication 37, pp. 507-508.
- Papandroulakis, N., Suquet, M., Spedicato, M.T., Machias, A., Fauvel, C., Divanach, P., 2004. Feeding rates, growth performance and gametogenesis of wreckfish (*Polyprion americanus*) kept in captivity. *Aquacult. Int.* 3: 1-13.
- Paperna, I., 1987. Systemic granuloma of sparid fish in culture. *Aquaculture* 67: 53-58.
- Peleteiro, J.B., others, others, others, 2010. First experiences with wreckfish culture (*Polyprion americanus*) in Galicia. Behaviour and ongrowing. *Proceedings of the Aquaculture Europe 2010*, Porto, Portugal.
- Peleteiro, J.B., Saavedra, C., Perez-Rial, E., Soares, E.C., Álvarez-Blázquez, B., Vila, A., 2011. Diversificación de especies en acuicultura marina. Desarrollo de técnicas de cultivo de la cherna (*Polyprion americanus*). XIII Congreso Nacional de Acuicultura, Castelldefels, Barcelona, Spain.
- Peng, L.I., Gatlin III, D.M., 2009. Dietary vitamin E requirement of the red drum *Sciaenops ocellatus*. *Aquaculture Nutrition* 15: 313–319.
- Peng, L.I., Wang, X., Gatlin III, D.M., 2008. *RRR*- $\alpha$ -Tocopheryl succinate is a less bioavailable source of vitamin E than *all-rac*- $\alpha$ -tocopheryl acetate for red drum, *Sciaenops ocellatus*. [Aquaculture](#) 280: 165–169.
- Pillay, T.V.R., 1993. *Aquaculture. Principles and Practices*. Fishing News Books, Oxford, UK, 575 pp.
- Pirozzi, I., Booth, M.A., 2009. The routine metabolic rate of mulloway (*Argyrosomus japonicus*: Sciaenidae) and yellowtail kingfish (*Seriola lalandi*: Carangidae) acclimated to six different temperatures. *Comp. Biochem. Physiol. A - Mol. Integr. Physiol.* 152: 586–592.
- Pirozzi, I., Booth, M.A., Allan, G.L., 2010a. A factorial approach to deriving dietary specifications and daily feed intake for mulloway, *Argyrosomus japonicus*, based on the requirements for digestible protein and energy. *Aquaculture* 302 (3-4): 235-242.
- Pirozzi, I., Booth, M.A., Allan G.L., 2010b. The interactive effects of dietary protein and energy on feed intake, growth and protein utilization of juvenile mulloway (*Argyrosomus japonicus*). *Aquaculture Nutrition* 16: 61-71.



- Pirozzi, I., Booth, M.A., Pankhurst, P.M., 2009. The effect of stocking density and repeated handling on the growth of juvenile mulloway, *Argyrosomus japonicus* (Temminck & Schlegel 1843). *Aquaculture Int.* 17: 199-205.
- Poli, B.M., Parisi, G., Zampacavallo, G., Lurzan, F., Mecatti, M., Lupi, P., Bonelli, A., 2003. Preliminary results on quality and quality changes in reared meagre (*Argyrosomus regius*): body and fillet traits and freshness changes in refrigerated commercial-size fish. *Aquac. Int.* 11: 301–311.
- Porter, M., 1985. *Competitive advantage; creating and sustaining superior performance*, Free Press, London, UK.
- Porter, M., 1998. *Competitive advantage of nations*, MacMillan press, London, UK.
- Pottinger, T.G., Carrick, T.R., 1999a. A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout. *Aquaculture* 175: 351–363
- Pottinger, T.G., Carrick, T.R., 1999b. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. *General and Comparative Endocrinology* 116: 122–132.
- Poulet, N., Balaesque, P., Aho, T., Bjorklund, M., 2009. Genetic structure and dynamics of a small introduced population: the pikeperch, *Sander lucioperca*, in the Rhône delta. *Genetica* 135: 77-86.
- Pruett, S.B., 2003. Stress and the immune system. *Physiopathology* 9: 133-153.
- Quémener, L., Suquet, M., Mero, D., Gaignon, J.-L., 2002. Selection method of new candidates for finfish aquaculture: the case of the French Atlantic, the Channel and the North Sea coasts. *Aquatic Living Resources* 15: 293-302.
- Ray, A.K., Ghosh, K., Ringø, E. 2012. Enzyme-producing bacteria isolated from fish gut: a review. *Aquaculture Nutrition* 18: 465-492.
- Rekecki, A., Dierckens, K., Laureau, S., Boon, N., Bossier, P., van den Broeck, W., 2009. Effect of germ-free rearing environment on gut development of larval sea bass (*Dicentrarchus labrax* L.). *Aquaculture* 293: 8-15.
- Rekecki, A., Gunasekera, RAYSA, Dierckens, K., Laureau, S., Boon, N., Favoreel, H., Cornelissen, M., Sorgeloos, P., Ducatelle, R., Bossier, P., van den Broeck, W., 2012. Bacterial host interaction of GFP-labelled *Vibrio anguillarum* HI-610 with gnotobiotic sea bass *Dicentrarchus labrax* (L.) larvae. *Journal of Fish Diseases* 35: 265-273.
- Rekecki, Am, Ringø, E., Olsen, R., Myklebust, R., Dierckens, K., Bergh, Ø., Laureau, S., Cornelissen, M., Ducatelle, R., Decostere, A., Bossier, P., Van den Brock, W., 2013. Luminal uptake of *Vibrio (Listonella) anguillarum* by shed enterocytes – a novel early defense strategy in larval fish. *Journal of Fish Diseases* 36: 419-426.
- Ren, T., Koshio, S., Teshima, S., Ishikawa, M., Panganiban, A., Uyan, O., Alam, M.S., 2008. Effectiveness of l-ascorbyl-2-monophosphate Na/Ca as a vitamin C source for yellowtail *Seriola quinqueradiata* juveniles. *Aquaculture Nutrition* 14: 416–422.
- Ribeiro, A.R.A., Ribeiro, L., Sæle, Ø., Hamre, K., Dinis, M.T., Moren, M., 2011. Iodine-enriched rotifers and *Artemia* prevent goitre in Senegalese sole (*Solea senegalensis*) larvae reared in a recirculation system. *Aquaculture Nutrition* 17: 248–257.
- Rigos, G., Katharios, P., 2010. Pathological obstacles of newly-introduced fish species in Mediterranean mariculture; a review. *Reviews in Fish Biology and Fisheries* 20: 47-70.
- Rigos, G., Pavlides, M., Divanach, P., 2001. Host susceptibility to *Cryptocaryon sp.* infection of Mediterranean marine broodfish held under intensive culture conditions: a case report. *Bulletin of the European Association of Fish Pathologists* 21: 33-36.
- Robaina, L., Izquierdo, M.S., Moyano, F.J., Socorro, J., Vergara, J.M., Montero, D., Fernández-Palacios, H. 1995. Soybean and lupin seed meals as protein sources in diets for gilthead sea bream (*Sparus aurata*). Nutritional and histological implications. *Aquaculture*, 130: 219-233.
- Robaina, L., Rodríguez-Lozano, A., Domínguez-Montesdeoca, D., Hernández-Cruz, C.M., Romero, J., 2013 (in press). Effect of the different dietary levels of vitamin E on the growth, fish composition, fillet quality and liver histology of on-growing meagre (*Argyrosomus regius*). *Aquaculture* (in press).
- Rocha, R.J., Ribeiro, L., Costa, R., Dinis, M.-J., 2008. Does the presence of microalgae influence fish larvae prey capture? *Aquaculture Research* 39: 362-369.
- Rodríguez, C., Perez, J.A., Diaz, M., Izquierdo, M.S., Fernandez-Palacios, H., 1997. Influence of the EPA/DHA ratio in rotifers on gilthead seabream (*Sparus aurata*) larval development. *Aquaculture* 150: 77-89.
- Rodríguez, C., Perez, J.A., Badia, P., Izquierdo, M.S., Fernandez-Palacios, H., Lorenzo Hernandez, A., 1998.



