



Palau Macaya and University Pompeu Fabra,
Barcelona, Spain
17-19 January 2017

Minutes of the Annual Coordination Meeting 2017 (for Y4)

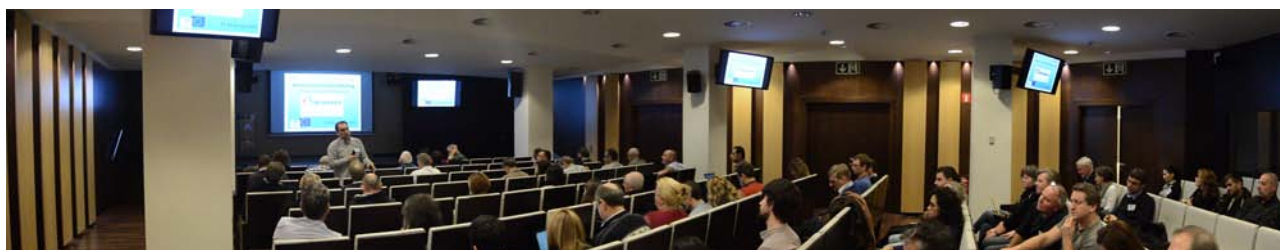


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Objectives

The objectives of the Annual Coordination Meeting (ACM) 2017 were to:

- (a) Present a large number of Task-specific results of the accomplished work during Y3 to the consortium members, as well as to a small number of invited guests,
- (b) Review and evaluate closely the work carried out in all Work Packages (WP) in the six Scientific Disciplines,
- (c) Plan the work to be implemented in all WPs in the following year,
- (d) Present the dissemination activities of the consortium (WP 31).

Description

The ACM 2017 was hosted by Dr. Alicia Estevez of the Instituto de Recerca y Tecnologia Agronomica (P3. IRTA) and was held at two venues between 17-19 January 2017. The task-specific presentations during Days 1 and 2 took place at Palau Macaya. The Group Work Package (GWP) workshops took place at the Campus Del Mar of the University Pompeu Fabra. In addition, a half-day meeting took place at the Hotel Ayre Rosellon on Friday 20 January 2017, for the participants of WP 30 Business model and marketing strategy development. The 3-day meeting was attended by 85 persons: 78 coming from the DIVERSIFY consortium and 8 invited guests from outside the consortium. No representative attended from three Beneficiaries (P26. GEI, P28. CANEXMAR and P37. EUFIC).

As for all previous ACMs, information regarding the meeting was uploaded continually on the project's web site (<http://www.diversifyfish.eu/2017-annual-coordination-meeting-jan.html>) to ensure that all participants had access to the most updated information. The Agenda (**Tables 1, 2 and 3**) was developed with assistance from GWP leaders and consisted of:

- (a) DAY 1 and 2: a common session for all participants (including invited guests) presenting Task-specific presentations from various WPs, and presentations from invited guests,
- (b) DAY 2: a presentation of the WP 31 Dissemination presenting the dissemination activities of the consortium, and organizing the preparation of Deliverables as well as of manuscripts for scientific articles, and
- (c) DAY 3: a common session dealing with Dissemination, Scientific and Financial Reporting, and Management.
- (d) In addition a brief meeting of WP 30 meeting was held on Friday 20 January

DAY 1 and 2 – Task presentations of implemented work and invited guests

The morning session started with a welcoming presentation (**Fig. 1**) by the Project Coordinator (PC), Dr. C.C. Mylonas, presenting the Agenda for the meeting, welcoming the invited guests from outside the consortium and explaining the intentions of the consortium (as presented in the DOW, WP1 Project Management) for including other scientists and stakeholders in these ACMs. Also, Dr Sergi Tudela, Director of Fisheries for the Catalonia government offered a welcoming. Dr. Tudela underlined the importance of DIVERSIFY for Spain and Catalonia, as the need for species diversification in the Mediterranean aquaculture has been recognized here as well.

The invited guests included Dr. Francesc Piferrer (Institute of Marine Sciences, CSIC, Barcelona, Spain), Dr. Francesc Padros (Autonomic University of Barcelona, Spain), Dr. Ignacio Gimenes (Rara Avis Biotech), Torre Remman (C-Feed S.A.), Dr Panos Christofilogiannis (AQUARK, S.A.), Mr Nigel Balmforth (5N Publishing), Mrs Rhiannon White (International Aqua Feed Magazine) and Mr. Javier Villa from a commercial aquaculture company (Andromeda SA from Greece/Spain).



Table 1. Agenda of DAY 1 of the Annual Coordination Meeting 2017, which took place on the 16-19 January 2017, at the Palau Macaya, Barcelona, Spain.

DAY 1		17-Jan	Tuesday (Open Day presentations)		
Start	End		Title	Presenter	Details
8.00	9.00		Registration		Pick up badges
9.00	9.30		Welcome-Logistics		Alicia Estevez & CC Mylonas
9.30	9.50		Welcome	Dr Sergi Tudela Casanovas	Director of Fisheries, Catalunya
9.50	10.10	1	Induced spawning of paired meagre with male rotation	Duncan, Neil	IRTA
10.10	10.30	2	Wreckfish reproduction status in Spain	Alvarez, Blanca	IEO
10.30	10.50	3	Some approaches to improve the nutrition and husbandry of DIVERSIFY's target species. A U La Laguna collaborative contribution	Rodriquez, Covadonga	ULL
10.50	11.30		Coffee		
11.30	11.50	4	Effect of background color and photophase on performance of larval greater amberjack and expression of genes related to the GH/IGF axis	Tsalafouta, Aleka	HCMR
11.50	12.10	5	Prospects for probiotics with Atlantic halibut larvae	Berg, Øivind	IMR
12.10	12.30	6	The effect of algal turbidity on larval performance and the ontogeny of digestive tract functionality in grey mullet	Koven, Bill	IOLR
12.30	12.50	7	Wreckfish ontogeny of the major organs related to feeding and digestion	Papadakis, Ioannis	HCMR
12.50	13.10	8	COLUMBUS Project – Knowledge Transfer for Blue Growth: Aquaculture knowledge outputs and case studie	Christofilogiannis, Panos	AQUARK (Invited)
13.10	13.30	9	Physical prototypes of new products from the selected DIVERSIFY species	Bou, Ricard and Robles, Rocio	IRTA/CTAQUA
13.30	15.00		Lunch		
15.00	15.20	10	Epigenetics in aquaculture	Piferrer, Francesc	ICM (Invited)
15.20	15.40	11	How can CFeed copepods help bring new marine species to the table	Remman, Tore	C-Feed (Invited)
15.40	16.00	12	Results on mullet grow out in farm conditions: a multi-partner trial	Robles, Rocio	CTAQUA
16.00	16.20	13	Parasitic infections in greater amberjack in Greece	Katharios, Pantelis	HCMR
16.20	16.40	14	Construction of a genetic linkage map in meagre and identification of genetic markers related to growth for use in marker-assisted breeding programs through QTL mapping	Tsigenopoulos, Costas	HCMR
16.40	17.00	15	Consumer sensory perceptions of the selected new products from DIVERSIFY species	Guerrero, Lluís	IRTA
17.00	17.30		Coffee		
17.30	17.50	16	What do we know about the immune system of meagre and amberjack?	Milne, Douglas	UNIABD
17.50	18.10	17	Behavioral analysis of intra-cohort cannibalism in young pikeperch	Colchen, Tatiana	UL
18.10	18.30	18	Wreckfish larval rearing trials	Vilar, Antonio	MC2
18.30	18.50	19	Feeding pattern for greater amberjack: effects on growth, feed utilization and welfare	Montero, Daniel	FCPCT
20.00			Dinner at Ayre Rosellon Hotel (consortium dinner)		



Table 2. Agenda of DAY 2 of the Annual Coordination Meeting 2017, which took place on the 16-19 January 2017, at the Palau Macaya, Barcelona, Spain.

	DIVERSIFY				
	7FP-KBBE-2013-603121				
	Meeting Agenda		2017 Annual Coordination Meeting	Barcelona 17-19 January 2017	Palau Macaya
DAY 2		18-lav	Wednesday (Open Day presentations)		
Start	End		Title	Presenter	Details
8.00	9.00		Registration		Pick up badges
9.00	9.20	1	Protocol for the strip spawning of meagre females and in vitro fertilization	Ramos, Sandra	IRTA
9.20	9.40	2	Spawning kinetics of greater amberjack in response to multiple GnRHa injections or implants	Fakriadis, Ioannis	HCMR
9.40	10.00	3	Effects of phosphoglycerides and HUFA levels on ontogenetic development and performance of pikeperch larvae	Lund, Ivar	DTU
10.00	10.20	4	Sensory characterization of DIVERSIFY species	Grigorakis, Kriton	HCMR
10.20	10.40	5	Influence of dietary combinations of vitamin e, c and k in the development of systemic granulomatosis in meagre	Montero, Daniel	FCPCT
10.40	11.00	6	Systemic granulomatosis in meagre	Katharios, Pantelis	HCMR
11.00	11.30		Coffee		
11.30	11.50	7	Meagre behaviour and response to feeding training stimuli	Papadakis, Ioannis	HCMR
11.50	12.10	8	The effect of cage depth in the performance of meagre	Tsalafouta, Aleka	HCMR
12.10	12.30	9	Experimental consumer test of the new products from DIVERSIFY	Krystallis, Thanassis	HRH/AU
12.30	12.50	10	Spermatogenesis and sperm characteristics in captive greater amberjack	Zupa, Rosa & Fauvel, Christian	UNIBA/IFREMER
12.50	13.10	11	Why I have come to hate meagre and why amberjack is a jinxed species: 25 years of feelings & experiences from health diagnostics	Padros, Sito	Uni Autònoma Barcelona (invited guest)
13.10	15.00		Lunch		
15.00	15.20	12	Launching the new DIVERSIFY products: business models, market tests and market diffusion	Nijssen, Ed and vd Borgh, Michel	TU/e
15.20	15.40	13	Comparison of programmed and auto-demand type feeding in tanks	Duncan, Neil	IRTA
15.40	16.00	14	Multifactorial nutrition experiment in pikeperch	Kestemont, Patrick	FUNDP
16.00	16.20	15	Maturation and spawning induction of grey mullet	Rosenfeld, Hanna	IOLR
16.20	16.40	16	Atlantic halibut larval nutrition and drivers of asymmetric pigmentation and eye migration in flounders	Hamre, Kristin	NIFES
16.40	17.30		Coffee		
17.30	17.50	17	Induction of gonadal maturation in teleosts by recombinant gonadotropins	Gimenes, Ignacio	Rara Avis Biotech (invited guest)
17.50	18.10	18	Nodavirus in Atlantic halibut and possible vaccine strategies	Patel, Sonal	IMR
18.10	18.30		Dissemination activities, articles and uploading on ECAS system - Rocio Robles		
18.30	18.50		Dissemination activities, articles and uploading on ECAS system - Rocio Robles		



Figure 1. The opening slides for the Annual Coordination Meeting 2017, held by P3. IRTA in Barcelona, Spain, explaining the Agenda of the meeting (upper right slide) and the slides explaining the organization of the DAY 1 & 2 presentations (lower left slide) and the DAY 3 GWP workshop with the four parallel sessions (lower right slide).

