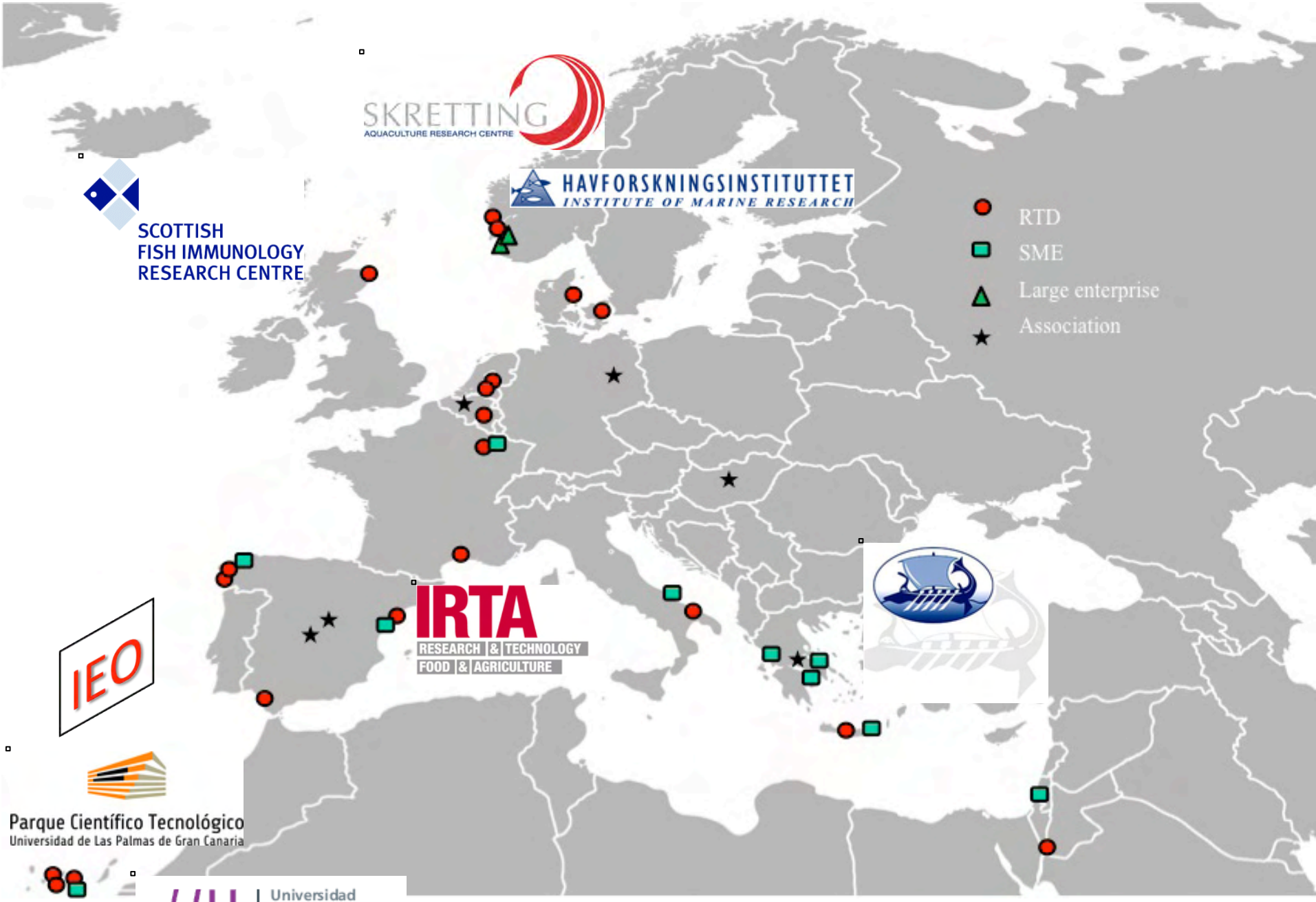
GWP
Fish Health

DIVERSIFY
7FP---KBBE---2013---603121
Coordination Meeting
Nancy 2-4 February 2016


SKRETTING
AQUACULTURE RESEARCH CENTRE
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Universidad de Las Palmas de Gran Canaria
ULL
Universidad
de La Laguna

HCMR (P1)
FCPCT
IRTA
UNIABDN
SARC

WP 24 Fish health - meagre

HCMR
FCPCT
UNIABDN (P5)
IEO
ULL

WP 25 Fish health - greater amberjack

IMR (P7)

WP 26 Fish health - Atlantic halibut

GWP Fish Health

*continues from previous page (3/3)