- The n-3 highly unsaturated fatty acids requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/EPA ratio in the diet. *Aquaculture* 169: 9-23.
- Rodríguez, J.L., Vega, E., Linares, F., 2011. Design and application of a recirculation aquaculture system for marine fish larvae rearing. *Actas World aquaculture, Aquaculture for a changing world*, Natal, Brasil.
- Rodríguez, J.L., Linares, F., Pazos, G., Soto, N., 2010. Larval culture of senegal sole, *Solea senegalensis*, in closed and open circuit. *Aquaculture Europe 2010: Seafarming Tomorrow*. European Aquaculture Society Publication, pp. 170.
- Rodríguez-Barreto, D., Jerez, S., Cejas, J.R., Martín, M.V., Acosta, N.G., Bolaños, A., Lorenzo, A., 2012. Comparative study of lipid and fatty acid composition in different tissues of wild and cultured female broodstock of greater amberjack (*Seriola dumerili*). *Aquaculture* 360–361: 1–9.
- Rodríguez-Villanueva, J.L., Peleteiro, J.B., Perez-Rial, E., Soares, E.C., Álvarez-Blázquez, B., Mariño, C., Linares, F., Mañanós, E., 2011. Growth of wreckfish (*Polyprion americanus*) in Galicia, Spain. *Aquaculture Europe 2011 (EAS)*, 18-21 October, Rhodes, Greece.
- Roiha Sunde, I., Samuelsen, O.B., Harboe, T., 2011. Efficacy of florfenicol in the treatment of bacterial infections in halibut, *Hippoglossus hippoglossus*, larvae. *Journal of Fish Diseases*. doi:10.1111/j.1365-2761.2011.01307.x.
- Rollin, F., Kennedy Nielsen, N., Smit, J., Wills, G.P., Guillen, J., 2011. Consumers and new food technologies. *Trends 2009. Market Integration of Fish in Food Science & Technology* 22: 99-111.
- Ronteltap, A., van Trijp, J.C., Renes, R.J., Frewer, L.J., 2007. Consumer acceptance of technology-based food innovations: lessons for the future of nutrigenomics. *Appetite* 49: 1-17.
- Roo, J., Fernández-Palacios, H., Hernández-Cruz, C.M., Mesa-Rodríguez, A., Schuchardt, D., Izquierdo, M., 2013 (in press). First results of spawning and larval rearing of longfin yellowtail *Seriola rivoliana* as a fast-growing candidate for European marine finfish aquaculture diversification. *Aquaculture Research*. doi: 10.1111/are.12007.
- Roo, J., Grossa, E., Schuchardt, D., Socorro, J., Hernández-Cruz, C.M., Izquierdo, M.S., Fernández-Palacios, H., 2010b. Potential of almako jack *Seriola rivoliana* as a fast-growing species for European aquaculture diversification. *Aquaculture Europe*, Porto, Portugal.
- Roo, J., Hernández-Cruz, C.M., Borrero, C., Fernández-Palacios, H., Schuchardt, D., 2007. Effect of rearing density and feeding regime in the larval rearing of meagre (*Argyrosomus regius*, Asso, 1801) during the first month. *XI Congreso Nacional de Acuicultura*, Vigo, Spain.
- Roo, F. J., Hernández-Cruz, C.M., Borrero, C., Schuchardt, D., Fernandez-Palacios, H., 2010a. Effect of larval density and feeding sequence on meagre (*Argyrosomus regius*; Asso, 1801) larval rearing. *Aquaculture* 302: 82-88. doi: 10.1016/j.aquaculture.2010.02.015.
- Roo, F.J., Hernández-Cruz, C.M., Fernández-Palacios, H., Schuchardt, D., Izquierdo, M.S., 2009. Effect of rearing system intensiveness on biological features, culture performance and larval quality of meagre (*Argyrosomus regius* Asso, 1801) larvae. *Europ. Aquacul. Soc. Spec. Publ.* 38: 371-374.
- Rosenfeld, H., Zlatnikov, V., Meiri-Ashkenazi, I., 2011. Fish gonadotropin agonists: applications in assisted reproductive technologies. *Indian Journal of Science and Technology*, 9th ISRPF Issue Vol. 4 No. S, pp. 272.
- Rosenlund, G., Corraze, G., Izquierdo, M.S., Torstensen, B., 2010. The effects of fish oil replacement on nutritional and organoleptic qualities of farmed fish. In: Turchini, G., Ng, W. and Tocher, D. (Eds.), *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*, CRC Press, Cambridge, UK, ISBN: 978-1-4398-0862-7, pp. 487-522.
- Ryan, J., 2004. Farming the deep blue. In: Mills, G., Maguire, D. (eds.), *Marine Inst.*, Dublin, Ireland, pp. 67.
- Sabate, F.S., Sakakura, Y., Shiozaki, M., Hagiwara, A., 2009. Onset and development of aggressive behavior in the early life stages of the seven-band grouper *Epinephelus septemfasciatus*. *Aquaculture* 290: 97-103.
- Saisa, M., Saliminen, M., Koljonen, M.L., Ruuhilarvi, J., 2010. Coastal and freshwater pikeperch (*Sander lucioperca*) populations differ genetically in the Baltic Sea basin. *Hereditas* 147: 205–214.
- Sakai, T., Murata, H., Endo, M., Shimomura, T., Yamauchi, K., Ito, T., Yamaguchi, T., Nakajima, H., Fukudome, M., 1998. Severe oxidative stress is thought to be a principal cause of jaundice of yellowtail *Seriola quinqueradiata*. *Aquaculture* 160 (3-4): 205–214.
- Sales, J., 2009. The effect of fish meal replacement by soya bean products on fish growth: A meta-analysis. *The British Journal of Nutrition* 102: 1709-22.
- Saliminen, M., Koljonen, M.L., Saisa, M., Ruuhilarvi, J., 2011. Genetic effects of supportive stockings on native pikeperch populations in boreal lakes – three cases, three different outcomes. *Hereditas* 149: 1–15.



- Sandel, E., Uni, Z., Koven, W., 2010. The effect of different dietary ratios of Phosphatidylcholine and Phosphatidylinositol fed to the gilthead sea bream (*Sparus aurata*) larvae on larvae and juvenile performance. *Aquaculture* 304: 42-48.
- Sandlund, N., Rodseth, O.M., Knappskog, D.H., Fiksdal, I.U., Bergh, Ø., 2010. Comparative susceptibility of turbot, halibut and cod yolk-sac larvae to challenge with *Vibrio* spp. *Diseases of Aquatic Organisms* 89 (1): 29-37.
- Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999. Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* 179: 217–229.
- Sarker, P.K., Fukada, H., Masumoto, T., 2009. Phosphorus availability from inorganic phosphorus sources in yellowtail (*Seriola quinqueradiata* Temminck and Schlegel). *Aquaculture* 289 (1-2): 113-117.
- Savoie, A., Le François, N.R., Cahu, C., Blier, P.U., Andreassen, I., 2006. Do protein hydrolysates improve survival and growth of newly-hatched spotted wolffish (*Anarhichas minor*), a non-metamorphic aquaculture fish species? *Aquaculture* 261: 782-788.
- Scabini, V., Roo, J., Hernández-Cruz, C.M., Schuchardt, D., Borrero, C., Fenrnández-Palacios, H., 2008. Effect of larval density and feeding sequence on meagre (*Argyrosomus regius* Asso 1801) larval rearing. XIII International Symposium on Fish Nutrition and Feeding, Florianopolis, Brasil.
- Schreck, C.B., Contreras-Sanchez, W., Fitzpatrick, M.S., 2001. Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture* 197: 3-24.
- Sealey, W.M., Gatlin, D.M., 2002. Dietary vitamin C and vitamin E interact to influence growth and tissue composition of juvenile hybrid striped bass (*Morone chrysops* x *M. saxatilis*) but have limited effects on immune responses. *J. Nutr.* 132: 748-755.
- Sedberry, G.R., Andrade, C.A.P., Carlin, J.L., Chapman, R.W., Luckhurst, B.E., Manooch, C.S. III, Menezes, G., Thomsen, B., Ulrich, G.F., 1999. Wreckfish *Polyprion americanus* in the North Atlantic: fisheries, biology and management of a widely distributed and long-lived fish. American Fisheries Society Symposium. Life in slow lane: ecology and conservation of long lived marine animals, nº23, pp. 27-50.
- Segner, H., Sundh, H., Buchmann, K., Douxfils, J., Sundell, K.S., Mathieu, C., Ruane, N., Jutfelt, F., Toften, H., Vaughan, L., 2012. Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. *Fish Physiology and Biochemistry* 38: 85-105.
- Shimeno, S., 1991. Yellowtail, *Seriola quinqueradiata*. In: Wilson, R.P. (Ed.), *Handbook of Nutrient Requirements of Finfish*, CRC Press, Boca Raton, FL, pp.181-191.
- Sorbera, L.A., Mylonas, C.C., Zanuy, S., Carillo, M., Zohar, Y., 1996. Sustained administration of GnRH $\alpha$  increases milt volume without altering sperm counts in the sea bass. *J. Exp. Zool.* 276: 361-368.
- Sonne, A.-M., Grunert, K.G., Olsen, N.V., Granli, B.-S., Szabó, E., Banati, D., 2012. Consumers' perceptions of HPP and PEF food products. *British Food Europe Journal* 114: 85 –107.
- Soula, M., Zamorano, M. J., Navarro, A., Sánchez, J.J., Neil, D., Alejandro, G., Afonso, J.M., 2011. Diseño de dos nuevas PCRs múltiplex para corvina (*Argyrosomus regius*). XIII Congreso Nacional Acuicultura, Book of Abstracts, Universitat Politècnica de Catalunya ESAB-Castelldefels, Barcelona, España, 21-24 de Noviembre de 2012 (In Spanish, abstract in English).
- STECF, 2012. Economic performance of the EU Aquaculture Sector, European Commission, Joint Research Centre, Ispra, Italy.
- Steenfeldt, S.J., Lund, I., 2008. Development of methods of production for intensive rearing of pikeperch juveniles. DTU Aqua Research Report no. 199-2008, Technical University of Denmark, Denmark (in Danish).
- Steenfeldt, S., Lund, I., Höglund, E., 2010a. Is batch variability in hatching time related to size heterogeneity and cannibalism in pikeperch (*Sander lucioperca*)? *Aquac. Res.* 42(5): 727-732.
- Steenfeldt, S.J., Vestergaard, M., Overton, J.L., Lund, I., Paulsen, H., Larsen, V.J., Henriksen, N.H., 2010b. Further development of intensive pikeperch rearing in Denmark. DTU Aqua Research Report no. 228-2010, Technical University of Denmark, Denmark (in Danish).
- Stephen, S.J., Savage, A., 2010. Two mortality events in sea-caged yellowtail kingfish *Seriola lalandi* Valenciennes, 1833 (Nannoperidae) from Western Australia. *Australian Vet J.* 88: 414-6.
- Stuart, K.R., Drawbridge, M., 2011. The effect of light intensity and green water on survival and growth of cultured larval California yellowtail (*Seriola lalandi*). *Aquaculture* 321: 152-156.
- Suquet, M., La Pomèlie, Ch., 2002. Le cernier (*Polyprion americanus*): biologie, pêche, marché et potentiel aquacole. Plouzané: IFREMER, cop. 2002. 279 h. (Ressources de la mer). ISBN 2-84433-075-4.