The extended format of task-specific presentations for DAY 1 & 2 allowed a large number of the RTD partners to present their work –which in many cases was done in collaboration with the SMEs and Large companies participating in the project, as well as work to be presented from all Scientific Disciplines. In total, 18 RTD partners presented their work, representing collaboration with the two large companies and six SMEs from the DIVERSIFY consortium (**Fig. 2**).

The presentations from the invited guests, which followed the presentations from consortium Task leaders and Partners, demonstrated both the interest of other organizations to participate in our ACMs and the interactions DIVERSIFY is trying to encourage with relevant researchers. Of great interest were the presentations of Dr. Francesc Piferrer (reproductive endocrinologist) on the recent knowledge of the epigenetic modification of gene expression in aquaculture, and the effects early rearing may have on sex differentiation. Also of specific interest to the DIVERSIFY consortium where the presentations of Dr Francesc Padros (fish pathologist) on his extensive experience with meagre and greater amberjack diagnostics, and of Dr Ignacio Gimenes (reproductive medicine physician) on the production of recombinant gonadotropins and their use in inducing gametogenesis in captive fishes exhibiting reproductive dysfunctions in captivity. Also, of great interest to the larval rearing scientists in the consortium was the presentation of Mr Torre Remman from C-Feed S.A., a commercial company specializing in the production of marine copepods for use as live food items for marine fish larvae. The participation of commercial aquaculture companies is also a clear indication of the relevance of DIVERSIFY to the EU industry, and the interest of



their technical management to be updated with the current developments in the project. The connection with these companies also provides a means for DIVERSIFY to obtain relevant feedback from the sector, as well as having the potential to try some of the developed methodologies before the completion of the project and the release of the results. Some of these companies, such as Andromeda SA who attended the meeting for the third year, continue to provide access to their facilities and fish stocks, and collaborate with DIVERSIFY as non-partners at no cost to the project. This ensures that expensive infrastructures and resources from outside the consortium are available to DIVERSIFY at no extra charge.



Figure 2. The opening slides from some of the task-specific presentations of some of the RTD partners of the consortium during DAY 1 & 2.



Dissemination

At the end of Day 2, there was a presentation by the WP 31 Dissemination leader, Dr Rocio Robles. The presentation begun with a brief reiteration of the WP's many objectives, emphasizing the need for all Partners to participate actively in the preparation of dissemination materials and activities (**Fig. 3**). Then there was a presentation of the various dissemination activities carried out in the last 2 years (2014-2015), which included the publication of four semester Newsletters that are uploaded at the website of the project and three species-focused articles published at the quarterly magazine of the European Aquaculture Society (for greater amberjack, meagre and pikeperch). A special "DIVERSIFY" session was held at the annual conference of the European Aquaculture Society (Deliverable 31.10). The Special Session was titled "New/emerging finfish species (EU Diversify project)" and was organized in the order of the species' work in the DOW. The session opened with a summary presentation for DIVERSIFY, given by the PC of the project -see *Deliverable 31.9 Annual presentation of DIVERSIFY (Y2) at a relevant conference*. Following each of the six Species Leaders summary presentations, presentations were also given by DIVERSIFY researchers on specific Tasks of the DOW. The Special Session lasted for the whole day (10:30 to 17:00) and an estimated of 30-120 persons were present at the different presentations in the designated room. The Species Leaders' presentations have been uploaded on the DIVERSIFY website.

OBJECTIVES

- ✓ Disseminate the knowledge acquired to scientific community and aquaculture sector.
- ✓ Promote implementation of new husbandry methods, protocols & products developed by DIVERSIFY to the aquaculture industry & the seafood processors.
- ✓ Enhance awareness of the diversification efforts of the project to the general public. Special attention to Food industry & Consumer's organizations.
- ✓ Promote investment opportunities making available the species feasibility studies to the industry.
- ✓ Documented information to fish producers, fish processors & consumers on the new farmed aqua products from DIVERSIFY.



ACM, Barcelona, 2017

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PROGRESS:

- ✓ Task 31.3 Presentation of DIVERSIFY at Aqua Europe meetings:
 - ✓ EAS 2014, San Sebastián (Spain) (D 31.6),
 - ✓ EAS 2015 Rotterdam (D 31.9), *Special Session AE 2015* (D 31.10),
 - ✓ EAS 2016 Edinburgh (D31.14)
 - ✓ EAS 2017 Dubrovnik, *Special Session AE 2017* (D31.19).



✓ Articles in Aquaculture Europe



ACM, Barcelona, 2017

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Task 31.7 Dissemination to the food industry & consumers

IMPACT magazine January 2017

- Distributed in printed and digital format in December to 35'000 readers worldwide
- Open access on IngentaConnect, Portico repository and receive a CrossRef DOI.
- supply impact metrics from the IngentaConnect distribution including downloads, shares and reads.
- Printed copies for project partners



ACM, Nancy, 2016

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Figure 3. Photos from the presentation of WP31 leader Rocio Robles on Day 2.

As regards the DIVERSIFY website, the partners were informed that the website of the project (www.diversifyfish.eu) is being modified in order to make it easier for the visitors to find recent findings of



the project, as well as the scientific articles that are now being produced and published (**Fig. 4**). In order to facilitate the production of short reports on implemented work and acquired results to be uploaded in our site, the Dissemination leader prepared in 2014 a format file to be used by all scientists to prepare dissemination materials, in a way that would be easy for the partners to fill. The format file is available in the INTRA page of the DIVERSIFY website. Unfortunately, not many such reports have been produced so far, and more effort must be dedicated to encourage DIVERSIFY scientists to start preparing these short dissemination material from their activities.

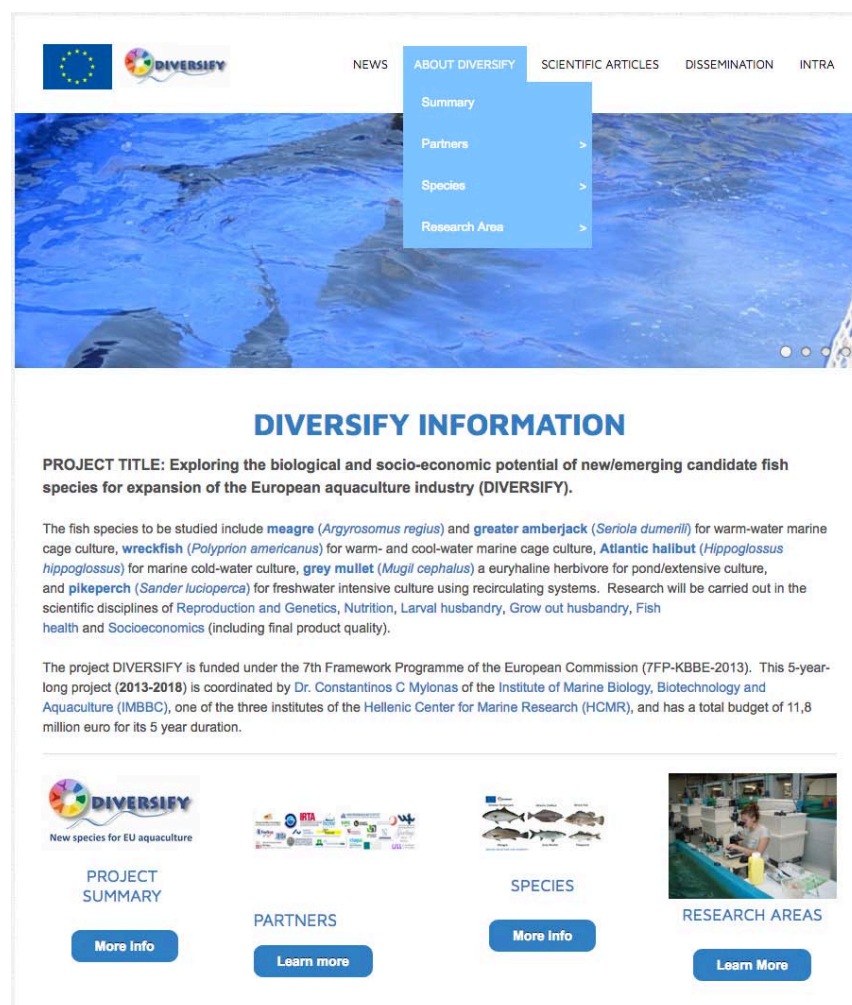


Figure 4. The new version of the project’s website, modified to give more emphasis on recent activities and news, as well as the easy dissemination of the scientific articles that are now being produced at a fast pace.

The Dissemination WP leader then discussed again the issue of uploading dissemination activities on the ECAS portal, as well as preparing the work done in DIVERSIFY for submission to scientific magazines (**Fig. 5**). Already 9 articles have been published and a number of manuscripts have been submitted for publication and many more researchers expressed their intention to start submitting their work. The contractual requirements of the DIVERSIFY are 2 articles per GWP per year, which makes for a total of 60 articles. Currently a total of 10 articles have been published from the areas of Reproduction and Genetics, Nutrition, Larval rearing, Fish health and Socioeconomics. As mentioned earlier, a change was done on the project’s website, by moving the “Scientific Publications” page to the main menu bar (**Fig. 6**), so that visitors will have a more rapid and direct access to the scientific work of the Consortium.

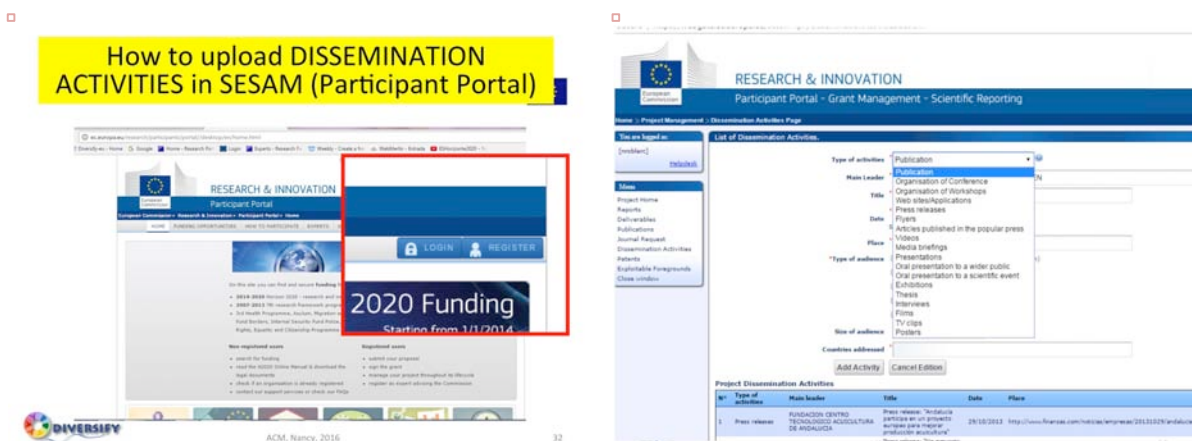


Figure 5. Representative slides from the discussion on uploading dissemination activities on the ECAS portal and about the preparation of scientific publications.



Figure 6. The new version of the project's website, modified to give more emphasis on the scientific articles that are now being produced at a fast pace.