GWP	Fish Health	Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
		Ma 3	Ju 6	Se 9	De 12	Ma 15	Ju 18	Se 21	De 24	Ma 27	Ju 30	Se 33	De 36	Ma 39	Ju 42	Se 45	De 48	Ma 51	Ju 54	Se 57	De 60
WP24	Fish health-meagre																				
	Task 24.1 Systematic Granulomatosis																				
	Task 24.2 Chronic Ulcerative Dermatopathy																				
	Task 24.3 Anti-parasitic treatments																				
	Task 24.4 *Mocarella* infection in meagre																				
	Task 24.5 First characterisation of the immune system																				
	Task 24.6 Monitor specific immune responses																				
	Task 24.7 Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in meagre																				
	Task 24.8 Diagnostic-recommendation manual for meagre health																				
WP25	Fish-health-greater amberjack																				
	Task 25.1 Study of Epitheliocystis during larval rearing																				
	Task 25.2 Promoting resistance to parasitic incidence on greater amberjack																				
	Task 25.3 Identification of immune markers																				
	Task 25.4 Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites																				
	Task 25.5 Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in greater amberjack																				
	Task 25.6 Diagnostic-recommendation manual for greater amberjack health																				
WP26	Fish-health-Atlantic halibut																				
	Task 26.1 Production of VNN capsid protein																				
	Task 26.2 Monitor and assess immune response and protection																				

WP24 Deliverables:

Month 20 (moved from Month 12)

D24.1 - The effect of vitamin D inclusions in diets in the development of Systemic Granulomatosis in meagre.

Month 30 (moved from Month 24 – now due for end May 2016)

D24.2 - The effect of Ca/P ratio in the diet in the development of Systemic Granulomatosis in meagre.

Month 26

D24.3 - Cloning of key marker genes of innate and adaptive immune responses in meagre.

Deliverable Report

Deliverable No:	D24.1	**Delivery Month:**	26
Deliverable Title	The effect of vitamin D inclusions in diets in the development of Systemic Granulomatosis in meagre		
WP No:	24	**WP Lead beneficiary:**	P1. HCMR
WP Title:	Fish health - meagre		
Task No:	24.1	**Task Lead beneficiary:**	P1. HCMR
Task Title:	Systemic Granulomatosis Subtask 24.1.1 Feeding trials. Trial 1		
Other beneficiaries:	P20.SARC		
Status:	Delivered	**Expected month:**	24
.....			

Lead Scientist preparing the Deliverable: Pantelis Katharios (HCMR)

Other Scientists participating: Tsertou, M. (HCMR), Cotou, E. (HCMR), Foundoulaki, E. (HCMR), Chatzifotis, S. (HCMR)

Objective

The objective of this study was to examine the role of vitamin D on the development of Systemic Granulomatosis (SG).

FP7-KBBE-2013-07, DIVERSIFY 603121

Deliverable Report

Deliverable No:	D24.3	**Delivery Month:**	26
Deliverable Title	Cloning of key marker genes of innate and adaptive immune responses in meagre.		
WP No:	24	**WP Lead beneficiary:**	P5. UNIABDN
WP Title:	Fish Health– Meagre		
Task No:	24.5	**Task Lead beneficiary:**	P5. UNIABDN
Task Title:	First characterization of the immune system		
Other beneficiaries:	P3. IRTA		
Status:	Delivered	**Expected month:**	26
.....			

Lead Scientist preparing the Deliverable: Secombes, C.J. (UNIABDN)

Other Scientists participating: Andree, K. (IRTA), Estévez, A. (IRTA)

Objective: The objective of this Deliverable was to develop quantitative PCR assays for investigating the development of the immune system during grow-out of meagre, *Argyrosomus regius*.

WP25 Deliverables:

None before Month 39!!

WP26 Deliverables:

Month 24

D26.1 - Assess the use of two eukaryotic expression systems; microalgae and a protozoa (*Leishmania tarentolae*) for production of nodavirus capsid protein.

Deliverable Report

Deliverable No:	D26.1	**Delivery Month:**	24
Deliverable Title	Assess the use of two eukaryotic expression systems; microalgae and a protozoan (*Leishmania tarentolae*) for production of nodavirus capsid protein		
WP No:	26	**WP Lead beneficiary:**	P7. IMR
WP Title:	Fish Health – Atlantic halibut		
Task No:	26.1	**Task Lead beneficiary:**	P7. IMR
Task Title:	Production of VNN capsid protein		
Other beneficiaries:			
Status:	Delivered	**Expected month:**	24
.....			

Lead Scientist preparing the Deliverable: Patel, S. (IMR)

Other Scientists participating: Nerland, A.H. (IMR)

Objective: The objective of this Deliverable is to assess the two eukaryotic systems -microalgae and a protozoan (*L. tarentolae*)- for the production of nodavirus (Viral Neural Necrosis, VNN) capsid protein, to be used for the development of an oral vaccine for Atlantic halibut (*Hippoglossus hippoglossus*). We have worked on several alternatives for the expression of nodavirus capsid protein in this task and we have two vaccine candidates: a vaccine with recombinant nodavirus capsid protein expressed in *E. coli*, which previously has been shown to elicit protection in turbot (*Scophthalmus maximus*) and to induce an immune response in Atlantic halibut with a protective character. Recombinant capsid protein has also been expressed in tobacco plants, from which it can be isolated as virus like particles (VLP) for integration in a vaccine.