- Szkudlarek, M., Zakęś, Z., 2011. Effect of stocking density on survival and growth performance of pikeperch. Czech J. Anim. Sci. 56 (11): 483–489.
- Takagi, S., Murata, H., Goto, T., Endo, M., Yamashita, H., Ukawa, M., 2008. Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate. Aquaculture 280: 198–205.
- Takakuwa, F., Fukada, H., Hosokawa, H., Masumoto, T., 2006. Optimum digestible protein and energy levels and ratio for greater amberjack *Seriola dumerili* (Risso) fingerling. Aquaculture Research 37: 1532–1539.
- Takeuchi, T., 2001. A review of feed development for early life stages of marine finfish in Japan. Aquaculture 200: 203–222.
- Talbot, C., Garcia-Gomez, A., De la Gandara, F., Muraccioli, P., 2000. Food intake, growth and body composition in Mediterranean yellowtail (*Seriola dumerilii*) fed isonitrogenous diets containing different lipid levels. Cah. Opt. Mediterr. 47: 259–266.
- Tamaru, C.S., Ako, H., Lee, C.S., 1992. Fatty acid and amino acid profiles of spawned eggs of striped mullet, *Mugil cephalus* L. Aquaculture 105: 83-94.
- Tapia-Paniagua, S., Lobo, C., García de la Banda, I., Balebona, M.C., Moriñigo, M.A., 2011. Impact of the intestinal microbiota species of Senegalese sole larvae and fries fed with *Shewanella putrefaciens* Pdp11 probiotic bioencapsulated in *Artemia*, effect of one or two intervals of application. Abstracts of the European Aquaculture Meeting, Rhodes (Greece), 622-623.
- Tapia-Paniagua, S.T., Díaz-Rosales, P., León-Rubio, J.M., García de la Banda, I., Lobo, C., Alarcón, F.J., Chabrilón, M., Rosas-Ledesma, P., Varela, J.L., Ruíz-Jarabo, I., Mancera, J.M., 2012. Use of the probiotic *S. putrefaciens* Pdp11 on the culture of Senegalese sole (*Solea senegalensis*, Kaup 1858) and gilthead seabream (*Sparus aurata*, L.). Aquacult. Int., <http://dx.doi.org/10.1007/s10499-012-9509-5>
- Teletchea, F., Fontaine, P., 2011. Particularities of early life stages in temperate freshwater fish species: comparisons with marine species and implications for aquaculture practices. Aquaculture Res. 42 (5): 630-654.
- Teletchea, F., Fontaine, P., 2012 (in press). Levels of domestication in fish: implications for the sustainable future of aquaculture. Fish and Fisheries (in press).
- Teletchea, F., Fostier, A., Kamler, E., Gardeur, J.-N., Le Bail, P.-Y., Jalabert, B., Fontaine, P., 2009b. Comparative analysis of reproductive traits in 65 freshwater fish species: application to the domestication of new fish species. Rev. Fish Biol. Fish. 19: 403-430.
- Teletchea, F., Gardeur, J.-N., Psenicka, M., Kaspar, V., Le Doré, Y., Linhart, O., Fontaine, P., 2009a. Effects of four factors on the quality of male reproductive cycle in pikeperch *Sander lucioperca*. Aquaculture 291 (3-4): 217-223.
- Ternengo, S., Agostini, S., Quilichini, Y., Euzet, L., Marchand, B., 2010. Intensive infestations of *Sciaenocotyle panzerii* (Monogenea, Microcotylidae) on *Argyrosomus regius* (Asso) under fish-farming conditions. Journal of Fish Diseases 33: 89–92.
- Thompson, B.A., Beasley, M., Wilson, C.A., 1999. Age distribution and growth of greater amberjack *Seriola dumerili*, from the north-central Gulf of Mexico. Fish. Bull. 97: 362-371.
- Thorsen, A., Kjesbu, O.S., 2001. A rapid method for estimation of oocyte size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. Journal of Sea Research 46 (3-4): 295-308.
- Tocher D.R., Bendiksen, E.Å., Campbell, P.J., Bell, J.G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture 280: 21-34.
- Tocher, D.R., Mourent, G., Van Der Eecken, A., Evjemo, J.O., Diaz, E., Wille, M., Bell, J.G., Olsen, Y., 2003. Comparative study of antioxidant defense mechanisms in marine fish fed variable levels of oxidized oil and vitamin E. Aquaculture International 11: 195–216.
- Toksen, E., Buchmann, K., Bresciani, J., 2007. Occurrence of *Benedenia sciaenae* van Beneden, 1856 (Monogenea: Capsalidae) in cultured meagre (*Argyrosomus regius* Asso, 1801) (Teleost: Sciaenidae) from western Turkey. Bull. Eur. Ass. Fish Pathol. 27(6): 250.
- Tovar, D., Zambonino, J., Cahu, C., Gatesoupe, F.J., Vazquez-Juarez, R., Lésel, R., 2002. Effect of live yeast incorporation in compound diet on enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 204: 113-123.
- Trabelsi, A., Gardeur, J.-N., Teletchea, F., Brun-Bellut, J., Fontaine, P., 2011. Hatching time effect on the intra-spawning larval morphology and growth in Northern pike (*Esox lucius* L.). Aquaculture Research (in press, doi :10.1111/j ;1365-2109-2011.03070x).



- Trabelsi, A., Gardeur, J.-N., Teletchea, F., Fontaine, P., 2011. Multifactorial analysis of effects of nutritional, environmental and populational variables on burbot *Lota lota* weaning performances. *Aquaculture* 316 (1-4): 104-110.
- Uyan, O., Koshio, S., Ishikawa, M., Yokoyama, S., Uyan, S., Ren, T., Hernandez, L.H.H., 2009. The influence of dietary phospholipid level on the performances of juvenile amberjack, *Seriola dumerili*, fed non-fishmeal diets. *Aquaculture Nutrition* 15 (5): 550-557.
- Vadstein, O., Bergh, Ø., Gatesoupe, F.-J., Galindo-Villegas, J., Mulero, V., Picchetti, S., Scapiglatti, G., Makridis, P., Olsen, Y., Dierckens, K., Defoirdt, T., Boon, N., De Schryver, P., Bossier, P., 2013 (in press). Microbiology and immunology of fish larvae. *Reviews in Aquaculture* (in press).
- Vallés, R., Estévez, A., 2013. Light conditions for larval rearing of meagre (*Argyrosomus regius*). *Aquaculture* 376-379: 15-19.
- Van der Meeren, T., Mangor-Jensen, A., Pickova, J., 2007. The effect of green water and light intensity on survival, growth and lipid composition in Atlantic cod (*Gadus morhua*) during intensive larval rearing. *Aquaculture* 265(1): 206-217.
- Van der Meeren, T., Olsen, R.E., Hamre, K., Fyhn, H.J., 2008. Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. *Aquaculture* 274: 375–397.
- Vassallo-Agius, R., Watanabe, T., Imaizumi, H., Yamazaki, T., 2002. Spawning performance of yellowtail *Seriola quinqueradiata* fed dry pellets containing paprika and squid meal. *Fish. Sci.* 68: 230–232.
- Vassallo Agius, R., Watanabe, T., Satoh, S., Kiron, V., Imaizumi, H., Yamazaki, T., Kawano, K., 2001. Supplementation of paprika as a carotenoid source in soft-dry pellets for broodstock yellowtail *Seriola quinqueradiata* (Temminck & Schlegel). *Aquaculture Research* 32: 263–272.
- Verakunpiriya, V., Mushiake, K., Kawano, K., Watanabe, T., 1997a. Supplemental effect of astaxanthin in broodstock diets on the quality of yellowtail eggs. *Fish. Sci.* 63: 816–823.
- Verakunpiriya, V., Watanabe, K., Mushiake, K., Kawano, K., Kobayashi, T., Hasegawa, I., Kiron, V., Satoh, S., Watanabe, T., 1997b. Effect of krill meal supplementation in soft-dry pellets on spawning and quality of egg of yellowtail. *Fish. Sci.* 63: 433–439.
- Verbeek, P., Iwamoto, T., Murakami, N., 2008. Variable stress-responsiveness in wild type and domesticated fighting fish. *Physiol Behav.* 93 (1-2): 83-88.
- Vermeirssen, E.L.M., Mazorra de Quero, C., Shields, R.J., Norberg, B., Kime, D.E., Scott, A.P., 2004. Fertility and motility of sperm from Atlantic halibut (*Hippoglossus hippoglossus*) in relation to dose and timing of gonadotropin-releasing hormone agonist implant. *Aquaculture* 230: 547-567.
- Vidal, A.T., De la Gandara, F., García, A., Jover, M., 2008. Effect of the protein/energy ratio on the growth of Mediterranean yellowtail (*Seriola dumerili*). *Aquaculture Research* 39 (11): 1141-1148.
- Villalta, M., Estévez, A., Bransden, M.P., Bell, J.G., 2007. Arachidonic acid, arachidonic/eicosapentaenoic acid ratio, stearidonic acid and eicosanoids are involved in dietary-induced albinism in Senegal sole (*Solea senegalensis*). *Aquaculture Nutrition* 13: 1-9.
- Waagbø, R., 2006. Feeding and disease resistance in fish. In: Mosenthin, R., Zentec, J., Zebrowska, T. (Eds), *Biology of nutrition in growing animals*, London, Elsevier, pp. 387-415.
- Walton, M.J., Cowey, C.B., Adron J.W., 1984. The effect of dietary lysine levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). *Br. J. Nutr.* 52: 115-122.
- Wang N., Gardeur J.-N., Henrotte E., Marie M., Kestemont P., Fontaine P., 2006. Determinism of the induction of the reproductive cycle in female Eurasian Perch, *Perca fluviatilis*: effects of environmental cues and modulating factors. *Aquaculture* 261: 706-714.
- Wang, N., Milla, S., Fontaine, P., Kestemont, P., 2008. Abstracts of the Percid fish culture workshop: From research to production, January 23-24, Namur, Belgium.
- Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. *Aquaculture* 124: 223–251.
- Watanabe, T., Izquierdo, M.S., Takeuchi, T., Satoh, S.Y., Kitajima, C., 1989. Comparison between eicosapentaenoic and docosahexaenoic acids in terms of essential fatty acid efficacy in larval Red Sea bream (Nippon Suisan Gakkaishi). *Bull. Japan. Soc. Scien. Fish.* 55 (9): 1635 -1640.
- Watanabe, K., Ura, K., Yada, T., Kiron, V., Satoh, S., Watanabe, T., 2000. Energy and protein requirements of yellowtail for maximum growth and maintenance of body weight. *Fisheries Science* 66: 1053-1061.
- Whitfield, A.K., Panfill, J., Durnd, J.-D., 2012. A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex. *Reviews in Fish Biology and Fisheries* 22 (3): 641-681.