In agreement with the intentions of the consortium to be as open as possible and to disseminate the results as promptly as possible, all the presentations of the ACM 2017 were uploaded on the website of the project within 2 weeks after the end of the meeting (end of January 2017), to be available to all interested stakeholders. In addition, it was agreed that all GWP leaders will submit a paragraph with the major highlights of the work implemented so far in their Scientific Disciplines, in order to prepare a 1-2 page flyer, which will then be translated to various languages by our Professional Association partners and disseminated to their members (*e.g.* in Greece, Spain, Hungary and Germany).

The next ACM is planned for 23-26 January 2018 in Tenerife, Spain. In the DOW, it was proposed that one of these meetings would be held in Norway, and would be organized by P7. IMR. However, due to the fact the time coincides with the mid of winter in this partner it was proposed by the PC, after communication with P8. IEO and P15. ULL to hold the next meeting in Tenerife, Canary Islands, Spain. This was received with enthusiasm by the Partners, therefore the next meeting will be hosted by the latter partners.

DAY 3 – Scientific Discipline-specific workshops

During Day 3 of the meeting, six Workshop Sessions were organized according to Scientific Disciplines with the objective of (a) reviewing and evaluating the work carried out and (b) planning the work to be implemented in the various scientific WPs during the fourth year (2017) of the project (**Table 3**).

Table 3. Agenda of DAY 3 of the Annual Coordination Meeting 2017, which took place on the 17-19 January, at the Campus del Mar of the University Pompeu Fabra, Barcelona, Spain.



DIVERSIFY

7FP-KBBE-2013-603121

Meeting Agenda

2017 Annual Coordination Meeting

Barcelona 17-19 January 2017

University Pompeu Fabra

DAY 3		Thursday (GWP Workshops)			
Start	End	ROOM 1	ROOM 2	ROOM 3	ROOM 4
9,00	9,30	GWP 2 Repro & Gen (amberjack)	GWP 6 Fish health (meagre)	GWP 3 Nutrition (mullet)	GWP 7 Socioeco -SMEs
9,30	10,00	GWP 2 Repro & Gen (amberjack)	GWP 6 Fish health (meagre)	GWP 3 Nutrition (amberjack)	GWP 7 Socioeco -SMEs
10,00	10,30	GWP 2 Repro & Gen (amberjack)	GWP 6 Fish health (meagre)	GWP 3 Nutrition (halibut)	GWP 7 Socioeco -SMEs
10,30	11,00	GWP 2 Repro & Gen (amberjack)	GWP 6 Fish health (halibut)	GWP 3 Nutrition (pikeperch)	GWP 7 Socioeco -SMEs
11,00	11,30	Coffee			
11,30	12,00	GWP 2 Repro & Gen (mullet)	GWP 6 Fish health (amberjack)	GWP 3 Nutrition (meagre)	GWP 7 Socioeco -SMEs
12,00	12,30	GWP 2 Repro & Gen (mullet)	GWP 6 Fish health (amberjack)	GWP 3 Nutrition (wreckfish)	GWP 7 Socioeco -SMEs
12,30	13,00	GWP 2 Repro & Gen (halibut)	GWP 6 Fish health (amberjack)	General discusion	GWP 7 Socioeco -SMEs
13,00	13,30	Lunch at student's restaurant or in the local area			
13,30	14,00				
14,00	14,30				
14,30	15,00	GWP 2 Repro & Gen (wreckfish)	GWP 4 Larval (mullet)	GWP 5 Grow out (amberjack)	GWP 7 Socioeco
15,30	16,00	GWP 2 Repro & Gen (wreckfish)	GWP 4 Larval (halibut)	GWP 5 Grow out (amberjack)	GWP 7 Socioeco
16,00	16,30	GWP 2 Repro & Gen (wreckfish)	GWP 4 Larval (pikeperch)	GWP 5 Grow out (amberjack)	GWP 7 Socioeco
16,30	17,00	GWP 2 Repro & Gen (wreckfish)	GWP 4 Larval (meagre)	GWP 5 Grow out (mullet)	GWP 7 Socioeco
17,00	17,30	GWP 2 Repro & Gen (wreckfish)	GWP 4 Larval (wreckfish)	GWP 5 Grow out (pikeperch)	GWP 7 Socioeco
17,30	18,00	GWP 2 Repro & Gen (wreckfish)	GWP 4 Larval (amberjack)	GWP 5 Grow out (meagre)	GWP 7 Socioeco
18,00	18,30	GWP 2 Repro & Gen (wreckfish)	GWP 4 Larval (amberjack)	GWP 5 Grow out (meagre)	GWP 7 Socioeco

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The workshops of DAY 3 were running in parallel (4 Scientific Disciplines at a given time) in an attempt to minimize the potential time conflict for most Beneficiaries. The duration of each session was decided by the



GWP leader based on the number of WP included in the Scientific Discipline, as well as the amount of work that needed to be presented and discussed, and the workload expected for the upcoming year. Therefore, GWP Reproduction & Genetics and GWP Socioeconomics requested full-day Workshops, so a room was dedicated to their work. In addition, the Workshops were organized in a way that the WPs dealing with the same species were planned at different times during the Workshops, to allow all scientists attending all the WPs of the same species. This was also achieved, to a degree, by the participation to the ACM 2016 of more than one scientist from some of the beneficiaries that are involved in many GWPs. For example, P3. IRTA was represented by eight researchers and P1. HCMR by nine researchers. Unfortunately, P2. FCPCT that has the third largest budget in the project was represented only by a single scientist (Dr Daniel Montero, the GWP leader for Nutrition), while the PI of the organization was not present at this ACM also.

Together with the minutes of the whole meeting, the minutes prepared by the GWP leader of each scientific discipline from the different GWP workshops (**Fig. 7**) were provided to the EU Scientific Officer (Dr. Marta Iglesias), and are presented below.



Figure 8. Photos from the DAY 3 Workshops in the various scientific discipline GWP.



Minutes of GWP Reproduction and Genetics workshop

Annual Coordination Meeting 2017, Day 3 (19/1/2017, 9:00-18:00)



By Dr. Neil Duncan, IRTA (GWP Leader)

Participants

Participants in the meetings

	Name	Institution	Amberjack	Mullet	Halibut	Wreckfish
1	Neil Duncan	IRTA	✓	✓	✓	✓
2	Constantinos Mylonas	HCMR	✓	✓	✓	✓
3	Ignacio Gimenez	RARA AVIS Biotech SL	✓	✓	✓	✓
4	Yannis Fakriadis	HCMR	✓	✓	✓	✓
5	Hanna Rosenfeld	IOLR	✓	✓	✓	✓
6	Rosa Zupa	UNIBA	✓	✓	✓	✓
7	Christian Fauvel	IFREMER	✓	✓	✓	✓
8	Keilliopi Tsakoniti	GMF	✓			
9	Blanca Alvarez-Blanquez	IEO-Vigo	✓			✓
10	Jose Luis Rodriguez	CMRM	✓			✓
11	Montse Perez Rodriguez	IEO-Vigo	✓			✓
12	Elena Chaves Pozo	IEO-Murcia	✓			
13	Marta Arizcun Arizcun	IEO-Murcia	✓			
14	Jose Antonio Perez	ULL	✓			
15	Salvador Jerez	IEO-Canarias	✓			
16	Tasos Raftopoulos	ARGO	✓			
17	Babis Sirigos	FORKYS	✓			
18	Antonios Ploumis	FORKYS	✓			
19	Birgitta Norberg	IMR		✓	✓	
20	Fatima Linares	CMRM				✓
21	Antonio Vilar	MC2				✓
22	Benjamin Geffroy	IFREMER				✓

Excellent progress, most Deliverables are being submitted in time, **work finished on pikeperch (WP4) and meagre (WP2)** and all Deliverables submitted.

WP 3 *Seriola*

Absent: FCPCT,

Deliverable status WP3:

D3.1) Establishment of quantitative PCR assays to measure transcript levels of target genes in greater amberjack (i.e., LH β , FSH β , leptin, Vg and Vg receptor): M12, **Delivered**

D3.2) Establishment of hormone specific ELISAs for measuring LH, FSH and leptin in greater amberjack: M18 **Delayed**, need time to finalise Leptin assay.

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: M24, **Delivered**



D3.4) Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of greater amberjack sperm: M32, **Delivered**

D3.5) Description of the process of oogenesis in captive greater amberjack: M46, in progress

D3.6) Description of the process of spermatogenesis in captive greater amberjack: M46, in progress

D3.7) Comparative effectiveness of a GnRHa injection vs GnRHa implant treatment for the induction of spawning of greater amberjack in the eastern Atlantic: M48, in progress

D3.8) Dose response of GnRHa implant therapy for the induction of spawning in F1 generation broodstock: M54, in progress

D3.9) Development of a spawning induction therapy for captive reared broodstock in the Mediterranean Sea: M54, in progress

D3.10) Method for inducing spawning and collecting greater amberjack eggs in sea cages: M54, in progress

Comments, presentations and discussions WP3

Overall WP3 is going ahead of schedule, all work in Las Palmas has been completed (FCPCT), but repeating some work. An introduction was given by CCM (substituting A. Corriero) on where we stand with the greater amberjack work. FCPCT has completed their task and they are currently preparing an article. The manuscript has been sent by Pipo to CCM, and it is currently under modifications.

Presentation by YF (HCMR)

Reported that so far tank-reared fish do not reach appropriate reproductive stage, so we will use the method for cage rearing and induction in tanks.

Additional project, spermiation induction in males (FORKYS). HR suggested to use 17aMT implants to induce spermiation, it would take 2 weeks. HR asked to get blood from the experiment to run FSH/LH. HR also suggested to do the experiments a bit earlier in May, based on the fact that in nature (Zupa et al. 2017; PLOS One doi: 10.1371/journal.pone.0169645) they are in spawning in May. But this was at 19°C and in Sicily. We should try in GMF to start the first treatment in 23 May to see the earliest time.

Two experiments will be held in the 2017 breeding season, different GnRHa doses in implants (ARGO) and timing of GnRHa application (GMF), starting in late May. There is a thought for earlier pre-treatments to induce spermiation in ARGO, but it has not been decided yet. Pre-treatments earlier to induce spermiation (experiment in FORKYS): GnRHa + another treatment perhaps MT or HCG. Males are difficult to strip sperm, CF suggests stripping sperm immediately after egg collection and spawning. Problems of pre-treating males is the stress caused to females that are in the same group and would be disturbed. Would be good to separate all the males from females or some to from a small group of males. Perhaps separate half the males (6) to another cage. Leave some males mixed with females. Two weeks before female induction the separated males will be hormonally treated and re-joined with the females.

Presentation by SJ (IEO)

Presented the proposed task (DoW), which was to test three different doses of GnRHa in implants, in an F1 stock of greater amberjack of the Atlantic stock. However, due to heavy mortality in the first year of the project, not enough fish were available. So it was decided to use the remaining



stock for a single dose trial, and change the dose in subsequent year. So far two doses have been tested (50 and 75 µg/kg). For next year we will have even less fish, but we will try the lower dose (25 µg/kg). So far, the 75 µg/kg seemed to be doing better, producing more eggs and of better quality. The induction in 2017 will be in June, as in 2016 best results were in June compared to May (and July). Possibly poor eggs are lost to bottom of tank and not collect resulting in 100% fertilization. Improved results in 2016, due to higher dose, improved handling, lower density, different temperatures?