DIVERSIFY

Meagre

WP 24. HEALTH - MEAGRE (*Argyrosomus regius*)

RESULTS OF THE EU DIVERSIFY PROJECT IN 2014 AND 2015

Christopher Secombes, Douglas Milne
(University of Aberdeen, Scotland)

Alicia Estévez, Neil Duncan, Karl Andree, Ana Roque, Cindy Campoverde
(IRTA, Spain)

Pantelis Katharios, Stavros Chatzifotis, Maria Ioanna Tsertou
(HCMR, Greece)

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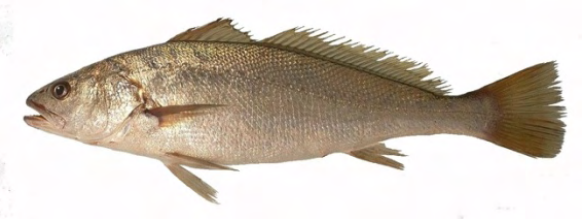
Meagre



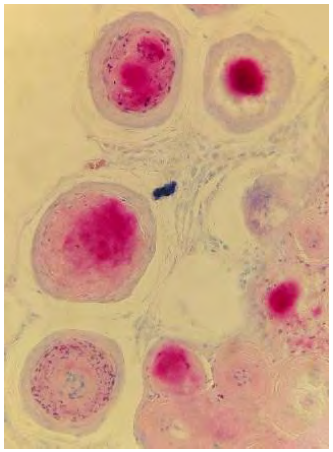
Task 24.1. Systemic granulomatosis and nutrition (vitamin D, Ca/P, plant protein, vit E, C and carotenoids, minerals)

Two trials carried out at HCMR using different levels of vitamin D and Ca/P to observe effects on the appearance of granulomatosis.

1st trial: Effect of Vitamin D – Jul- Oct 2014

- ✓ **Samplings every month**
  - •12 tanks (500 L)
  - • 600 fish
  - • Evaluation of granulomas, histology
  - • Histology
  - • Vitamin D determination & plasma analysis

Diet	IU/Kg
D0	4550
D1	7000
D2	10000
D3	20000



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Meagre

Task 24.1. Systemic granulomatosis and nutrition (vitamin D, Ca/P, plant protein, vit E, C and carotenoids, minerals)

2nd trial: Effect of Ca/P ratio

- June – October 2015
- 9 diets
- 27 tanks
- 1350 fish

	1	2	3	4	5	6	7	8	9
Ca(g/Kg)	5,49	6,44	12,83	5,49	10	20	5,55	15	30
P (g/Kg)	6,44	6,44	6,42	10	10	10	15,58	15	15

✓ **Samplings every month**

- Evaluation of granulomas
- Histology
- Ca/P and specific biomarkers determination
- Growth performance
- Plasma analysis

Results under analysis

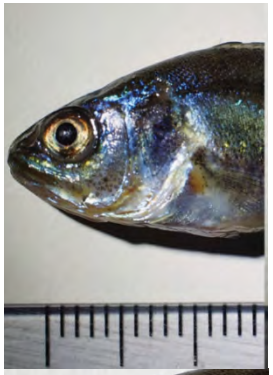
DIVERSIFY

Meagre

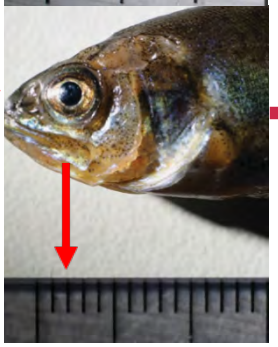
24.2.-Chronic Ulcerative Dermatopathy (CUD) associated with the use of borehole water

- May – July 2015
- 2 parallel larval rearings (B.H. water vs seawater)
- 4 tanks (40 m^3)
- monitoring of pH, CO_2, salinity

Seawater



Borehole water



✓ Samplings (1 - 56dph)

- Histology
- SEM
- Histochemistry
- RT - PCR

First results

All fish reared on borehole water had lesions from CUD. Lab analysis is under way.

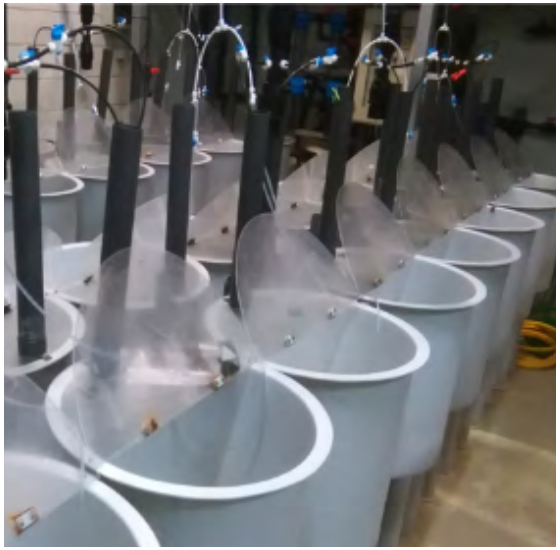
Meagre

24.3.-Anti-parasitic treatments

- Preliminary experiment carried out at IRTA indicated that fish will eat pellets containing essential oils of bergamot, mint and cinnamon out of 15 different essential oils with antiparasitic properties.