- Wilson, R.P., 2003. Amino acid requirements of finfish and crustaceans. In: D'Mello, J.P.F. (Ed.), *Amino Acids in Animal Nutrition*, CAB International, Wallingford, Oxon, United Kingdom, pp. 427–447.
- Wilson, R.P., J.E. Halver. 1986. Protein and amino acid requirements of fishes. *Annual Reviews of Nutrition* 6: 225-244.
- Wilson-Leedy J.G., Ingermann, R.L., 2006. Development of a novel CASA system based on open source software for characterization of zebrafish sperm motility parameters, *Theriogenology*, doi:10.1016/j.theriogenology.2006.10.003.
- Wirth, T., Robert, S.L., Louis, B., 1999. Isolation and characterization of microsatellite loci in the walleye (*Stizostedion vitreum*) and cross-species amplification within the family Percidae. *Molecular Ecology* 8: 1957-1969.
- Woolley, L.D., Jones, C.L.W., Britz, P.J., 2010. Effect of dietary protein to energy ratio on growth and nitrogenous waste production of cultured dusky kob *Argyrosomus japonicus*. *African Journal of Marine Science* 32(3): 625–631
- Yamamoto, T., Teruya, K., Hara, T., Hokazono, H., Hashimoto, H., Suzuki, N., Iwashita, Y., Matsunari, H., Furuita, H., Mushiake, K., 2008. Nutritional evaluation of live food organisms and commercial dry feeds used for seed production of amberjack *Seriola dumerili*. *Fisheries Science* 74: 1096–1108.
- Yamamoto, T., Teruya, K., Hara, T., Hokazono, H., Kai, I., 2009. Nutritional evaluation of rotifers in rearing tanks without water exchange during seed production of amberjack *Seriola dumerili*. *Fisheries Science* 75: 697-709.
- Yaron, Z., Bogomolnaya, A., Drori, S., Biton, I., Aizen, J., Kulikovsky, Z., Levavi-Sivan, B., 2009. Spawning induction in the carp: past experience and future prospects - a review. *Israeli Journal of Aquaculture – Bamidgeh* 61 (1): 5-26.
- Yashouv, A., 1969. Preliminary report on induced spawning of *M. cephalus* (L.) reared in captivity in fresh water ponds. *Bamidgeh* 21: 19–24.
- Yokogoshi, H. and Oda, H., 2002. Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. *Amino Acids* 23: 433-439.
- Yokoyama, M., Takeuchi, T., Park, G.S., Nakazoe, J., 2001. Hepatic cysteinesulphinatase decarboxylase activity in fish. *Aquac. Res.* 32: 216–220.
- Yoshimatsu, T., Hayashi, M., Toda, K., Furuichi, M., Kitajima, C., 1995. Preliminary experiment on the requirement of larval redlip mullet (*Mugil cephalus*) for essential fatty acids and the supplemental effect of *Nannochloropsis* to rearing water. *Nippon Suisan Gakkaishi* 61: 912-918.
- Zaiss, M.M., Papadakis, I.E., Maingot, E., Divanach, P., Mylonas, C., 2006. Ontogeny of the digestive tract in shi drum (*Umbrina cirrosa* L.) reared using the mesocosm larval rearing system. *Aquaculture* 260: 357–368.
- Zambonino Infante, J.L., Cahu, C., 2001. Ontogeny of gastrointestinal tract of marine fish larvae. *Comp. Biochem. Physiol. C* 130: 477-487.
- Zarski, D., Kucharczyk, D., Targonska, K., Palinska, K., Kupren, K., Fontaine, P., Kestemont, P., 2012. A new classification of pre-ovulatory oocyte maturation stages in pikeperch, *Sander lucioperca* (L.), and its application during artificial reproduction. *Aquaculture Research* 43: 713–721.
- Zhan, A., Wang, Y., Brown, B., Wang, H.P., 2009. Isolation and characterization of novel microsatellite markers for yellow perch (*Perca flavescens*). *Int. J. Mol. Sc.* 10: 18-27.
- Zohar, Y., Harel, M., Hassin, S., Tandler, A., 1995. Gilt-Head Sea Bream (*Sparus aurata*). In: Bromage, N.R. and Roberts R.J. (Eds.), *Broodstock Management and Egg and Larval Quality*, Blackwell Science, Oxford, UK, pp. 94-117.
- Zouiten, D., Ben Khemis, I., Besbes, R., Cahu, C., 2008. Ontogeny of the digestive tract of thick lipped grey mullet (*Chelon labrosus*) larvae reared in mesocosms. *Aquaculture* 279: 166-172.
- Zouiten, D., Masmoudi, A.S., El Abed, A., Helal, A.N., Ben khemis, I., 2004. Co-feeding and early weaning of the European sea bass (*Dicentrarchus labrax*) under semi-extensive conditions in "mesocosms" in Tunisian winter geoclimatic context. *Biologia Marina Mediterranea* 11(2): 754-757.
- Zuberi A., Ali S., Brown C. A., 2011. Non-invasive assay for monitoring stress responses: A comparison between wild and captive-reared rainbowfish (*Melanoteania duboulayi*). *Aquaculture* 321: 267-272.



## ***ANNEX 1 . List of relevant projects currently implemented by Partners***

*Recommendation 25. A list with relevant national and international projects in which the participants of DIVERSIFY are involved should be provided. The main objectives of these projects and the role of the DIVERSIFY participants should be indicated. Means for creating synergies with relevant on-growing projects should be proposed. Any potential risk of duplication with previous and/or on-going projects should be indicated and avoided.*

### **Partner: 1. HCMR**

#### **Project n.1**

**Project acronym:** Kranios

**Full title of the project:** Development of reproduction and rearing methods for meagre (*Argyrosomus regius*) as a measure for increasing competitiveness of the aquaculture by introducing new species

**Funding Agency:** National, General Secretariat of Research and Technology, Ministry of Education and Lifelong learning

**Funding amount to partner:** 318000€

**Main objective:** Study the life cycle of meagre including evaluation of the final product

**Role/Activity:** Coordinator / Involved in different tasks

**Means of creating synergies with DIVERSIFY:** KRANIOS is ending on February 2014. As a result no direct synergies with DIVERSIFY will be possible. However, this project has provided a substantial amount of knowledge, on which DIVERSIFY builds on. For example, the study of reproduction and the development of spawning induction methods, enables the reliable production of eggs and larvae for the nutritional experiments planned for DIVERSIFY. Also, the health problems to be investigated in DIVERSIFY were identified first in the course of the experiments of KRANIOS

**Potential risk for duplication:** NONE

#### **Project n.2**

**Project acronym:** Kripis

**Full title of the project:** Marine Biology, Biotechnology and Aquaculture Section3: Diversification of the Aquaculture production with new fast growing species (the greater amberjack)

**Funding Agency:** National, General Secretariat of Research and Technology, Ministry of Education and Lifelong learning