Presentation by JAP (ULL)

Presented a summary of the finding so far, which indicate that some lipids (EPA and LA) are lower in the gonads of captive reared fish, compared to wild. Changes in protein content existed during the spawning season, between captive and wild, with wild having significantly higher values. Almost 70% of the work has been completed. Some analyses will be completed and will be used for the preparation of articles. Work on comparing nutritional status of sinking and floating eggs needs more samples.

Presentation by RZ (UNIBA)

Three of the six deliverables in which UNIBA is involved have been completed. The other three are making good progress, with some analyses remaining to be completed. An additional sampling completed in the summer of 2016 was presented. This sampling was made to obtain smaller individuals, in order to determine the age/size of puberty of the species. Greater amberjack females appear to start maturation at age 3, and are 100% mature by age 4. Age 3 males have been found in advanced spermatogenesis; however, it is not sure they are capable to spawn at this age. Moreover, in 2016, body condition index was compared between wild and captive-reared greater amberjack, demonstrating no difference of this parameter between the two groups. Plans for 2017: vitellogenin, gonadotropin and leptin ELISAs and egg biochemical composition.

Presentation by HR, (IOLR)

The analyses of GtH have been completed, both in pituitary content and plasma levels. Some different results from what was expected in relation to other species, with LH being higher during the early season, and with FSH not showing much cycling during the sampling periods (in the blood), but increasing over the time course. Peak in steroids does not agree with LH levels, which were low. IG - Possibly related to receptor numbers. HR – Perhaps, but levels are low compared to other species.

A suggestion was made to use recombinant FSH to treat the HCMR tank stock, in order to induce gametogenesis. We will use the tuna rFSH, which is very potent and a single treatment should be enough. For the males 17a-MT implants should then be given 2 weeks later. The exact protocol (doses, time of treatment) will be discussed and arranged. For the females, treatment could be given at 300 µm oocytes (April) and then after 5-6 weeks a check for maturation. We will discuss and agree the protocol.

The Leptin is still problematic with the ELISA not showing enough sensitivity, so the deliverables have been delayed further. No problem to complete but will be delayed.

CCM: Based on the findings from the spawning experiments so far, it seems that there are significant differences in reproductive biology characteristics between the stocks in Las Palmas/Tenerife and the Mediterranean. These include maturing well in tanks, spawning for a much longer period (May-September), responding much better to GnRHa injections vs implants, and they spawn spontaneously in small tanks without much of a problem (Las Palmas). This prompted us to question if the stocks are different, and fin clips have been obtained from all the



available stocks from the Partner, plus some others (Cyprus and Turkey) to examine the existence of different stocks. The analyses have been made at HCMR, without any additional funding and will be presented in the near future.

Mullet WP 7

Deliverable status WP7:

D7.1) Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of grey mullet sperm: M12, **Delivered**

D7.2) Production of recombinant bioactive LH and FSH assay for grey mullet: M 18, **Delivered**

D7.3) Comparative effectiveness of hormonal treatments for spawning induction in captive grey mullet: M 24, **Delivered**

D7.4) Protocol for shipping grey mullet eggs: M24, **Delivered**

D7.5) Description of the process of oogenesis in captive-reared vs hatchery-produced grey mullet: M48, in progress

D7.6) Culture procedure that identifies the ongrowing period for the production of grey mullet roe (bottarga): M54, in progress

D7.7) Development of a breeding protocol for captive reared grey mullet broodstock based on optimized hormonal treatment: M60, in progress

Comments, presentations and discussions WP7

Presentation by HR (IOAP)

Stimulating gametogenesis with rFSH + DA

Two GnRH α + DA 22.5 hours apart, spawning 22.5 hours after 2nd injection

Work in IRTA depends on fish numbers, plans include 1) test IOLR protocol, applying rFSH a month before spawning, 2) test rGtH from Rara-avis, many possibilities, with sole rGtH, bass rGtH. HR suggested making a test to confirm species in IRTA. ND will send samples when available.

Discussion on rGtH: HR indicated that over evolution teleosts have reduced glycosylation to reduce half-life. Therefore, HR does not focus on having high glycosylation. IG agrees that glycosylation has decreased with evolution and says for this reason he has glycosylation on the linker and not on the beta sub-unit. This does not affect the action of the gonadotropin (with the receptor) but increases the half-life as the linker has a stronger bond. HR says the single application stimulates the endocrine system including pheromone and social aspects and effects can be observed in the pituitary. ND asked if the protocols with a single rGtH injection will work in other centres with different breeders and different husbandry (stress) procedures, is the advance in gametogenesis prior to the injection critical to the success of the treatment?

Atlantic halibut WP5

Deliverable status WP5:

D5.1) Documentation of reproductive performance in wild-captured vs cultured female Atlantic halibut: M30, **Delivered**

D5.2) An optimised GnRH α therapy protocol to improve spawning performance of F1/F2 Atlantic halibut, M30, **Delivered**



D5.3) Identification of potential disturbances in reproductive development in F1/F2 Atlantic halibut females: M48, in progress

Comments, presentations and discussions WP5

Presentation by BN (IMR)

A new addition to WP5 will be the analysis of circulating LH and FSH for the D5.3.

Wreckfish WP6

Deliverable status WP6:

D6.1) Computer Assisted Sperm Analysis (CASA) for wreckfish sperm: M24, **Delivered**

D6.2) Cryopreservation method for wreckfish: M24, **Delivered**

D6.3) Spawning induction methods with in vitro fertilization of wreckfish: M36, **Delayed**, need more time to increase result set and success of in vitro fertilisations.

D6.4) Establish reliable collection methods and protocols to form new wreckfish broodstocks: M36, **Delayed**, need more time to increase number of capture which are becoming very rare despite of fishing efforts.

D6.5) Description of the reproductive cycle of wreckfish: M48, in progress

D6.6) An in vitro fertilization protocol to be employed by the industry to spawn wreckfish: M48, in progress

D6.7) Spawning induction method for spontaneous spawning of wreckfish in large tanks: M54, in progress

Comments, presentations and discussions WP6

Presentation by BAB (IEO)

In the Aquarium Finisterre (A Coruña) aquarium they can identify which female spawns by the visual tag that can be seen from outside the tank and related to changes in swelling of the abdomen. The spawning behaviour is characterized by males chasing the females and spawning is the same behaviour, but with liberation of gametes. Spawning is during the night or very early in the morning. GnRHa implant was unsuccessful at oocyte size of 1200 µm and successful at 1400 µm. Fish stripped four days after GnRHa implant. Correct oocyte size for implant should be 1200+ µm, but best 1300-1400+ µm.

Amazing results in terms of steroid variations during vitellogenesis and post-vitellogenesis that may be due to instability in the assays. This is not exploitable at this time, but such an approach would be profitable to assess the effect of recombinant gonadotropins and particularly the association of both beta-subunits (LH and FSH).

Natural spawning has been giving better results than strip spawning. Both females that are encountered naturally ovulated and females that are implanted to ovulate. What time are ovulated eggs ready for stripping? Need to be methodical and start to collect the time when fish are checked. Set up groups of females, implant and make various checks to each female to determine the correct time. Need to first examine the timing from implants to spawning. AV has data on spawning times and will edit it and send it to help identify stripping time. AV thinks to put cameras, IGAFa will put cameras. These could be used to identify changes in behaviour associate with ovulation, i.e. chasing, this change in behaviour could be used to identify stripping time for *in vitro* fertilization.

The wreckfish does not grow well at +16°C and perhaps 15°C is too high for the maturation? Normally live at depth. Never go to surface. However, small temperature increases 15 to 16°C



increases natural spawning. What is best temperature for egg incubation (and therefore maturation)? BAB presented an experiment that showed slightly better hatching in eggs incubated at 17°C compared to 14°C. However, all hatching rates in this experiment were low and differences between groups were small and probably not significant. Eggs are usually spawned, collected and incubated at 15-16°C and good hatching rates have been obtained (65%), but there is a large variation (2-65%).

Presentation by YF (HCMR)

HCMR need input from Galicia to fine tune timing for stripping. This year will make some more stripping attempts to find the right stripping time.

HR needs the samples to analyse for LH and FSH to describe the reproductive cycle? It is thought all samples were sent to HCMR and HCMR should send excess samples to IOLR. HCMR later confirmed that all samples from Spain are in their freezers and will forward to Israel. Hormone analysis would be interesting for the fish injected with rGtH. However, according to the DOW samples are needed for describing the reproductive cycle not effects of hormone induction.

Why were no eggs sent to Crete? The egg quality was bad and, therefore, no eggs were sent. Need to complete with the DOW and increase our chances of success by sending eggs to other hatcheries. BAB suggests that it is better to send larvae than eggs, because the risk of the eggs is the bacterial infections.

What is the plan to identify stripping time for in vitro fertilization? There are two deliverables for in vitro fertilization, first a protocol and 2nd an industrial protocol.

Plans for 2017

Leave all fish that have spawned naturally in good conditions to spawn naturally.

Other fish should be used to be induced and strip spawned.

CMRM

1 tank for 6 females for work with Ignacio → strip fish

2nd tank (3 fish) left to spawn naturally

IEO

Two tanks different diets, fish left for natural spawning. Possibility for strip spawning? BAB, the group are thinking about this to fixed a method to control the females.

MC2

Best approach is natural spawning, strip spawning has not work in the past. Some fish that are swollen each year, but do not spawn could be used for induced spawning for natural tank spawning. However, it may be best to try in vitro with these fish?

Antonio presented an excel file that could be a good tool to judge the modifications of fish status as well as a way to show the observation and handling work of the team. Such a file filled by all wreckfish stock owners would provide a valuable biological information and a proof of what was done by the partners. It would be interesting that Antonio sent the file to each wreckfish partner for a regular filling along the last 2 years of the project. IEO-Vigo have a similar file with all



actualized data of the reproduction work. This file was initially elaborated by CCM can be provided at request.

Comment by ND, WP6 should propose joining the following deliverables into one deliverable: **D6.3)** Spawning induction methods with in vitro fertilization of wreckfish and **D6.6)** An in vitro fertilization protocol to be employed by the industry to spawn wreckfish. BAB and group are considering what to do about delayed deliverables.



Minutes of GWP Nutrition workshop

Annual Coordination Meeting 2017, Day 3 (19/1/2017, 9:00-13:00)



By Dr. Daniel Montero, P2. FCPCT (GWP Leader)

Nutrition for grey mullet (WP13)

Participants

Daniel Montero, Kristin Hamre, Bill Koven, Ivar Lund, Patrick Kestemont, Najlae El Kertaoui, Fatima Linares, Salvador Jerez and Virginia Martin. Jose Perez, Grethe Rosenlund (SARC), Ramon Fontanilla (SARC),

Also present in the room: Nikos Papandroulakis, Alicia Estevez

WP8-Meagre

In general the work planned is as in DOW

T8.1 Improvement of larval weaning feeds. D8.1 completed.

Additional results on the role of Vitamin K in meagre larval development were presented by Daniel Montero.

Highlights:

Microdiets with different graded levels of Menadione SB (from 0 to 35 mg/kg) were formulated and levels of menadione were analyzed in diet with a correlation between added/analyzed menadione in diet.

Among the different levels studied, 5.90 mg/kg of menadione in diet shows a trend to increase growth and survival of meagre larvae, however, large amount seems to decrease growth and survival, possibly due to its toxic potential. Low levels of vitamin K may interfere with lipid utilization, increasing intestinal and hepatic steatosis. Increasing vitamin K levels in the diet increase MGP gene expression and decrease the incidence of anomalies (Scored as described by Boglione et al., 2014) in meagre larvae.