  - 4 groups, (12 fish each - Avg 60.96g)
  - fed 2g daily, 45 min/day, for 6 weeks

- Diets:
  - control- fish oil (control)
  - praziquantel (in fish oil)
  - mint essential oil
  - cinnamon essential oil



Consumption was estimated everyday collecting leftover food, in the end of the experiment, fish were sacrificed and samples were taken for histology and immunology.

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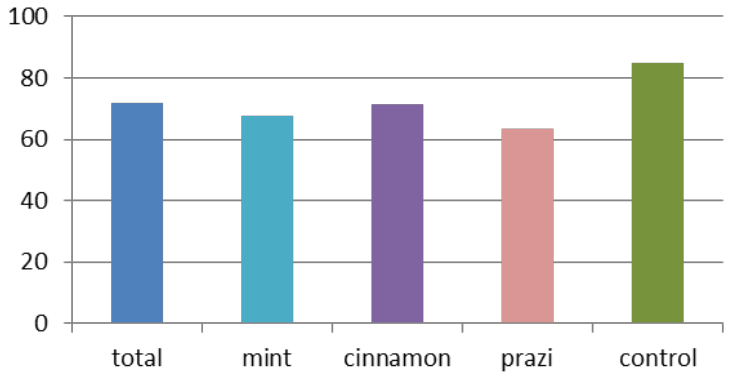
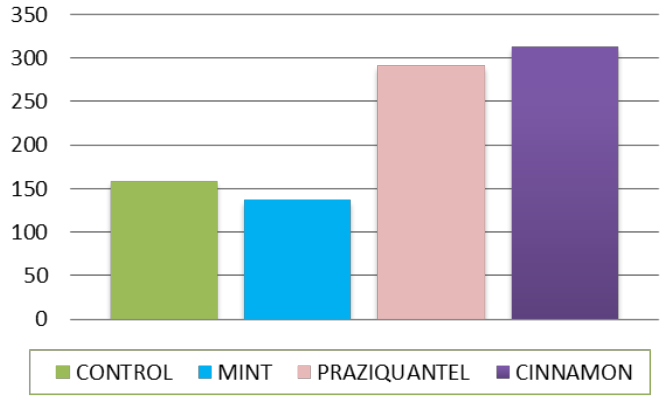
Meagre