**Funding amount to partner:** 727245€

**Main objective:** Study of the critical stages for the development of the amberjack aquaculture including:

**Reproduction:** environmental control, development of spawning methods in tanks and cages; **Larval rearing:** improving rearing methods in terms of environmental parameters; **Nutrition:** definition of requirements at larval stages, improve palatability and acceptance of weaning diets, define optimum protein/energy and protein/lipid levels for on growing diets; **Genomics:** total genome expression and genome sequencing; **On growing:** optimize rearing methods in terms of density and volume of cages; **Pathology:** study of epitheliocystis and parasites of the species; **Final product** evaluation: filet composition, organoleptic evaluation

**Role/Activity:** Coordinator / Involved in different tasks

**Means of creating synergies with DIVERSIFY:** This project is expected to have a great number of synergies with DIVERSIFY. Especially, as this project covers the development of infrastructures, it will allow the completion of the facilities necessary to maintain the broodstock, larvae and juveniles in cages at HCMR. These facilities will be also used for DIVERSIFY experiments, which are different than the ones planned for KRHPIS. Specific examples are reported below:

**Reproduction:** KRIPIS will pay for the completion of the environmental control system (photoperiod and temperature) to maintain broodstock in closed recirculation systems. It will also cover the development of an egg collection system for cages. The cost of this (20,000) has been now removed from DIVERSIFY. The





study of environmental parameters during **larval rearing** performed in DIVERSIFY and in particular the hydrodynamics work will be performed at FCPCT. In the frame of KRIPIS a similar study will be performed at HCMR. It is expected that the results will be complementary and will facilitate the better understanding of the studied parameters. The **nutritional** studies planned in KRIPIS will provide additional and complementary information by determining different nutrient requirements at different fish age and size in order to develop appropriate diets for the amberjack. In particular while in DIVERSIFY the nutritional value (PUFAs, AA, vitamins, minerals, carotenoids) will be studied, in KRIPIS the palatability and acceptability characteristics of the diet will be the main target (texture analysis, moisture content, binders, extrusion conditions and use of attractants). Furthermore some studies performed in DIVERSIFY without the participation of HCMR will be complemented with the studies planned in KRIPIS as well. Information obtained from the **genomics** study of KRHPIS could potentially serve the various studies on the species planned in DIVERSIFY, where no such studies have been planned. Studies for **on growing** planned in the two projects are complementary (pilot scale in KRIPIS, industrial scale in DIVERSIFY) creating significant synergies. **Pathological** studies of KRIPIS will include preliminary studies on screening/identifying the parasitic fauna of greater amberjack in Greek farms. This work will complement the studies in DIVERSIFY, which are focusing on developing prevention methods (e.g., epitheliocystis). Planned studies on **final product** evaluation in the two projects are complementary. In KRIPIS, organoleptic analysis will be limited to difference test, i.e. whether untrained taste panelist can detect potential difference among fish from different diets. Chemical evaluation of quality includes fatty acids and volatile compounds of these specific dietary groups. On the other hand, the organoleptic analysis in DIVERSIFY includes analytical descriptive analysis. Analytical descriptive analysis is a complicated and time consuming method that includes a) long training of panelists in order to create a group of “specialists” that will evolve a specific vocabulary in order to analyse – describe all organoleptic differences. Chemical analysis will include fillet composition and fatty acids of the species in order to compare with the other species, correlate this quality with technological ability of the fish (i.e. how processing will take place) and to outline what marketing potentials the different species have (e.g commercializing differently fatty species from non fatty species etc)

**Potential risk for duplication:** A potential overlapping for the development of spawning methods in cages is addressed by a reduction in HCMR budget in DIVERSIFY of 20.000 € in WP2.2.5 (Reproduction). The study on the light conditions during larval rearing is planned both in DIVERSIFY and KRIPIS but with different objectives-evaluation methods. It is proposed that the larval rearing trials will serve both projects for the required samples. With this synergy the HCMR budget in DIVERSIFY will be reduced by an additional amount of 20.000 € for WP 4.2.3 (Larval husbandry)



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**Partner: 2. FCPCT**

**Project n.1**

**Project acronym:** Proinmunoil

**Full title of the project:** Complementos dieteticos en la utilizacion de lipidos alternativos al aceite de pescado en acuicultura marina: herramienta para maximizar el potencial de resistencia a patogenos: Proinmunoil. AGL2012-39919

**Funding Agency:** Spanish Ministry of Economy and Competitiveness

**Funding amount to partner:** 165000€

**Main objective:** 1) To check dietary complements to increase the gut health of sea bass when using alternative ingredients to fish meal and oil; 2) To check strategies of the use of those nutritional strategies; 3) To increase the knowledge of gut health of European sea bass

**Role/Activity:** Project Coordinator / 1) Formulation and analysis of the diet; 2) Biomarkers of gut health; 3) Protocols for challenge test with pathogens; 4) Knowledge on the enhancement of intestine mucus production

**Means of creating synergies with DIVERSIFY:** The increase of knowledge on health mechanisms of European seabass, the determination of health bio-indicators, the determination of the enhancement of mucus production could help to understand similar processes in the species proposed in the present project

**Potential risk for duplication:** NONE

**Project n.2**

**Project acronym:** MBS (2013)

**Full title of the project:** Estación de Bio-seguridad (biosecurity station)

**Funding Agency:** Spanish Ministry of Economy and Competitiveness through innplanta projects

**Funding amount to partner:** 442515€

**Main objective:** Starting and implementation of a Bio-security station

**Role/Activity:** Project Coordinator / Biosecurity station to conduct challenge test against different pathogens

**Means of creating synergies with DIVERSIFY:** The results permit to use an important tool to make challenge test for DIVERSIFY

**Potential risk for duplication:** NONE

**Project n.3**

**Project acronym:** Sadcomp (2013)

**Full title of the project:** Sistema de adquisicion de datos de comportamiento y control del bienestar en instalaciones acucolas (Sadcomp).

**Funding Agency:** Spanish Ministry of Economy and Competitiveness through innplanta projects

**Funding amount to partner:** 162100€

**Main objective:** Starting and implementation of a welfare control system

**Role/Activity:** Project Coordinator / Station of control and monitoring welfare of aquaculture fish

**Means of creating synergies with DIVERSIFY:** The results will permit the increase of welfare for the species studied in DIVERSIFY

**Potential risk for duplication:** NONE

**Project n.4**

**Project acronym:** Arraina

**Full title of the project:** Research initiatives for nutrition and aquaculture: Arraina

**Funding Agency:** EU (7<sup>th</sup> Framework Program)

**Funding amount to partner:** 450000€

**Main objective:** 1) To determine new strategies on the use of dietary ingredients alternative to fish meal and oil; 2) To increase the knowledge on nutrition and health relationship; 3) To determine biomarkers to be used in nutritional strategies

**Role/Activity:** Partner, leader of WP, leader of tasks / 1) Formulation and analysis of the diet; 2) Biomarkers of fish health; 3) Determining new insights for fish metabolism and nutrition and health relationship; 4) New strategies to promote larval quality; 5) New strategies to increase spawning quality



**Means of creating synergies with DIVERSIFY:** The increase of knowledge on metabolic, health and larval development mechanisms of the target species (European seabass & gilthead sea bream), the determination of bio-indicators, can help to understand similar processes in the species proposed in DIVERSIFY

**Potential risk for duplication:** NONE

#### **Project n.5**

**Project acronym:** Aquatrans (finishing in 2013)

**Full title of the project:** Cooperacion transfronteriza para el desarrollo sostenible de la acuicultura. Aquatrans 2a

**Funding Agency:** EU through “Programa Operativo de Cooperación Transfronteriza España Fronteras Exteriores”

**Funding amount to partner:** 795666€

**Main objective:** 1) Implementation of techniques for meagre culture in African coast (among other objectives related with international cooperation); 2) Transfer of knowledge

**Role/Activity:** Project Coordinator / 1) On-growing; 2) Culture techniques; 3) Reproduction ; 4) Dietary formulation; 5) Transfer of knowledge; 6) Training and educational programs

**Means of creating synergies with DIVERSIFY:** The results of this project can help to understand specific points to be studied in DIVERSIFY

**Potential risk for duplication:** NONE

#### **Project n.6**

**Project acronym:** Emcria (2011-2013)

**Full title of the project:** Estacion de estabulacion, monitorizacion y control de reproductores de especies de interes acuícola (Emcria)

**Funding Agency:** Spanish Ministry of Economy and Competitivity through innplanta projects

**Funding amount to partner:** 111349€

**Main objective:** Starting and implementation of a broodstock plant

**Role/Activity:** Project Coordinator / Station and facilities for fish reproduction

**Means of creating synergies with DIVERSIFY:** Facilities necessary to keep and broodstock of the species studied in DIVERSIFY

**Potential risk for duplication:** NONE

#### **Project n.7**

**Project acronym:** Metcser (2011-2013)

**Full title of the project:** Mejora de las técnicas de cría de larvas de (*Seriola rivoliana*): determinación de requerimientos de ácidos grasos esenciales en su etapa larvaria y optimización de la secuencia alimentaria (Metcser)

**Funding Agency:** Canary Islands government

**Funding amount to partner:** 35000€

**Main objective:** 1) Improvement of larval rearing techniques of *Seriola rivoliana*; 2) Determination of requirements of essential fatty acids; 3) Feeding strategies to improve larval culture

**Role/Activity:** Project Coordinator / 1) Diet formulation; 2) Larval rearing techniques; 3) Analysis; 4) Biochemical studies