T8.2 Determination of nutritional requirements to promote feed utilization. D8.2 Month 48, expected to be in time.

Daniel Montero presented preliminary results on the essential fatty acid (EFA) requirements of meagre juveniles.



- A diet with graded levels of EFAs was formulated by SARC and FCPCT, and analyzed accordingly.
- Preliminary results on growth were presented, juveniles increasing growth as fatty acid increase in diets.

WORKING PLAN FOR 2017 IN WP8. To continue analysis from T8.2, define growth curves and other parameters (health, histology, etc).

DISSEMINATION ACTIVITIES IN WP8.

- Master Thesis: Sara Ramirez (FCPCT)
- ISFNF 2016, Sun Valley, Idaho, USA
- 2nd International Conference of Fish and Shellfish Immunology, June Maine, USA
- European Aquaculture 2016, Edinburg, Scotland, September 2016

* some comments in the discussion: to unify criteria to name different anomalies and deformities.

WP9. Greater Amberjack

In general the work planned is as in DOW.

T 9.1. Improved larval enrichment products to enhance production of larvae and juveniles.

Subtask 9.1.1. The optimum essential fatty acid for enrichment products for live prey. D9.1. Submitted and approved in time.

Subtask 9.1.2. Combined effect of PUFA-rich lipids and carotenoids. D9.1. Submitted in time and approved.

IEO presented the summarized results of the previous studies done in deliverable D 9.1.

T 9.2. Development of diets for grow-out of amberjack to maximize growth.

Subtask 9.2.1. Lysine requirements for juvenile amberjack. D9.2. To be submitted in January 2017. Results couldn't be presented because Y. Kotzamanis (HCMR) could not attend the meeting, but an overview was sent and is included within these minutes.

Highlights:

- Six different diets with graded levels of Lysine were formulated by SARC and HCMR, from 0.0 up to 0.52% added lysine, 1.93 up to 2.29 analyzed lysine (%)).
- The results from the present study indicated that the dietary lysine requirements based on the Broken-line model, which can support maximum weight gain of greater amberjack juveniles fed on a diet based mainly on plant ingredients, containing 45% protein, 18% lipid and 25% fish meal inclusion was 2.11% of diet.
- The highest HSP90 levels of expression observed at L2, L3 and L6 lysine levels compared to L1, L4 and L5. HSP70 levels remained equal at all dietary groups with a tendency to lower levels in L2 and L4 groups. Lysine supplementation affected the specific activity of CAT in liver and intestine of greater amberjack.
- The data presented in the current study will be useful in developing balanced commercial diets for greater amberjack particularly when fishmeal is replaced by plant protein blends. Evaluation of other EAA requirements should also be conducted.

Subtask 9.2.2. Grow-out diet defined will be tested and reported in D9.3. Month 58



No results presented by CANEXMAR, who were not present in the workshop

T 9.3. Design adequate feeding regimes for broodstock to optimize reproduction

Subtask 9.3.1. Optimum ARA, EPA, DHA levels for reproductive success. D 9.4. Month 58.

Some results commented by Daniel Montero (not presented as he needed to leave the room to assist health workpackage for some minutes). Daniel Montero commented that analysis from previous studies on aminoacids in broodstock diets were finished (data available in the 2nd Periodic Report sent to EU in June) and new diets have been formulated to conduct the experiments in 2017 (next spawning season) to determine EFA levels in broodstock diets.

Subtask 9.3.2. Lipid and Carotenoids D9.4 expected in time (month 58)

Virginia Martin (IEO) presented the working plan for 2017 in which the background for designing the diets was presented, to be used in the next spawning season.

Highlights:

- Cultured females displayed lower proportion of arachidonic acid (20:4n-6, ARA) and higher proportions of linoleic acid (18:2n-6) and eicosapentaenoic acid (20:5n-3, EPA) than wild specimens for all tissues
- Nutritional status of captive vs. wild broodstock. Proportions of total polar lipids differed among wild and captive-reared fish. Testes and ovaries of captive fish displaying around 30-40% less DHA and ARA, and higher contents of 18:2n-6. DHA/EPA and ARA/EPA ratios are lower in the gonads of the captive fish
- Based on these findings, experimental diets with optimized EFA and carotenoid contents will be designed during this year. The estimated requirements for the experimental diets will be: Broodstock biomass about 140 kg per treatment, fish fed three times a week, estimated daily ration of 1.0 -1.5 %, duration of trial 6 - 12 months, weekly feed: 4.2 - 6.3 kg, monthly feed: 16.8 – 25.2 kg, annual feed: 201.6 – 302.4 kg

There is currently a new stock of greater amberjack broodstock available for the implementation of this task at the Culture Unit of Canary Islands Oceanographic Centre (IEO). The fish stock will be the potential broodstock group to assay the experimental diets, with an average weight of 10-12 Kg and between 4 and 5 years old. The trial with experimental diets will be started in January 2018.

WP10. Pikeperch

Task 10.1. Effect of selected dietary nutrients on pikeperch larval development and performance. D10.1 (month 36) will be delayed until month 41

Ivar Lund (DTU) and Patrick Kestemont (FUNDP) presented the results done in 2016. As both presented their results in previous days in the meeting, they presented here some selected results and graphics and some highlights on the multifactorial trial with 16 diets:

- The 16 experimental diets differed by their levels of fatty acids, vitamins and minerals as expected. Multifactorial experiment.
- Larval growth was significantly lower in larvae fed high Ca/P diet, but a high Ca/P improved survival rate.
- Results also showed that high dietary ARA levels significantly decreased the activities of digestive enzymes (i.e. pepsin; leucine-alanine peptidase; aminopeptidase and alkaline phosphatase). A high vitamin E level significantly increased amylase activity.



- High levels of EPA + DHA seemed to increase prevalence of lordosis and prehaemal scoliosis, while low levels of the EPA+ DHA had the opposite effect. Whether a negative effect of high dietary ARA; 20:4n-6 needs confirmation of larval n-3 HUFA/n-6 HUFA content
- Results on gene expression and histology are still being analyzed.

Two confirmatory experiments with selected levels and ratios of minerals, vitamins and HUFAs will be performed in the spring of 2017.

Task 10.2. Effects of pikeperch early fatty acid nutrition on long-term stress sensitivity. D 10.2, month 36 (delayed until month 41); D10.3 month 48.

Ivar Lund presented some highlights. The trial was performed from September to November 2016 at DTU facilities. Diets were formulated and produced by SPAROS, with increasing levels of phospholipids and /or HUFAs, from EPA+DHA = 0.41+0.66 up to 0.75 + 3.0) and phospholipids from 3.7 up to 14.5.

Highlights:

- Growth and survival was significantly highest by the highest PL inclusion level (14 %), and a positive effect of supplemented n-3 HUFA level, however not significant
- The combination of low PL level (3%) with and without n-3 HUFAs caused low survival and slow growth.
- Escape response: No effects on latency time, (defined as the time elapsed between stimulus breaking the water surface and the first detectable escape motion of the fish), or peak velocity during the escape response (bl sec⁻¹), nor any effects on distance covered during the first 80 ms of the escape response, and peak acceleration (m sec⁻²).
- Stress response to a stressor: No significant difference between dietary groups. For all groups, cortisol and glucose levels rose subsequent to 2 min of chasing the fish. After 5 hours, levels had not returned to control values.
- Rheotaxis: Larvae exposed to highest levels of phospholipids + HUFAs exposed a higher sensitivity.

General comments after presentations: Some suggestions on the role of ARA acid in intestine development. Some comments on checking other levels of ARA in pikeperch diets. Patrick Kestemont also pointed out the importance of measure the ratio Ca/P in diets not only varying one of the two minerals, but also varying both (as mentioned above, two trials will be performed on this in spring 2017).

WP11. Halibut

Kristine Hamre (NIFES) presented the results obtained in 2016.

Task 11.1 Early weaning of Atlantic halibut larvae. Deliverable D11.2. Month 36. Presented and accepted

- The feed was chosen based on experiment from 2015
- Weaning started on day 15, day 22 or day 28 after first feeding and lasted for 5 days
- Triplicate 50 L tanks with 15-20 larvae per tank



- Gut fullness was registered visually every morning and evening, using a torch

Highlights:

- the number of larvae with filled guts was very low when weaning started on 15 dpff, concomitant with almost total mortality during the five days the experiment lasted.
- When weaning started on 22 dpff, both feed intake and survival were higher than on 15dpff.
- Weaning on 28 dpff resulted in a mortality of only 3 ± 1 out of 17 ± 2 larvae and all surviving larvae were full on day 3 of the experiment.
- The low success rate at the two early time points may be connected to the rearing system which may have been suboptimal for pelagic larvae or to the quality of the feed

Task 11.2 Development of a production strategy for on-grown *Artemia*. D. 11.1. Month 24. Reported and accepted. Results previously presented as activities of 2015

Task 11.3 Nutrient retention and digestive physiology in Atlantic halibut larvae fed *Artemia* nauplii and on-grown *Artemia*. D11.2. Month 36. Reported and accepted. Results previously presented as activities of 2015

Task 11.4 Atlantic halibut larvae reared on *Artemia* nauplii in RAS vs FT system. D11.4 Month 36. Submitted and accepted.

Kristin Hamre also presented some highlights on the experiment conducted in 2016 to cover Deliverable 11.4. Some highlights on the comparison of RAS to FT:

- Less than 50% final weight
- 30% less lipid in whole body
- 10% less protein
- More free amino acids except glycine. Indicate inhibition of protein synthesis?
- More vitamin K (MK6) as expected. Bacterial origin?
- More iodine (not expected). Iodine uptake from sea water may be inhibited in RAS
- No differences in digestive enzymes except that Amylase was higher in larvae from the FT system.

Task 11.5 Effect of dietary PL on digestion, absorption and metabolism of lipids in Atlantic halibut juveniles. D 11.5. Month 48.

Kristin Hamre pointed out that this experiment gave no differences in growth and the analyses are in progress.

WP12 Wreckfish.

Fatima Linares (CMRM) presented a general overview of both tasks to be done with wreckfish and also the working plan for 2017, in which special effort will be done to obtain larvae.

Task 12.1. Live preys and enrichments for wreckfish larvae. D12.1. Month 54

Highlights:



- Preliminary data of fatty acid profile of wreckfish were obtained from 1dph to 26 dph to complete the data obtained in 2015.
- A new enrichment product for live food was developed. The formula is based on soybean meal, krill phospholipids, soy lecithin, fish oil, microalgae and mixtures of vitamins and minerals. It could not be tested because the amount of larvae was not sufficient to perform the experiments.

Task 12.2. Influence of broodstock feeding regimes for fecundity and spawn quality. Month 58.

Highlights:

- Regarding wreckfish broodstock feeding regimes, a specific dry food for wreckfish broodstock was formulated. The results obtained so far demonstrated that most of commercial dry food has too much fat for wreckfish broodstock, so the level of fat should be much lower than in commercial food containing a large amount of n-3 HUFA and the EPA/ARA ratio must be around 1.5, similar to that obtained previously in wild fish.
- Fatty acid profiles of oocytes were obtained at different stages of development.
- There is a clear relationship between the fatty acid profile of broodstock diets (semi moisture and dry food) and the fatty acid profile of oocytes and larvae, which is very remarkable in n-6 PUFA content. These results confirm those obtained in 2015.