24.3.-Anti-parasitic treatments

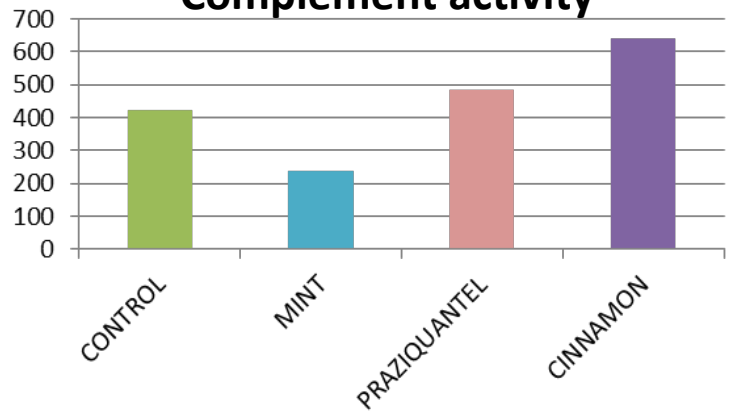
Final weights



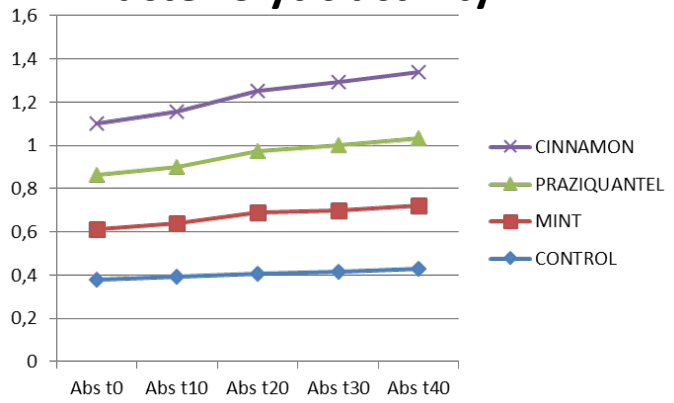
Lysozyme



Complement activity



Bacteriolytic activity

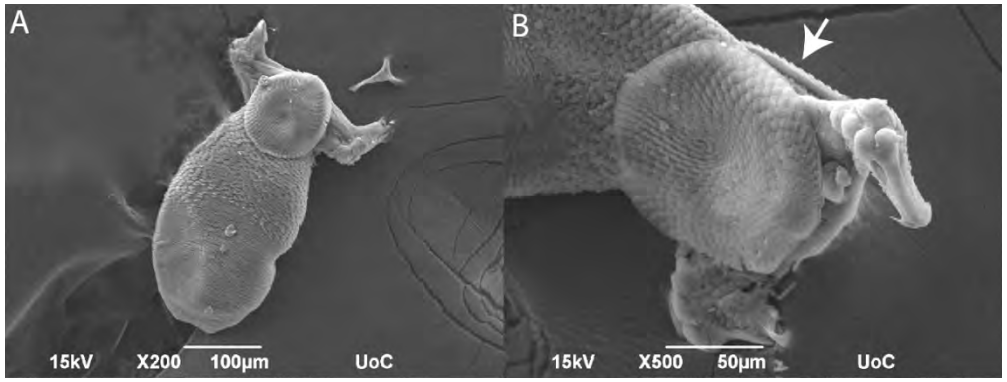


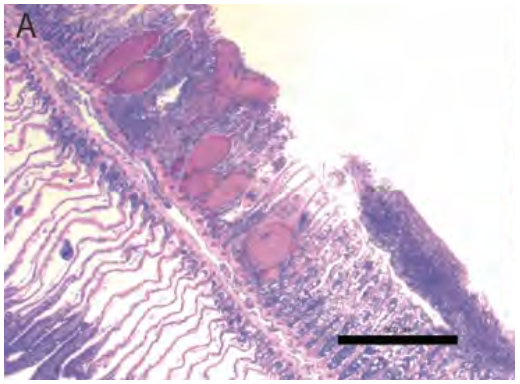
24.3.- Manuscript in preparation: parasite description

Repeated gill infections with a monogenean and deaths of some broodstock lead to development of a case report for publication.

Title:

Diplectanum sciaenae (Van Beneden & Hesse, 1863) (Monogenea) infecting meagre, *Argyrosmus regius* (Asso, 1801) broodstock. A case report.





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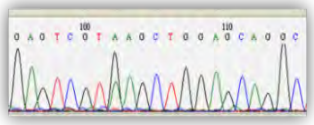
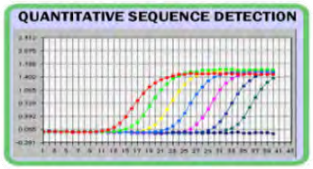
Meagre

24.5.- Characterization of the Immune System

Specific growth rate calculated during grow-out, and duplicate sets of chronological samples for the immune ontogeny were collected from relevant tissues. Tissues collected were spleen, head kidney, gills, and intestine.





Degenerate/consensus primers designed using homologous sequences of other species obtained from GenBank, successfully amplified gene fragments from meagre cDNA. **A total of 28 gene expression assays (23 immune genes and 5 endogenous control genes) are now ready** for analysis in the coming year (2016).

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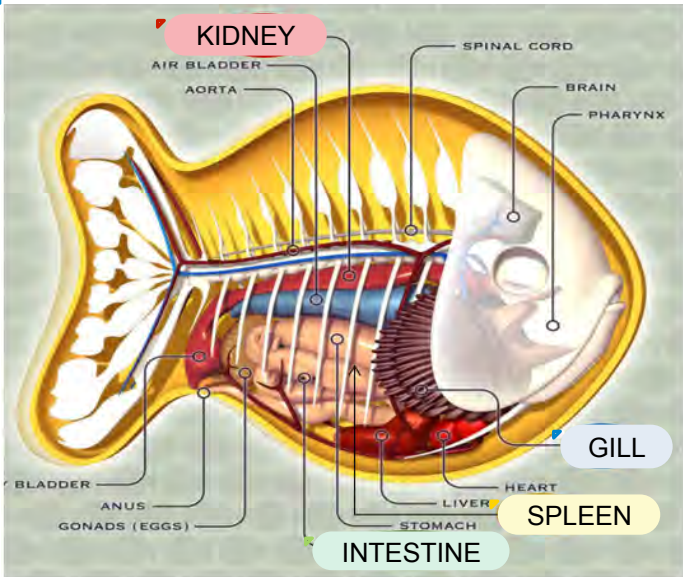
Meagre

24.5.- Characterization of the Immune System

	Immune function	Amplicon Size (bp)	Efficiency % E
Endogenous Controls	18S ribosomal RNA	140	100.10
	elongation factor 1a (EF1a)	186	100.80
	glyceraldehyde phosphate dehydrogenase (GAPDH)	109	100.00
	Beta Actin	212	100.05
	Hypoxanthine-guanine phosphoribosyltransferase (HPF	160	100.95
Target Genes	Metallothionein	70	102.20
	Lysozyme	148	100.0
	Hepcidin	140	95.90
	piscicidin	111	101.95
	defensin	138	102.30
	C3 complement (C3)	120	100.10
	MX protein (MXP)	187	98.20
	nucleotide oligomerizing domain 2 (NOD 2)	~	~
	nucleotide oligomerizing domain 3 (NOD 3)	154	100.80
	tumor necrosis factor 1a (TNF1a)	104	99.20
	Immunoglobulin M (IgM)	304	99.85
	Immunoglobulin T (IgT)	104	100.00
	recombination activating gene (RAG1)	144	100.40
	Myeloid differentiation primary response 88 (MyD 88)	120	102.20
	T-cell receptor beta, heavy chain (TCRb)	281	103.15
	cyclooxygenase (COX2)	211	100.50
	Interleukin 1B (IL-1b)	103	99.60
	Interleukin 4/13 (IL-4/13)	107	99.00
	Interleukin 10 (IL-10)	187	102.15
	Interleukin 17 (IL-17)	91	96.10
	Interleukin 22 (IL-22)	146	101.75
	Interferon type 1 (INF 1)	178	100.10
	Interferon gamma (INF g)	171	99.85



RNA extractions and cDNA synthesis of samples for immune ontogeny gene expression study are underway.