**Means of creating synergies with DIVERSIFY:** To increase the knowledge of species similar to those included in DIVERSIFY

**Potential risk for duplication:** NONE

#### **Project n.8**

**Project acronym:** Aquaexcell

**Full title of the project:** Aquaculture infrastructures for excellence in European fish research. Aquaexcell

**Funding Agency:** EU FP7-infrastructures-2010-1

**Funding amount to partner:** 397454€

**Main objective:** Implementation of aquaculture facilities and integration of a network of excellence

**Role/Activity:** Partner, WP leader / 1) Improvement of infrastructures; 2) Improvement of techniques

**Means of creating synergies with DIVERSIFY:** Facilities and methodologies to be used with the rearing of the different species studied in DIVERSIFY



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**Potential risk for duplication:** NONE



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**Partner: 3. IRTA - NO PROJECTS RELATED TO DIVERSIFY**

**Partner: 4. IOLR**

**Project n.1**

**Project acronym:**

**Full title of the project:** Improving grey mullet stocking success in Lake Kineret & evaluating survival and growth performance of wild and cultured stocks via genetic markers

**Funding Agency:** Israel Ministry of Agriculture 2011-2014 (894-0186-11)

**Funding amount to partner:** 150000\$

**Main objective:** 1) Establishing genetic diversity of grey mullet using random amplified polymorphic DNA markers; 2) Evaluating grey mullet stocking success in Lake Kineret (Israel)

**Role/Activity:** Coordinator

**Means of creating synergies with DIVERSIFY:** There are no direct implications, yet the developed DNA markers may have long term contribution, upon implementation of sound genetic improvement programs for this species

**Potential risk for duplication:** NONE



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**Partner: 5. UNIABDN****Project n.1****Project acronym:** TargetFish**Full title of the project:** Prevention of important diseases of farmed fish species**Funding Agency:** EC**Funding amount to partner:** 317208€**Main objective:** To reduce the impact fish diseases are having on the European aquaculture industry through the development of targeted vaccination strategies in carp, trout, salmon, turbot, seabass and seabream**Role/Activity:** Partner / Mechanisms of disease resistance in vaccinated salmonids**Means of creating synergies with DIVERSIFY:** Information on salmonid immune genes and assays will inform studies in Diversify**Potential risk for duplication:** NONE**Project n.2****Project acronym:****Full title of the project:** Prophylactic measures in rainbow trout aquaculture: Further development of a DNA vaccine for proliferative kidney disease**Funding Agency:** BBSRC**Funding amount to partner:** 230467£**Main objective:** To determine the protective efficacy of DNA vaccines against PKD**Role/Activity:** Principal Investigator / Development of a vaccine to PKD in trout**Means of creating synergies with DIVERSIFY:** Knowledge of approaches to fish vaccine development will be of use in new species**Potential risk for duplication:** NONE**Project n.3****Project acronym:****Full title of the project:** Biochemical characterisation of the translocation process of RxLR-like effector proteins via tyrosine-O-sulphate modified cell surface receptors**Funding Agency:** BBSRC**Funding amount to partner:** 361065£**Main objective:** To dissect the molecular mechanisms that enable pathogenic oomycetes to translocate their effector repertoire into host cells, thus enabling a successful infection and to use this information to develop alternative control methods**Role/Activity:** Co-Principal Investigator / Understanding infection processes in *Saprolegnia* sp.**Means of creating synergies with DIVERSIFY:** Knowledge of how fish are infected by diseases will be of use in new species**Potential risk for duplication:** NONE**Project n.4****Project acronym:****Full title of the project:** Development of a non lethal sampling method to monitor immune response and disease progression in salmonid fish**Funding Agency:** NC3Rs**Funding amount to partner:** 178715£**Main objective:** 1) To develop a novel experimental design to monitor immune response and evaluate virulence in fish based on a non-lethal sampling method; 2) To develop an experimental design that will contribute to a reduction in the number of individuals used in fish experimentation**Role/Activity:** Co-Principal Investigator / As in the title**Means of creating synergies with DIVERSIFY:** Knowledge of how best to sample fish to monitor immunity will be of use in new species**Potential risk for duplication:** NONE



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**Partner: 6. LEI**

**Project n.1**

**Project acronym:**

**Full title of the project:** Fish on demand

**Funding Agency:** Ministry of Economic Affairs

**Funding amount to partner:** 50000€

**Main objective:** Market development for a relatively new aquaculture fish species (another species than the species in this project)

**Role/Activity:** Project Leader / Data mining, quality advise, (international) market analysis (Germany, Austria, Poland, Hungary and Czech Republic)

**Means of creating synergies with DIVERSIFY:** Methodology of market analysis

**Potential risk for duplication:** NONE

**Project n.2**

**Project acronym:**

**Full title of the project:** Market intelligence animal products

**Funding Agency:** Ministry of Economic Affairs

**Funding amount to partner:** 426000€

**Main objective:** Providing market intelligence for all animal sectors including fish

**Role/Activity:** Project Leader / Data mining and consumer research

**Means of creating synergies with DIVERSIFY:** Methodology of market analysis

**Potential risk for duplication:** NONE



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**Partner: 7. IMR**

**Project n.1**

**Project acronym:** KveiteCop

**Full title of the project:** Copepods in first feeding of Atlantic halibut

**Funding Agency:** Nord-Trøndelag County council

**Funding amount to partner:** 58000€

**Main objective:** 1) Large scale production of eggs from the copepode Temora; 2) Use of Temora as supplement feed for halibut larvae

**Role/Activity:** Participant / Facilities and expertise for experimental feeding of halibut larvae

**Means of creating synergies with DIVERSIFY:** Increased knowledge in halibut larval nutrition and general experience in larval husbandry

**Potential risk for duplication:** NONE

**Project n.2**

**Project acronym:** All female halibut

**Full title of the project:** Large scale production of all female halibut

**Funding Agency:** Sterling White Halibut

**Funding amount to partner:** 269000€

**Main objective:** Large scale production of all female halibut juveniles

**Role/Activity:** Project Coordinator / Experimental work, diet formulation, histology, hormone assays

**Means of creating synergies with DIVERSIFY:** General experience in larval husbandry

**Potential risk for duplication:** NONE





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**Partner: 8. IEO**

**Project n.1**

**Project acronym:** Rodagen

**Full title of the project:** Determinación del efecto de la temperatura de cultivo sobre la proporción de sexos en las progenies de rodaballo

**Funding Agency:** Ministerio de Economía y Competitividad

**Funding amount to partner:** 60500 €

**Main objective:** Control sex differentiation in Turbot

**Role/Activity:** Coordinator / Involved in different tasks

**Means of creating synergies with DIVERSIFY:** Fish reproduction

**Potential risk for duplication:** NONE

**Project n.2**

**Project acronym:**

**Full title of the project:** Sustainable diets for Senegalese sole (*Solea senegalensis*)

**Funding Agency:** Regional Government of Galicia

**Funding amount to partner:** 31066 €

**Main objective:** Assessment of the feasibility of macroalgae as feed ingredients for Senegalese sole

**Role/Activity:** Task leader / 1) Culture in recirculation system of *Solea senegalensis*; 2) Biochemical analyses

**Means of creating synergies with DIVERSIFY:** Progress obtained could benefit nutrition research of species included in DIVERSIFY project

**Potential risk for duplication:** NONE

**Project n.3**

**Project acronym:** Umbrina

**Full title of the project:** Study of environmental and nutritional factors on larval rearing and on-growing of shi drum, *Umbrina cirrosa*

**Funding Agency:** Fundación Séneca – Regional government of Murcia

**Funding amount to partner:** 43635€

**Main objective:** 1) Environmental needs of larvae, fry and/or juvenile; 2) Optimal culture and feed conditions

**Role/Activity:** Project Coordinator / 1) Larval culture; 2) Skeletal malformations; 3) Nutritional requirements; 4) Determination of digestive and antioxidant enzymes

**Means of creating synergies with DIVERSIFY:** Same methodologies will be applied to determine malformations and oxidative status

**Potential risk for duplication:** NONE

**Project n.4**

**Project acronym:** Contacui

**Full title of the project:** Environmental contaminants: effect on humoral immune response and hormone levels in gilthead sea bream

**Funding Agency:** Spanish government (MICINN), National projects

**Funding amount to partner:** 49278€

**Main objective:** 1) Potential effect on the production of *Sparus aurata* of exposure to Tamoxifen (TMX) and 17 $\alpha$ -ethynylestradiol (EE<sub>2</sub>), common environmental contaminants which act as endocrine disruptors; 2) Study the effects of TMX and the combination of TMX and EE<sub>2</sub> on the immune response and reproduction; 3) Identify the mechanisms of action of estrogenic and androgenic substances and plasmatic parameters and/or levels of gene expression which might be used as markers of estrogenic or androgenic contamination of the water

**Role/Activity:** Participant / 1) Functional analysis of the humoral immune response; 2) Experiment of exposure to contaminants and assess of reproductive parameters; 3) Analysis of plasmatic parameters

**Means of creating synergies with DIVERSIFY:** Same methodologies will be applied to assess the humoral immune responses

**Potential risk for duplication:** NONE




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**Partners 9 to 14: NO PROJECTS RELATED TO DIVERSIFY**
**Partner: 15.ULL**
**Project n.1**
**Project acronym:**