Research Plan 2017:

- Special effort will be done to obtain larvae to perform the experiments of feeding.
- Experiments of broodstock feeding with semi moist diet and dry food will continue in 2017 in IEO facilities.
- Additionally, samples of gonads from wild wreckfish and all the diets supplied to different wreckfish broodstocks were analyzed.

As a general discussion, the major concern commented was the difficulty to obtain larvae to check enrichment products. Although larval analysis were done until 26 dph larvae, it was possible that these larvae were not feeding and a general discussion was done on the importance (or not) to obtain data from larvae that were not feeding. On one hand, no other information is available for this species. Results must be discussed when larvae will be available. Besides, feeding regime of broodstock has been suggested to be standardized as much as possible when the feeding experiments have finished.

WP13. Grey Mullet.

Task 13.1. Improvement of larval performance. D13.1. & 13.2. (Month 18) Delayed to June 2017.

Subtask 13.1.1. Improvement of larval performance through adequate first feeding regimes.

An experiment was conducted to test DHA requirements in juvenile grey mullet reared at 15 and 40‰. Six treatments tested in replicates of five 400 l tanks/treatment. Some of the highlights presented by Bill Koven (IOLR) as preliminary results:



- 15 % significantly ($P=0.047$) increased growth over 40 %
- DHA does not significantly ($P>0.05$) affect growth.
- Significant ($P=0.025$) combined salinity-diet DHA (0.8%) effect.
- Current sampling for gene expression, DT enzyme, fatty acid, amino acid and Na⁺-K⁺ATPase analyses.

Sub-task 13.1.2 (IOLR) Using the most effective DHA-aurine diet and salinity to investigate the effect of supplemental arachidonic acid (ArA; 20:4n-6) on larval growth, survival, presence of urinary crystals, as well as synchrony in “silvering” during metamorphosis.

Bill Koven commented that this study is planned for the Fall of 2017.

Task 13.2. Determining mullet nutritional needs for improved weaning to a dry diet. D13.3
(Month 36) Delayed to February 2018.

Sub-task 13.2.1 Determine expression of Tau rate limiting enzyme; cysteine sulfonate decarboxylase (CSD) at various stages (larval and grow out).

Sub-task 13.2.2 Determine expression of rate limiting enzyme of bile salt synthesis, cholesterol 7 α -hydroxylase (CYP7A1) at various stages (larval and grow-out).

Bill Koven pointed out that working primers for these enzymes have been prepared and samples from different stages of the taurine experiment are being analyzed presently.

Task 13.3. Determining mullet nutritional needs for a most cost-effective productions. D13.4
Month 48.

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.

Bill Koven commented that this study is planned for Fall of 2017.



Minutes of GWP Larval Husbandry workshop

Annual Coordination Meeting 2017, Day 3 (19/1/2017, 15:00-18:30)



By Dr. William (Bill) Koven, P4. IOLR (GWP Leader)

WP14 Meagre

This WP was not presented at this session as their tasks have been completed and all deliverables submitted.

WP15 Greater amberjack

In larval amberjack, the results suggested that rotifers supplemented with immune modulator substances such as Echium oil or black cumin oil was beneficial. The sub-task 15.3.1 “Effect of tank hydrodynamics” found marked differences in current profiles when comparing 40,000 l cylindrical and 2,000 l cylindro-conical tanks. In addition, when amberjack eggs were stocked at two different densities (10 and 20 eggs l⁻¹) in four 2000 l tanks (each density was tested in duplicate tanks) and 10 eggs l⁻¹ in two 40,000 l tanks, the results showed survival was significantly higher ($P < 0.05$) in 2,000 l tanks, independent of density, compared to 40,000 l tanks. Moreover, the larvae of 2,000 l tanks, which were stocked with 10 eggs l⁻¹, exhibited the highest total length and body weight compared to the other treatments.

The sub-task 15.3.2 “Effect of light intensity and duration on larval rearing” was repeated in 2016. A non-significant ($P < 0.05$) trend of improved growth and survival in fish reared under a 18L:6D photoperiod was observed as well as increased gene expression of IGF-I, IGF-BP1, IGF-BP3 and IGF-BP5 compared to the 24L:00D photoperiod at specific developmental stages. Different background colors of the rearing tanks (white, green and black) did not have an effect on larval growth (total length and body weight). On the other hand, larvae in the white tanks exhibited the highest survival rate compared to the black and green tanks in both the 2015 and 2016 trials as well as increased expression of genes in the growth axis system. An industrial protocol for greater amberjack will be developed during 2017 led by IEO. The deliverables D15.1, D15.2 and D15.3 have been sent for review and will be submitted shortly.

WP16 Pike perch

Pike perch studies in 2016 included a multifactorial experiment, that determined the effects of four nutritional factors (1 – the onset of weaning at 10 or 16 dph; 2 - continuous or discontinuous diet distribution; 3 - implementing co-feeding or not and 4 - the weaning duration of 3 or 9 days)



and their interactions on pikeperch larval rearing. It was demonstrated that weaned juveniles of 1.0-1.5 g could be produced with good survival (10-13%). This is the first time larval pikeperch have been successfully reared in large tanks (700 l) and over an extended period (53 dph), including the initial phase of feeding on live prey and the weaning period. The multifactorial analysis showed that discontinuous feeding was the most effective while the longer weaning duration (9 days) increased mean swim bladder levels (67%) and final biomass increase (62%) as well as reducing deformities in pikeperch populations. The corresponding deliverable (D16.2) was submitted in 2016. In order to study the effects of population variables (1 - larvae density, 50 or 100 larvae l⁻¹; 2 - geographical origin, Hungarian or Czech strains; 3 – removal or not of cannibals from the population), a second trial was planned in April-May 2016. However due to disease (*Rhabdovirus*), the trial was stopped and will be repeated in 2017.

WP17 Atlantic halibut

In the larval husbandry of halibut, task 17.2 “Recirculation (RAS) vs Flow through (FT) systems during yolk sac and first feeding stages and the effects on larval survival, quality and growth” showed survival and larval quality were similar in RAS and FT systems. However, larval growth was less in the RAS system and this is most likely due to elevated levels of un-ionized ammonia (NH₃). In 2017, the RAS system will run approximately two months before the start of the experiment in order that environmental parameters will stabilize. Task 17.3 “The effect of probiotics on larval microbiota and survival and development of an industrial protocol” is on-going while the Task 17.4 “Comparison of feeding on-grown *Artemia* versus *Artemia* nauplii on larval performance” showed no differences in larval performance between these two groups despite marked differences in their nutrient content. It was concluded that *Artemia* nauplii with its current enrichment and feeding protocols were sufficient to produce fully pigmented halibut fry with proper eye migration. A productive discussion of the importance of hygiene in live feed production followed.

WP18 wreckfish

Progress during the last year was made towards the optimization of the environmental parameters in the rearing of wreckfish. Taking advantage of the improved spawns and the availability of eggs, a number of trials were performed that tested different incubation temperatures. It was shown that the optimal incubation temperature was 16±0.8°C, which elicited normal embryonic development and a hatching rate of 65%. On the other hand, larval survival never exceeded 27 dph and malformed individuals were observed. There was a discussion among the participants on how to improve wreckfish larval rearing. Much of the discussion centered on egg and live food disinfection, which the Norwegian-halibut group have studied extensively. As the yolk stage is very long, it was suggested that the wreckfish researchers try a similar approach to Atlantic halibut rearing where the yolk sac larvae are in the dark. Possibly lower temperatures could also be beneficial and that the reported deformities were very much like those observed in halibut before hygiene protocols were implemented.

PC comments (24 March 2017): It was asked if it makes sense, and would the Norwegian partners (IMR) be interested in receiving some wreckfish eggs for trials in the next 2 years. The PI from IMR replied that this is not possible, as it requires extensive legal documentation, quarantine, etc. But they suggested exchanging personnel (from IMR to Spain, and from IEO-MC2-CMRM to



Norway) during the period of egg incubation for Atlantic halibut and wreckfish, in order to try to exchange ideas about improving the larval rearing protocol for wreckfish.

WP19 Grey mullet

Enzyme analysis in 2016 from task 19.1 “The effect of algal turbidity on larval performance and the ontogeny of digestive tract functionality in the grey mullet (*Mugil cephalus*)” was completed and a manuscript based on the findings of task 19.1 was submitted to the journal of Aquaculture and is presently being reviewed. Previous studies showed that an algal produced turbidity of 1.25 NTU in the rearing tanks was more effective than the no-algae control (0.25 NTU) and low turbidity (0.75 NTU). In 2016, a clay treatment testing the same turbidity was performed, which showed significantly ($P < 0.05$) reduced growth, survival and rotifer ingestion in this treatment compared to larvae exposed to the best performing algal turbidity. This suggests that the algae were providing an unidentified factor that promoted larval performance. Enzyme analysis carried out in 2016 indicated that the ontogeny and activity of the pancreatic and intestinal enzymes were largely genetically programmed with development. In addition, the alkaline protease, amylase and leu-ala peptidase activities indicated a transition from a carnivorous to omnivorous mode of feeding between 40-79 dph. Taking these results into account, weaning diets, designed for a carnivorous, herbivorous or omnivorous mode of feeding, were tested. Fish weaned onto the omnivorous diet demonstrated significantly ($P < 0.05$) improved growth, tank biomass and survival than the other diet treatments. Taken altogether the results suggest larvae should be exposed to an algal produced turbidity of 1.25 NTU in the rearing tanks and weaned, due to sufficient activities of amylase and alkaline proteases, onto a omnivorous weaning diet from 24 to 38 dph.



Minutes of GWP Grow out husbandry workshop

Annual Coordination Meeting 2017, Day 3 (19/1/2017, 15:00-18:30)



By Dr. N. Papandroulakis, P1. HCMR (GWP Leader)

Participants

Nikos Papandroulakis (HCMR; npap@hcmr.gr), Alicia Estevez (IRTA; alicia.estevez@irta.cat), Daniel Montero (FCPTC; daniel.montero@ulpgc.es), Salvador Jerez (IEO; salvador.jerez@ca.ieu.es), Elena Chaves Pozo (IEO; elena.chaves@mu.ieu.es), Patrick Kestemont (<patrick.kestemont@fundp.ac.be>), El Kertaoui Najlae (FUNDP), Bossuyt Jiri (Fish2B), Rocio Robles (CTAqua; r.robles@ctaqua.es), Yiannis Papadakis (HCMR), Babis Syrigos (FORKYS; b.sirigos@yahoo.gr), Antonis Ploumis (FORKYS), Kaliopi Tsakoniti (GMF).

Not present: UL, GEI, DORAQUA, CANEXMAR

The discussion was organized according to the GWP structure.

Not present: GEI, DORAQUA, CTAQUA

WP20 Meagre

T20.1 Deliverable completed

T20.2 HCMR presented the work implemented and planned

T20.2.1 The experiment and the analysis are completed. Presentation Tsalafouta (Day 2). No deviations from DOW.

T20.2.2 First experiment completed. Second is implemented. No deviations from DOW

T20.3 HCMR presented the work done

T20.3.1, 2, experiments and analysis are completed. Presentation Papadakis (Day 2). No deviation from DOW.