Sequence analysis

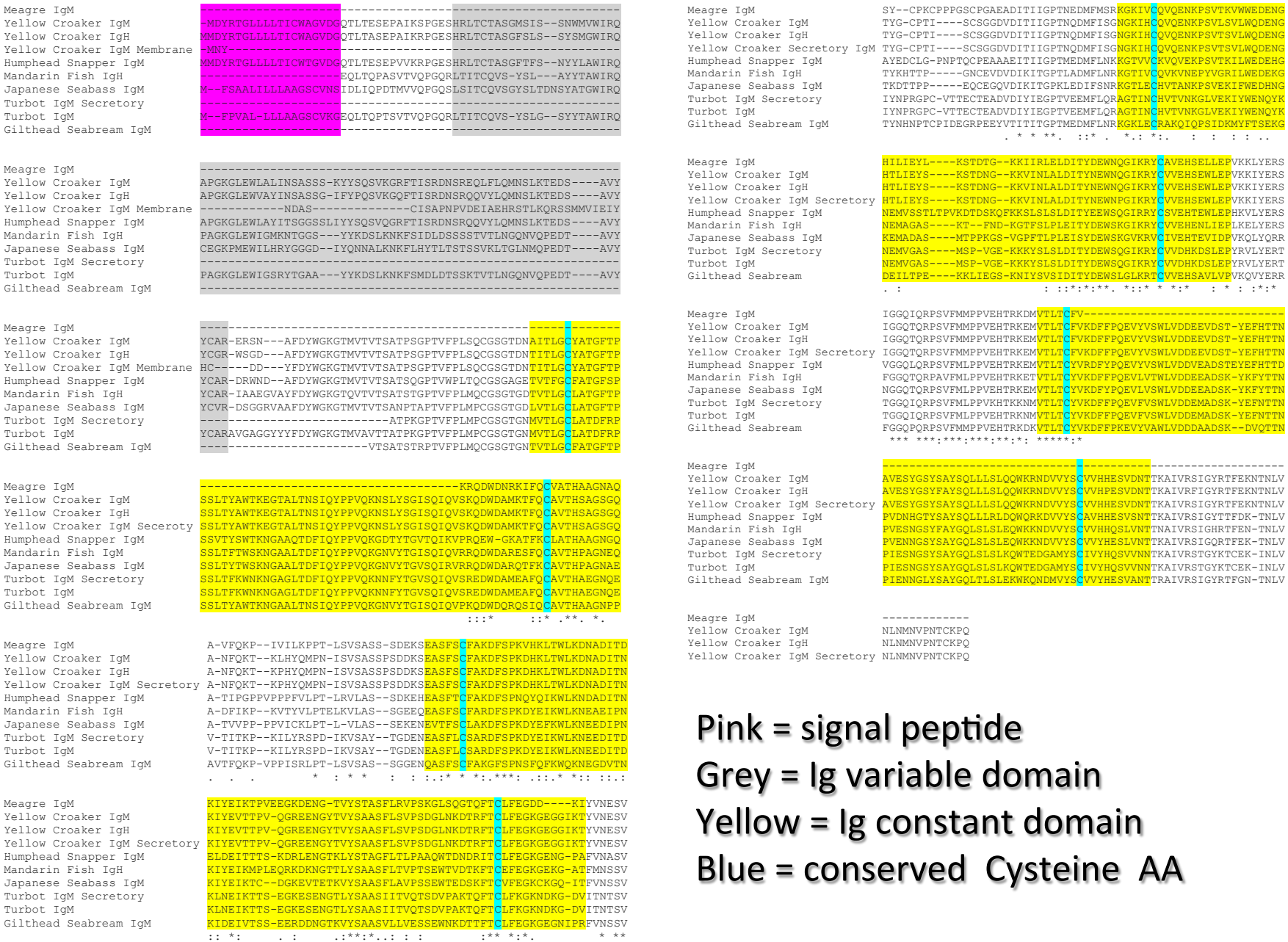
Using Meagre IgM as an example, gene sequences were analysed as follows :