**Full title of the project:** Fish meal and fish oil substitution by vegetable oils in *Seriola dumerili* diets. Analytical study: lipids (Ref.: AGL2011-30547-C03)

**Funding Agency:** Spanish Government: Ministry of Education and Science (CICYT)

**Funding amount to partner:** 24200€

**Main objective:** Determine the effects of different replacement levels of fish meal and fish oil by vegetable sources on growth parameters, body composition, nutrient utilization and retention, and health status

**Role/Activity:** Project Coordinator / 1) Diet formulation; 2) Analytical studies of diets and fish samples; 3) Study of dietary lipids and fatty acids incorporation to fish tissues; 4) Analysis of plasmatic indicators of fish welfare

**Means of creating synergies with DIVERSIFY:** This project provides technical and preliminary information that will be essential and very useful for the setup and achievement of tasks proposed in DIVERSIFY, specifically with nutrition aspects of greater amberjack

**Potential risk for duplication:** NONE

**Project n.2**
**Project acronym:**

**Full title of the project:** Assessment on the essential fatty acid requirements and carotenoids of *Seriola dumerili* broodstock (Ref.: AGL2008-05014.C02 (CICYT)/ PI2007/016 (ACIISI))

**Funding Agency:** Spanish Government: Ministry of Education and Science (CICYT) and Canary Island Government (ACISII)

**Funding amount to partner:** 78650€ (CICYT) and 30000€ (ACIISI)

**Objectives:** 1) Obtain information to formulate a diet more suitable for *Seriola* broodstock; 2) Test and assess the nutritional efficiency of the experimental diet

**Role/Activity:** Project Coordinator / 1) Analytical studies, comparison of the biochemical composition of ovary, muscle, and liver from wild and cultured (G1 fed a non-specific diet) specimens; 2) Diet formulation; 3) Analytical studies, comparison of the fatty acid profiles of animals fed the new experimental diet in contrast to the non-specific diet

**Means of creating synergies with DIVERSIFY:** This project provides technical and preliminary information that will be essential and very useful for the setup and achievement of tasks proposed in DIVERSIFY, specifically with aspects of nutrition in greater amberjack broodstock

**Potential risk for duplication:** NONE




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**Partner: 16. FUNDP - NO PROJECTS RELATED TO DIVERSIFY**

**Partner: 17. NIFES**

**Project n.1****Project acronym:** CODE**Full title of the project:** Cod development**Funding Agency:** Research Council of Norway**Funding amount to partner:** 430000€**Main objective:** 1) Gain a deeper understanding of developmental processes in cod and effects of environmental factors (nutrition, temperature); 2) Identify mechanisms underlying differences in larval quality; 3) Recommend improved rearing protocols**Role/Activity:** Second in charge of consortium / In charge of activity on lipid metabolism, bone development and redox balance in cod larvae**Means of creating synergies with DIVERSIFY:** Similar basic knowledge on larval development and nutrition**Potential risk for duplication:** NONE**Project n.2****Project acronym:** Leppeprod**Full title of the project:** Produksjon av Berggylt**Funding Agency:** The Norwegian Seafood Research Fund (FHF)**Funding amount to partner:** 650000€**Main objective:** 1) Establish the knowledge needed for formulation of diets (larval, grow out, broodstock); 2) Feeding regimes for Ballan wrasse**Role/Activity:** Partner / In charge of development of ongrowing and broodstock diets, diets for rotifers and investigation of gut physiology and absorption and metabolism**Means of creating synergies with DIVERSIFY:** The project has developed an approach to take new species into aquaculture, which one could take advantage of in Diversify**Potential risk for duplication:** NONE**Project n.3****Project acronym:** Arraina**Full title of the project:** Advanced Research Initiatives for Nutrition & Aquaculture**Funding Agency:** EU, FP7**Funding amount to partner:** 615000€**Main objective:** Updating nutrient requirements in European commercial fish species in view of the changes in feed ingredients**Role/Activity:** Partner and leader of WP3 / Determining nutrient requirements. In charge of multivariate nutrient requirement studies in salmon juveniles and smolts and nutrient metabolism and epigenetic effects of feeding plant based diets during the life cycle of Atlantic salmon**Means of creating synergies with DIVERSIFY:** Similar designs are used in some of the tasks in DIVERSIFY**Potential risk for duplication:** NONE



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## Partner: 18. CTAQUA

### Project n.1

**Project acronym:** Biomass

**Full title of the project:** Total biomass estimation in off-shore fish production facilities

**Funding Agency:** Ministry of Environment, marine and rural environment. Spain

**Funding amount to partner:** 124996€

**Main objective:** 1) Implement a combined system of eco-sound and video for biomass estimation; 2) Promote technological development to implement the system in cage farms; 3) Improve production management; 4) Improve financial management in fish farms; 5) Increase control on escapees

**Role/Activity:** Scientific and technological responsible (RTO) / Involved in different tasks

**Means of creating synergies with DIVERSIFY:** Most of DIVERSIFY species are farmed in cages; once the grow-out phase is controlled, biomass estimation will be very useful for feed management, escapees and excess of nutrient leakage to the environment

**Potential risk for duplication:** NONE

### Project n.2

**Project acronym:** SumergI+Dos

**Full title of the project:** Development of IMTA (integrated multitrophic aquaculture) systems using advance submarine technology

**Funding Agency:** CDTI (Centre for industrial and technological development), Spain, and EU (European Regional Development Funds)

**Funding amount to partner:** 495942€

**Main objective:** 1) Integration of different trophic levels in aqua production; 2) Improve cage fish farming environmental sustainability; 3) Improve quality, efficiency and safety of underwater jobs; 4) Develop and test a new submarine vessel for underwater operations in off-shore farms; 5) Develop new and more compact equipment for underwater operations

**Role/Activity:** Scientific and technological organization (RTO) / Involved in different tasks

**Means of creating synergies with DIVERSIFY:** As mentioned for Biomass project, the results of this project will have a direct application for the farming of the DIVERSIFY species

**Potential risk for duplication:** NONE

### Project n.3

**Project acronym:** ReTCETEC

**Full title of the project:** Transboundary Cooperation among technology centres from Andalusia and Morocco to foster business and entrepreneurship

**Funding Agency:** Cross Border Cooperation Programme Spain-External Borders (POCTEFEX)

**Funding amount to partner:** 180625€

**Main objective:** 1) To promote cross-border cooperation in technology transfer between Technological Centres and enterprises in both territories; 2) Promote the Spanish-Moroccan cooperation for development of new products, methods and processes obtained as a result of technological cooperation; 3) Disseminate experiences and good practices in the field of cross-border cooperation to technological innovation system agents in both territories; 4) Support the creation of a cooperation network among Technological Center in both territories

**Role/Activity:** Scientific and technological organization (RTO) / Involved in different tasks

**Means of creating synergies with DIVERSIFY:** As a result of this project, we have identified suitable areas for aquaculture farming in the Mediterranean coast of Morocco in which to implement new production projects related to the development of new species, such as the ones of DIVERSIFY

**Potential risk for duplication:** NONE



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**Partner: 19. CMRM****Project n.1****Project acronym:** IMTA**Full title of the project:** Integrated Multitrophic aquaculture**Funding Agency:** Regional Government of Galicia**Funding amount to partner:** 30636€**Main objective:** 1) Develop pilot scale IMTA systems in the open sea; 2) Validate the use of *Sacharina latissima* as a biofilter**Role/Activity:** Project Coordinator / 1) Culture in cages of blackspot seabream (*Pagellus bogaraveo*); 2) Biochemical analyses related with the experiments of the project**Means of creating synergies with DIVERSIFY:** IMTA is an option for diversification in aquaculture and some of the species included in Diversify could be cultured in an IMTA system**Potential risk for duplication:** NONE**Project n.2****Project acronym:****Full title of the project:** Sustainable diets for Senegalese sole (*Solea senegalensis*)**Funding Agency:** Regional Government of Galicia**Funding amount to partner:** 31066€**Main objective:** 1) Assessment of the feasibility of macroalgae as feed ingredients for Senegalese sole: influence on growth, nutritional, immunological and sensory value of the fish; 2) Influence of fish oil substitution by vegetable oils on growth, immune status, stress response, gene expression and sensory value of Senegalese sole**Role/Activity:** Project Coordinator / 1) Culture in recirculation system (RAS) of Senegalese sole (*Solea senegalensis*); 2) Biochemical analyses related with the experiments of the project**Means of creating synergies with DIVERSIFY:** Progress in achieving sustainable Senegalese sole diets can be very useful in the nutrition of some species included in DIVERSIFY project**Potential risk for duplication:** NONE



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**Partner: 22. SWH**

**Project n.1**

**Project acronym:** KveiteCop

**Full title of the project:** Copepods in first feeding of Atlantic halibut

**Funding Agency:** Nord-Trøndelag County council

**Funding amount to partner:** 58000€

**Main objective:** 1) Large scale production of eggs from the copepode Temora; 2) Use of Temora as supplement feed for halibut larvae

**Role/Activity:** Participant / Involved in different activities

**Means of creating synergies with DIVERSIFY:** Increased knowledge in halibut larval nutrition and general experience in larval husbandry