T20.3.3 As decided the task is re-organize. A trial with one size class is now implemented for testing efficiency of night feeding. A second trial for testing feeding method will start in Spring 2017.



T20.3.4 IRTA presented the work performed until now. Presentation Duncan (Day2). The experiment will continue for 1 year. It was mentioned that an extra check of the experimental conditions should be done especially in relation to feed losses by installing a camera at the bottom of the tank. No deviation following the amendment.

Deliverables

D20.2: Cannot be delivered as in DOW at M39 because the second trial at ARGO has not finished. The Deliverable should be postponed to end of 2017 (M49)

D20.3: Cannot be delivered as in DOW at M42 because it is required the implementation of the trials currently performed planned by HCMR and IRTA. The Deliverable should be postponed to the beginning of 2018 (M54)

WP21 Greater Amberjack

In general the work performed/planned is as in DOW. A general concern from the partners exists in terms of fry availability. IEO presented the work performed during the last year in Task 21.2.

T21.1

T21.1.1 The trial started with 30,000 individuals delivered by HCMR to FORKYS in August 2016. Of them 12,000 individuals were transferred in cages in September 2016 (8x8x6m; 8x8x10m). In Nov 2016 during sampling 9600 individuals were found and due to high size variability a size selection was performed creating two size classes of MW 458 ± 20 g; 263 ± 19 g. The trial will continue until July 2017. Second trial planned for September 2017.

T21.1.2 CANEXMAR finally resolved the administrative issues for the installation of the submerged cage. A group of 5,000 individuals was lost due to the delays. In January 2017 a second batch was delivered by FCPTC. The implementation of the trial will be monitored by FCPTC.

T21.2

Results on Task 21.2 (Development of feeding methods. Test of different feeding methods including estimation of daily rhythm and frequency. Definition of feeding pattern for 5 g fish reared in 500 l-tanks for 4 months), were presented the first day of the meeting by FCPCT.

21.2. Implementation as in DOW.

21.3.1 The task is implemented by the institutions involved. Three are the remaining trials

- a. temperature of 200 g individuals by HCMR
- b. temperature of 500 g individuals by FCPTC; growth curves presented, final analysis pending
- c. density with 150 g individuals by IEO

Deliverables

Deliverables are expected to be ready on time

WP22 Pike perch.

FUNDP presented the work implemented and planned. Presentation Kestemont Day2.

P39. Fish2B was presented as new partner.



T22.1. The Experiment was focused on light and husbandry practices with a multifactorial/screening. Data were presented last year and was part of the D22.1. A new confirmation experience with challenge test is planned with red light being the main factor improving challenge.

T22.2 The task will start in March 2017 until Dec 2017. ASIALOR left the project and Fish2B is the new partner. Deliverable 22.2 is expected to be delayed by 12 months.

T22.3 HCMR made the genetic analysis and discovered 3 populations: Czech (wild) and two Hungarian (F0, F4). For the trial, the availability of different broodstocks is secured and the larval rearing will be performed at UL. There is no issue with the availability of facilities. The trial is to start on Sept 2017 and will end May 2018. Only critical point the quality of eggs/pre larvae. 5-6 months delay for D22.3 (Mo55).

WP 23 Grey Mullet

T23.1 Deliverable was submitted.

T23.2, 3, 4

CTAQUA presented the work done in Greece, Israel and Spain. Concerns were expressed with the results obtained in the Greek trial (no growth observed in cement tanks) and after a better analysis of the data the trial might need to be repeated. In the case of Israel, several trials have been performed and the results show a clear influence of culture density of fish growth. In the case of earthen ponds in Spain, same results were found in terms of lower growth at high densities; regarding survival in this trial, no complete data are available since the ponds have not been totally harvested. Nevertheless, no delay is expected in this deliverable (D23.3) M40.

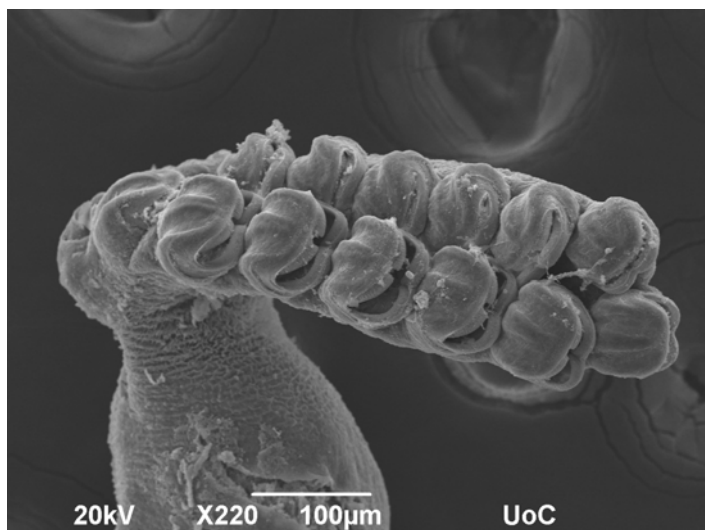
PC comments (24 March 2017): After communicating with the GWP leader, it was realized that most of the relevant partners for the submission of this deliverable (GEI, DOR and HCMR), as well as the WP leader (IOLR) were either not present in the ACM 2017 (GEI, DOR, HCMR) or were missing from the room at the time of the above discussion (IOLR), so the above decision (*i.e.* repeating the trial) was taken with the understanding that it would be confirmed with them. At the time the minutes have been completed, it is still not clear what will happen with this Deliverable and whether will be submitted now as it is, or the trial will be repeated.

Deliverable 23.2, due for May 2016 will be late for approximately 12 months.



Minutes of GWP Fish Health workshop

Annual Coordination Meeting 2017, Day 3 (19/1/2017, 9:00-13:00)



By Dr. C. Secombes, P6. UNIABDN (GWP Leader)

Present

Chris Secombes, Douglas Milne, Cindy Campoverde, Daniel Montero, Pantelis Katharios, Karl Andree, Sonal Patel, Ramon Fontanillas, Aleka Tsalafonta, Maria Ioanna Tsertou, Maria Smyrli, Angeliki Antonakaki and Stavros Chatzifotis.

CS welcomed everyone to the meeting. The discussions were organized per WP/fish species, with a review of progress made against milestones/deliverables (D), with a particular focus on Ds due in the coming 12 months.

WP24 meagre

D. Montero kicked off the discussions with a talk on the latest diet trials to attempt to reduce the development of systemic granulomatosis (SG). Trials had included vitamin C, E and K supplementation. The highest levels of vitamins C and E seemed to reduce the incidence of SG, but the studies were confounded by a high initial incidence (45%), such that the reduced levels were still very high (80%).

Discussion was had as to whether SG could be reversible. It was thought to be unlikely in adults but may be possibilities with larvae. It was noted that the histology suggests the disease begins as a vasculitis.

PK next outlined work in Greece. An association with crystals in the centre of the granulomas has been noted, and overall the disease is similar to sarcoidosis in humans. Large numbers of rodlet cells occur in the peritoneal membranes, which contain piscidin. Vitamin D supplementation (4 inclusion levels) had no effects on SG (or growth). The dietary Ca/P ratio was also studied, in diets where $\text{Ca} > \text{P}$, $\text{Ca} = \text{P}$ and $\text{Ca} < \text{P}$. Some effects of P were noted. Plant protein containing diets were also investigated, but had a negative effect on SG.



A possible link of SG with *Nocardia* was investigated, but no association was found. *Nocardia* was found, and shown to cause characteristic lesions and granulomas but always in association with bacteria unlike the case in SG. The strain was sequence verified and has shown to have a single nucleotide difference to the reference strain.

Some studies of meagre immune responses were also reported by AT, in studies of shallow vs deep nets.

DM reported on a *Nocardia* challenge model. With a dose $>10^6$ bacteria/fish (70g fish), the fish started to die from day 39, reaching ~16% mortality. *Nocardia* was recovered from all the fish injected with higher doses (10^7 or 10^8) and ~25% of fish at lower doses. Granulomas were apparent in 25-50% of the fish.

KA reported on a vaccine trial against vibriosis. The vaccine was presumed polyvalent (proprietary info from company prevents knowing with certainty), containing multiple strains. 420 fish were used, and sampling (immune tissues) post-vaccination was performed ahead of an anticipated challenge at week 8. Unfortunately at week 7 fish began to die and were found to be infected with *Cryptocaryon irritans* (or *Amyloodinium ocellatum?*), and the experiment was terminated. It will be repeated in the autumn of 2017, with inclusion of sentinels. As a consequence D24.6 and D24.10 have been pushed back to month 48, however in further discussion it was realised that D24.10 will need additional time, to allow the analysis to be completed. **Request a delay to month 52 for D24.10.**

KA also reported on a study of anti-parasitic treatments, where inclusion of cinnamon had been particularly effective.

WP25 Greater amberjack

PK reported results on studies of epitheliocystis. Two new bacterial species were associated with the disease, that appears to be due to a pathogen complex. Can be a problem for the grow-out fish but is not host specific. Is a particular problem when combined with monogenean infections. These results will go into the manual (D25.8). PK also reported on a major *Cryptocaryon* outbreak in amberjack that killed large fish within 1 day!

CS reported on the cloning of the immune markers for D25.1 and studies in collaboration with P2 on modulation of these genes by stimulation with PAMPs in vitro and in vivo.

D. Montero reported on a temperature rearing experiment with fish held at 17°C, 22°C or 26°C. No parasites were detected at 17°C, but the prevalence was 29% at 22°C and 62% at 26°C. Fish density experiments were also undertaken (low vs high). Higher cortisol was seen in the high-density groups (~120 ng/ml plasma vs control of ~20 ng/ml). Similar results were found with mucus cortisol levels. Dietary trials with Biomos, Actigen or both combined were also undertaken. Sampling occurred at days 60 and 90, for histology and gene expression analysis. A parasite challenge was also performed 15 days after the final sampling. Eggs were collected and used for infection of fish, to generate infected fish for cohabitation trials. Whilst no effects on growth were found with the different diets there was a positive effect of Actigen (+/- Biomos). It will be interesting to see if this is also apparent in the gene expression analysis (on-going in collaboration with P5).



In relation to D25.2 it was reported that proteomic analysis of mucus by an external source was still not back, and that a student visit from P2 to P5 was due February to April inclusive, and would contribute results to this D. So a delay to month 43 (June 2017) would be requested.

Lastly, bacterial/viral screens had been carried out over the last year, with bacterial species being identified, namely *V. harveyi* and *V. alginolyticus*, as well as *Photobacterium damsela* spp. *piscicida*.

WP26 Atlantic halibut. SP next reported on progress with multiple expression systems being studied for production of the capsid protein of VNN. However, there had been a few delays getting the recombinant capsid protein made in tobacco, and some aquarium issues that have led to **D26.2 and D26.3 being pushed back to month 48**. Once the tobacco leaves are received they will be frozen, ground and then fed to *Artemia*, ready for vaccination trials (using *Artemia* as delivery system). Researchers in TargetFish project have been contacted and the best candidate that has shown promising results in sea bass will be possibly delivered to IMR for inclusion in halibut trial during spring 2017. After challenge of vaccinated fish neutralising antibodies and viral load will be measured.

The meeting was closed at 13.00.