1) Sequence was subjected to blast analysis and the top hits noted

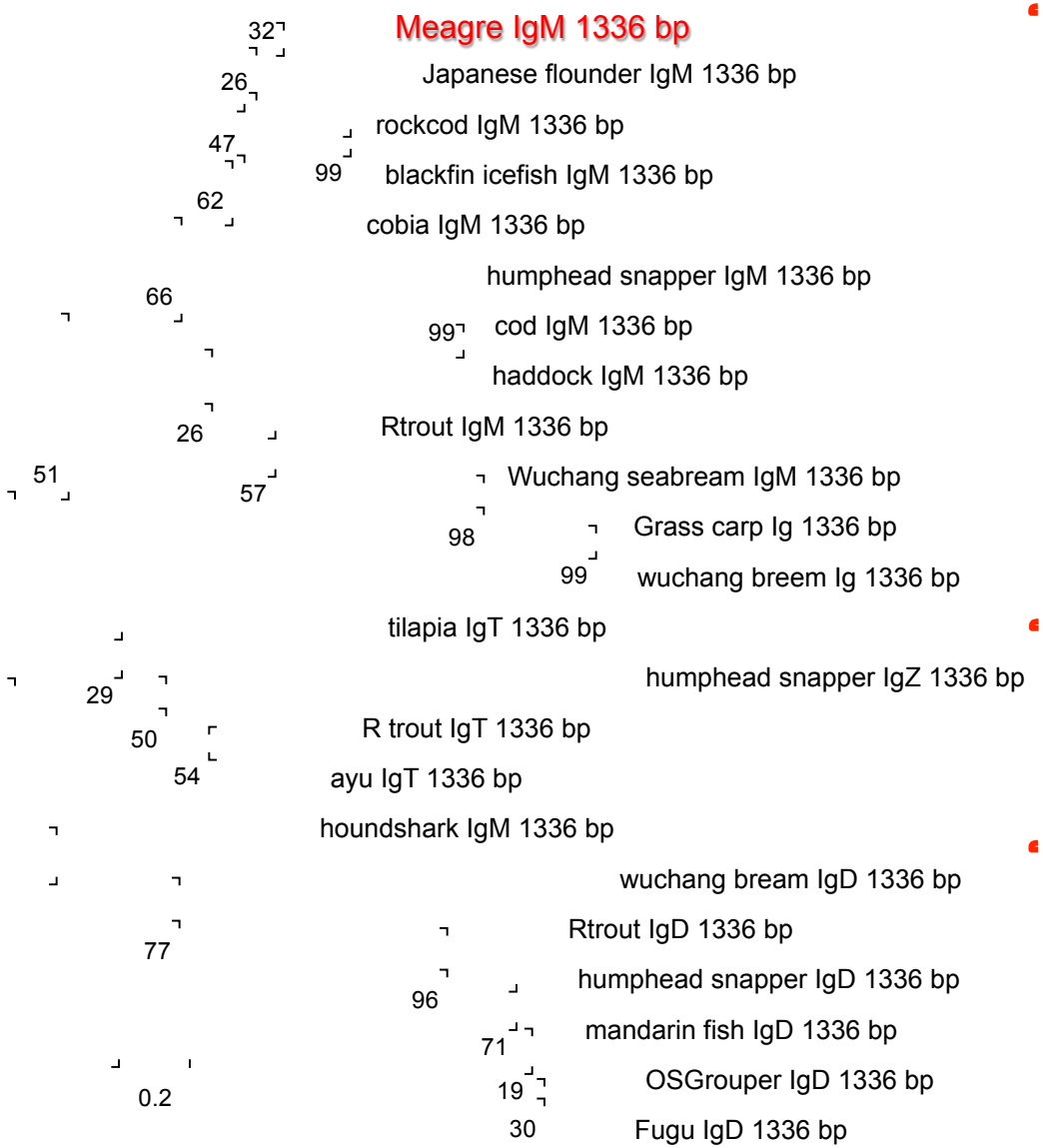
=

Species	Gene	Max Score	Total score	Coverage (%)	E-Value	Identity (%)	Accession
Large Yellow Croaker	IgM Heavy Chain	406	406	99	4e-135	73	ACM24795.1
Large Yellow Croaker	Ig Heavy Chain	405	405	99	8e-135	73	ACF22903.1
Large Yellow Croaker	IgM constant region membrane bound	402	402	99	1e-134	73	KKF16383.1
Mandarin fish	Ig Heavy Chain	304	304	100	1e-95	56	AAQ14862.1
Humphead snapper	IgM heavy chain	293	293	99	3e-91	55	ADX01345.1
New Zealand Grouper	IgM heavy chain variable region	274	274	87	1e-87	59	AEX93386.1
Turbot	IgM heavy chain secretory	279	279	99	3e-87	51	AII00835.1
Turbot	IgM heavy chain	283	283	99	4e-87	52	AGE84011.1
Gilthead Seabream	IgM heavy chain	275	275	99	1e-85	54	AFN20639.1
Japanese Sea Bass	IgM heavy chain	272	272	100	4e-83	52	AGT99002.1

2) Sequence is aligned with top Blast hits and protein domains Identified



Meagre



Teleost IgM

Teleost IgT/Z

Teleost IgD

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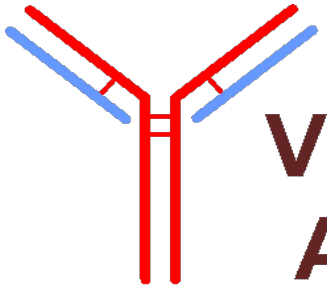
Meagre

24.5.- Characterization of the Immune System

Production of antibodies to Meagre Ig, for future determination of specific antibody titres following vaccination.