**Potential risk for duplication:** NONE

**Project n.2**

**Project acronym:** All female halibut

**Full title of the project:** Large scale production of all female halibut

**Funding Agency:** Sterling White Halibut

**Funding amount to partner:** 269000€

**Main objective:** Large scale production of all female halibut juveniles

**Role/Activity:** Participant / Involved in different activities

**Means of creating synergies with DIVERSIFY:** General experience in larval husbandry

**Potential risk for duplication:** NONE

**Partners 21, 23-38 - NO RELEVANT PROJECTS**



## ANNEX 2. Table of bottlenecks addressed and the expected outputs

*Recommendation 26. A table indicating the bottlenecks addressed and the expected outputs for each species considered in DIVERSIFY should be provided.*

Meagre bottlenecks	Expected output
<ul style="list-style-type: none"> <li>- Limited genetic variation of current broodstocks</li> </ul>	<ul style="list-style-type: none"> <li>- Characterize genetic diversity in current domesticated meagre broodstocks.</li> <li>- Tools to establish genetic improvement programs that will enable the industry to control broodstock genetic variability.</li> <li>- Development of genetic markers for an assisted breeding program (SNP library).</li> <li>- Protocol for paired spontaneous tank spawning and <i>in vitro</i> fertilization procedures, sperm quality evaluations and stripping ovulated eggs.</li> </ul>
<ul style="list-style-type: none"> <li>- Variable growth rates during the pre-growing phase</li> </ul>	<ul style="list-style-type: none"> <li>- Improvement of current larval weaning feeds, protocols and feeding practices to reduce initial size variation.</li> <li>- Genetic characterisation of fast and slow growing meagre and provide the tools necessary to genetically improve growth and reduce size variability.</li> <li>- Grow-out improve rearing practices, particularly study cage feeding behaviour and improve cage feeding systems.</li> <li>- Determination of nutritional requirements to promote feed utilization, consistent growth rates and fish welfare to reduce size variation.</li> </ul>
<ul style="list-style-type: none"> <li>- Variable growth and not developed feeding methods during on-growing phase in cages</li> </ul>	<ul style="list-style-type: none"> <li>- Development of an appropriate feeding method that respects the specific behaviour of meagre.</li> <li>- Modification of existing methodologies for cage culture related to volume and light conditions in order to maximize the performance.</li> <li>- Development of specific diets.</li> </ul>
<ul style="list-style-type: none"> <li>- Fish health, emerging diseases and parasites</li> </ul>	<ul style="list-style-type: none"> <li>- Improvement of prophylactic measures or chemical treatments in culture systems for particularly parasites.</li> <li>- Evaluation of the causes (bacteria and/or nutritional) of systemic granulomatosis and screening of potential solutions.</li> <li>- Increased knowledge of meagre immune responses.</li> </ul>
<ul style="list-style-type: none"> <li>- Niche market in Europe</li> </ul>	<ul style="list-style-type: none"> <li>- New meagre product development</li> </ul>
Greater amberjack bottlenecks	Expected output



- Lack of reliable reproduction and of egg availability	- Description of normal and dysfunctional maturational development. - Development of an appropriate broodstock diet - Development of species-specific hormonal spawning induction protocols and solve the reproductive dysfunction. - Development of a methodology for collecting eggs in sea cages.
- Limited production of larvae and juvenile	- Improvement of larval rearing conditions (biotic and abiotic), protocols, enrichment products and on-growing diets. - Development of a larval rearing protocol for the industry. - Optimization of current feeding practices and cage rearing conditions during on-growing.
- Scarce information on nutritional requirements during grow-out	- Development of diets for grow-out in order to maximize growth potential and enhance fillet quality.
- Lack of specific husbandry methods	- Development of appropriate techniques for cage rearing by identify the adequate volume and density for the rearing. - Test the application of submersible cages. - Definition of species-specific thermal ranges.
- Fish health, emerging diseases and parasites	- Improvement of prophylactic measures or chemical treatments in culture systems. - Set-up of early diagnosis tools and veterinary solutions. - Increase the immune status of the animal by means of the diet. - Increased knowledge of amberjack immune response.
- Limited market size in Europe	- Introduction of a consumer-oriented market and development of a market strategy.
<b>Pikeperch bottlenecks</b>	<b>Expected output</b>
- Lack of knowledge of the genetic variability of current broodstocks and variable or unpredictable growth rate during grow-out.	- Characterize genetic diversity in current domesticated pikeperch broodstocks and the available wild genetic variability. - Provide the genetic tools that will enable the industry to control broodstock genetic variability.
- Low larval survival; high incidence of skeletal deformities	- Development of species-specific diets considering the nutritional requirements of the species. - Improvement of larval rearing conditions (biotic and abiotic) and protocols for diets, in order to reduce cannibalism and aggressive behaviour among fish.
- High sensitivity to stressors, handling and	- Reduction of stress sensitivity through the





husbandry practices, inducing diseases and sudden mortalities	improvement of husbandry practices, nutrition, environmental factors and increase of immune resistance.
- Limited market size in Europe	Product and market development.
<b>Atlantic halibut bottlenecks</b>	<b>Expected output</b>
- Irregular supply of fry particularly from F1/F2 broodstock; long production cycle	- Documentation of reproductive performance of wild and F1/F2 broodstock. - Improvement of reproductive performance of captive broodstocks (wild, domesticated F1/F2). - Optimization of hormonal spawning induction protocols.
- Nutrition during first feeding and after weaning	- Characterisation of diets suitable for early weaning. - Documentation of nutrient uptake in RAS vs FTS rearing systems. - Documentation of nutrient content in on-grown <i>Artemia</i> vs <i>Artemia nauplii</i> . - Description of the role of phospholipids in development and growth post-weaning.
- Unstable survival rates at early life stages due to bacterial infections	- Development of specific protocols to improve fry rearing/production (RAS vs FTS). - Development of industry-scale protocol for probiotic treatment of larvae. - Development of specific protocols for on-growing <i>Artemia</i> as live prey for larvae.
- Mortalities during early life stages due to VNN (nodavirus)	- Evaluate the effect of delivering recombinant capsid protein during late larval stages on protection to VNN.
- No real bottleneck in the short run but possible bottlenecks in the long run due to potential numerous new entrants in a high-added value market	- Development of market strategy for long term.
<b>Wreckfish bottlenecks</b>	<b>Expected output</b>
- Lack of reproduction control and egg availability	- Describe the reproductive cycle of wreckfish. - Development of species-specific hormonal spawning induction protocols. - Development of in vitro fertilization procedures, sperm quality and stripping ovulated eggs - Recommendations for feed formulation for broodstock diets. - Develop protocols to form new wreckfish broodstock.
- Lack of larval rearing protocols	- Determine the effect of rearing system (RAS, FTS and mesocosm) on larval production. - Develop species-specific larval rearing and feeding protocols.



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| - Lack of availability of cultured fish on market | - Identification of market position in relation to other species in the short run and identification of market potential in the long run. |
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<b>Grey mullet bottlenecks</b>	<b>Expected output</b>
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| - Lack of control of the reproductive cycle; low and irregular egg quality | <ul style="list-style-type: none"> <li>- Improvement of broodstock management and nutrition.</li> <li>- Describe the reproductive cycle and gamete quality of mullet.</li> <li>- Development of species-specific hormonal spawning induction protocols and photo-thermal regime for large-scale egg production.</li> <li>- Protocol for shipping mullet eggs.</li> </ul> |
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| - Lack of standardized larval rearing protocols | <ul style="list-style-type: none"> <li>- Determine the species-specific nutritional requirements on key nutrients (e.g. FA, taurine).</li> <li>- Develop and standardize larval rearing protocols at a commercial scale.</li> </ul> |
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| - Lack of an economically sustainable grow-out feeds | <ul style="list-style-type: none"> <li>- Develop feed formulations based on low or absent fishmeal levels.</li> <li>- Developmental of specific grow-out protocols for different European regions.</li> </ul> |
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| - Niche market in the Mediterranean | <ul style="list-style-type: none"> <li>- Development of market for the species and for high valued products such as roe ('bottarga' in Italian).</li> <li>- Culture protocols for the production of roe.</li> </ul> |
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<b>Socioeconomics (all species) bottlenecks</b>	<b>Expected output</b>
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| - Limited markets and diversification of provided products | - Identify external environmental factors that affect the production chains of the species considered in the proposal, exploring the market chances of new species and a report on current certification schemes and standards and their business dynamics in the fish supply chain. |
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| - More product demand than offer in the EU markets and EU consumer's negative attitude towards aquaculture fish and products | <ul style="list-style-type: none"> <li>- Perform international survey on industrial buyers' attitudes and perceptions regarding cultured fish.</li> <li>- detection of critical success factors for market acceptance.</li> </ul> |
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| <ul style="list-style-type: none"> <li>- Low demand for new aquaculture products</li> <li>- Low added value of the aquaculture products</li> <li>- Improve the sustainability of the industry</li> </ul> | <ul style="list-style-type: none"> <li>- Consumer based report a list of ideas for new product development.</li> <li>- Develop physical prototypes of new products from the selected species meagre, greater amberjack, pikeperch and grey mullet.</li> <li>- Provide dataset of consumers' perceptions, attitudes, buying intentions, consumption, and willingness to buy and pay, and value perceptions towards the selected species.</li> <li>- Evaluate the effect of communication stimulus and evaluation of their effectiveness in changing</li> </ul> |
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consumer's attitudes and behaviour towards the products coming from the selected species.

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