Minutes of GWP Socioeconomics workshop

Annual Coordination Meeting 2017, Day 3 (19/1/2017, 9:00-18:30)



By Dr. G. Tacken, P5. DLO (GWP Leader)

Attendees:

Present: Gemma Tacken (chairman), Rocio Robles, Hellas Saltavarea, Kostas Larentzakis, Martina Ferreira Novio, Richard Bou, Lluís Guerrero, Anna Claret, Oxana Lazo, Karen Brunsø, Marija Banovic, Thanasis Krystallis, Cornelis van der Lelie, Kriton Grigorakis, Ed Nijssen and Machiel Reinders (minutes)

Dissemination:

- GWP Socio-economics has most accepted scientific papers of all GWPs! Congratulations!
- Total aim: 10 scientific papers! There are already some others in the pipeline, so we expect to reach that. Please don't forget to upload all dissemination activities (including seminars, presentations, etc.) in ECAS.
- We are successful in scientific papers, but we have to publish more in magazines for professionals in the sector, articles for magazines aimed at public policy makers, consumers and business (aquaculture sector). For example, see article in Aqua Feed International 2016.
- New dissemination opportunity: EAS-meeting in Dubrovnik in October 20-23rd, 2017.
- As dissemination activity to local chain partners in May, a workshop in Bremen, Germany will be organised by Rocio Robles. Possibility to disseminate our activities or give presentations based on the Deliverables in this GWP. Gemma stresses that for consumer issues first people in the project have to be asked, starting by first authors of deliverables.

WP28:

Update activities:

- Tasks 28.1 and 28.2 are finished. Now focus on Task 28.3.
- Activities are ahead of schedule: Sensory analysis of the WP is finished.
- Sub-task 28.3.2 (nutritional-rearing history): Cova (P15. ULL) has asked to check whether there will be enough testing materials for all species to do this task? The reaction is that pikeperch, greater amberjack, grey mullet and meagre are available. Wreckfish was for all other tasks in



WP 28 not available, while Atlantic halibut was not considered in this WP. So these two species will not be considered in 28.3.2.

WP29:

Deliverables to be expected:

- D29.6 (Report on the experimentation with product mock-ups) was planned for November 2016
> will be end February 2017

Action: Gemma informs the PC about the delay of Deliverable 29.6

- D29.7 and D29.8, regarding the communication experiment, are expected for end of May and July 2017 respectively. Specific aspects regarding this task:
 - o Test the effect of species on choice: fillet with the different types of species (meagre, greater amberjack, pikeperch and grey mullet);
 - o Decision based on discussion, we have 3 products:
 - Fresh fillet > sampling on existing criteria (involved innovators and involved traditional).
 - Fillet in olive oil > sampling on existing criteria (involved innovators and involved traditional).
 - Ready-to-eat salad > other sampling criteria: people that are health and convenience orientated, but they also have to eat fish at least once a month.
 - o Target segments: Given the questions in the plenary sessions we have to consider whether we do stick to the segments we have used so far? Based on the mock-up experiment we can look whether there are other segments too. Also the data set of the consumer survey can be used to check whether there are other interesting segments. Another option is that we stick to the two segments (involved innovators and involved traditional) that we used so far and enlarge the screening criteria by incorporating other aspects, like health orientation or convenience orientation, and relax some of the behavioural criteria (fish consumption).
 - o Suggestion by Thanasis: drop the division between innovativeness and traditional and keep only involvement (and consumption of at least once per month) and extend this with a short version of the food choice questionnaire (and health and convenience orientation) to allow to get insight in food choice motives.
 - Customize the recruitment per country on fish consumption, involvement, etc. If budget allows to do this.
 - o Think of the type of communication stimuli that we want to experiment with.
 - Convenience
 - Country-of-origin
 - Sustainability
 - Variations on communication means
- **Deadline of delivery WP 29: August 1st 2017**

Action: Thanasis and Marija make a data based choice whether new segments are introduced



WP30:

Task 30.1 Business modelling

- Task 30.1 is split up into 3 sub-tasks. Sub-task 30.1.1 = value proposition and target segments; sub-task 30.1.2 = resources necessary and key partners; sub-task 30.1.3 = price and cost structures and possibilities to further drive down the costs.
- Focus is on the 4 species (meagre, pikeperch, grey mullet and greater amberjack) in sub-task 30.1.1
- Focus is on the products (fish fillet, fish in olive oil and salad) in sub-tasks 30.1.2 and 30.1.3. So, the description of D30.4 is not correct: it should be products instead of species, because of the funnelling approach that we adopted in this GWP.

Action: Gemma informs the PC that the description of D30.4 is not right. It should be product instead of species

Task 30.2 New product marketing strategy development (test markets)

Three options:

- If there are physical products available we can do a real-life test market. But we then have to make available all these products in the five countries available.
- Make use of the virtual supermarket (as used by Wageningen University). Possibility for eye tracking etc. But we then have to travel with a bus with this supermarket to all the countries, which is very time consuming and expensive.
- Create an online shopping environment, where participants can choose from different fish products.

Manipulations:

- Store: supermarket or specialty store/ monger
- Promotion: yes or no
- Price: high or low

Phases in in-store experiments:

1. Observation: do people notice that there is a new product?
2. Ask questions: did they notice the new product? Did they like it?
3. Are they considering buying it? For example, if people have chosen another product: are you willing to switch this product for the new product?

Balancing between a more restrictive designed experiment (but how realistic is this as compared to a real market launch) and a real market simulation (with the risk that people will not buy the new product).

Thanasys: can we merge this test market with the communication experiment?

First ask background questions, expose participants to communication, give them an amount of money and let them go shopping (in a virtual environment) with questions afterwards (did you notice the new product, did you buy it, why (not), and are you willing to switch when confronted with the new product?). If you don't merge the tasks 29.4 and 30.2.2, how would you then link these tasks?



All pro's and con's of integration and not integration are discussed and the conclusion is:

- 29.4 and 30.2.2 will not be merged

Special Session on Socioeconomics-market testing

Present: Gemma Tacke (GWP-leader), Hellas Saltavarea, Kostas Larentzakis, Lluís Guerrero, Marija Banovic, Thanasis Krystallis, Ed Nijssen and Machiel Reinders

The indication is that it is not realistic to perform the test as stated in the text of the DoW. However, we first must make sure if products can be delivered and when products can be delivered.

We have a request for a case study with pikeperch in Carrefour in Belgium (Cornelis van der Lelie). For pikeperch we have products, but we have to discuss with partners in Spain (Xavier, Miguel, Martina) and Germany (Matthias), if it is possible to roll out on a broader scale for pikeperch.

If it is not possible to conduct the study completely as stated in the DoW, but we can do a real-life experiment on a smaller scale, and next to that we can do a virtual, online experiment. However, we first have to check whether this is possible given the budget.

Action Gemma asks the PC: can we use the budget of the SMEs that have withdrawn from the project for the experiment or not?

Comment by PC (23 March 2017): Gemma, at this stage the money have been re allocated to other partners during the 3rd Amendment. Depending on the amount that you are talking about, and the partner that will receive the money (it has to be an SME to keep their share >15%), then it may be possible to get some money from another SME, as I foresee that we will make further modifications in the coming year. BUT, I cannot promise you this right now. I will know more in 2-3 months

We agree on the following procedure:

- First check whether we can perform the market test in the original way as stated in the DoW: experimental design/ checklist for the SMEs with what we need for a real market test.
 - o Ed already has this design (Taguchi-experiment)
 - o We need an answer of the SMEs to have a go/ no go:
 - Availability of products
 - Availability of partners
 - Whether things can be settled in time
 - o Decision should be made by the 1st of May.

Action before by Gemma: Brussels should have been informed about the alternative, which has to be approved

- Parallel to this, we start designing the online experiment: online questionnaire that resembles an online shopping environment conducted in the 5 countries for those species/products that at this moment are in such stage that they are/ become in the market.



- Participating institutes should check their finances whether it is possible to re-allocate travel budget to material costs for the experiment.

Action: Gemma informs the PC whether re-allocation of budget of e.g, travelling costs to market research for online test

Comment by PC (23 March 2017): Yes, it is possible to reallocate money (within Partners). If it is going to be spent by the same partner (“material costs” through regular invoices), there is no problem, but if it is going to be subcontracted, then this is different and we need an amendment and prior justification and approval.

- APROMAR is involved in Task 30.1 with more than 8 men-months: we can ask them to survey farmers in Task 30.1, but not for doing something in Task 30.2

Design of the online market test experiment:

3 or 4 products:

- Fresh fillet
- Smoked fillet
- Fish in olive oil
- Fishburger or ready-to-eat salad

4 species: meagre, greater amberjack, pikeperch, grey mullet, or a selection

Procedure:

- Provide fictitious budget of e.g. €40 and ask participants to compile products for a meal for 4 persons with fish.
- First let them shop in a free way (unguided).
- Then provide some information/ stimuli (guided shopping).
- After they decide on a product, and if it is another product than one of the new species, ask them whether they are willing to switch.
- Ask other questions afterwards.

We propose to have an additional meeting on 18 May 2017 (Amsterdam Schiphol or Brussels) to further discuss the design of the online experiment.

Action point: TU/e chooses a location and organizes the meeting

Alternative idea by Lluís:

Qualitative study/ ethnographic approach > go shopping with consumers in one shop. You need only one shop and it is possible to get the products there (by IRTA, CTAQUA or a fish monger organisation like the one of Miguel).

Disadvantage is that it is qualitative data and that this is relatively expensive (in terms of recruiting consumers).



Task 30.3

Activities:

- It is good to already start with sub-task 30.3.1. Therefore, Wageningen first searches for the feasibility study criteria. Contributions from Eindhoven and IRTA:
 - o IRTA: new product development (Richard Bou)
 - o TU/e: input based on cost structure of Task 30.1/ farmers
- It is also good to start with the end-report of 30.3.2 (Recommendations) by gathering the recommendations that we already have based on the tasks done.

This concludes the minutes of the ACM 2017.



A group photo of some of the participants of DIVERSIFY ACM 2017 at the beautiful staircase of Palau Macaya, Barcelona, Spain.

Deviations from the DOW

The ACMs were planned in the DOW to consist of 2-days of open presentations and 1 for consortium activities. However, the previous ACM 2014 (Bari, Italy) and ACM 2016 (Nancy, France) contained only 1 open day and 2 days reserved for consortium activities. This was considered necessary because of the large



number of Work Packages in the project, and the need for as much time as possible to be allocated to the discussion of obtained results and future planning of the work, as well as the preparation of the 1st and 2nd Periodic Reports (Mo 12 and Mo 36). However, as this time there was no periodic report due, until a year later (January 2018), we returned to the originally planned format of having 2-days of open presentations and 1 for consortium activities and planning of upcoming activities. This decision was already taken after the previous ACM (2016) and was reported in ***Deliverable 1.6 Annual Coordination Meeting for Y3***. This format allowed all Partners to have a detailed view of the progress of the project after 3 years and will disseminate the information to a larger invited guest audience.

There were no other major deviations from the DOW at this time. Some delays in the uploading of the Deliverables have been discussed (and mentioned in the minutes of the GWP Workshops), but they are not considered major in kind. Also, there are a number of expected delays in some of the upcoming deliverables, but so far there is no expectation of any Deliverables not been completed within the lifespan of the project. These expected delays have been mentioned within the minutes of the specific GWP workshops reported in the previous pages.