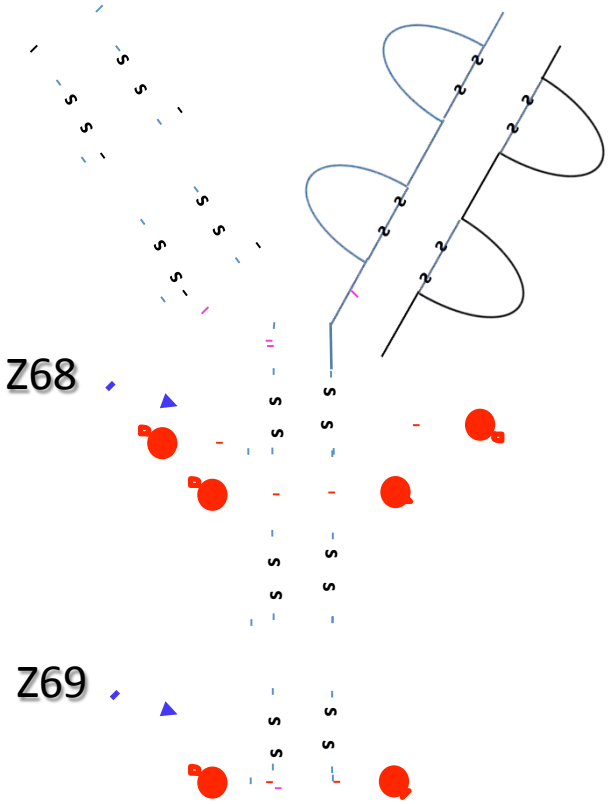


VERTEBRATE ANTIBODIES

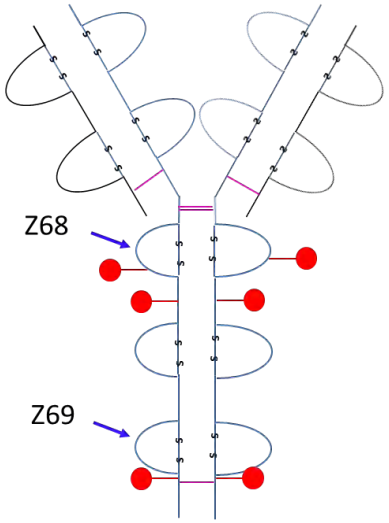
>Meagre IgM

MDYRTGLLLLTLCWAGVDGQTLTESEPAIKRPGESHRLTCTTSGFTFSSYHMNWVRQAPGKGLEWVAWVENDND
RKYYSQSVKGRFTISDNSRQQVFLQMNSLKTEDSAVYYCAREPGWGVGGGGGGFDYWGKGTMVTVTSATSTKPT
VFPLMQCGSGTNTVTLGCYATGFTPSSLTYAWTKEGTALTTSIQYPPVQKNNLYTGISQIQVSKQDWDNRKIFQ
CVATHAAGNAQAVFQKPIVILKPPTLSVSASSSDEKSEASFSCFAKDFSPKVHKLTWLKDNADITDKIYEIKTP
VEEGKDENGTVYSTASFLRVPSKGLSQGTQFTCLFEGDDKIYVNESVSYCPKCPPPGSCPGAEADITIIGPTNE
DMFMSRKGKIVCQVQENKPSVTKVWWEDENGHILIEYLKSTDTGKKIIRLELDITYDEWNQGIKRYCAVEHSEL
LEPVKKLYERSIGGQIQRPSVFMMPPVEHTRKDMVTLTCFVKDFFPQEVYVSWLVDDEEVDSTYEFHTTNPVES
YGSYSAYSQLLLSLEKWKRNDVVYSCVVHHESVANTTKAIVRSIGYRTYEKNTNLVNLNMNVPETCKPQ

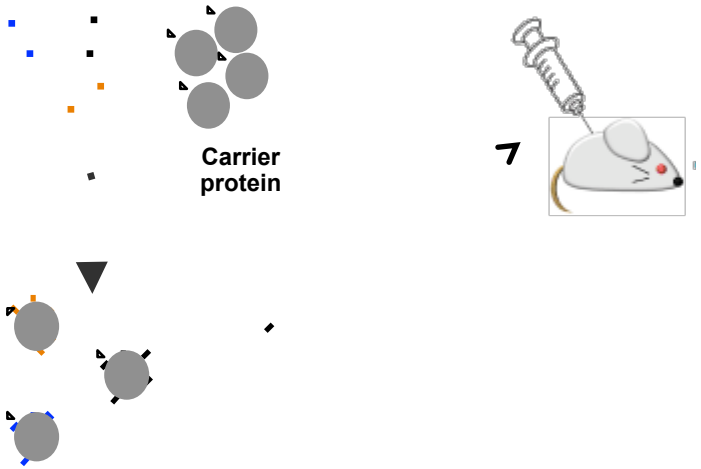
IgM(Meagre)	Z68	ITDKIYEIKTP
IgM(Meagre)	Z69	STYEFHTTNPV



Regions Selection & peptide Design



Carrier Conjugation, Immunisation & fusion



Peptides selection & synthesis:

- One peptide per selected region (Z68, Z69)

- High antigenicity & solubility
- Unique blast
- Multiple peptides leads to generation of more than one antibody per target – developing highly desirable assays such as Sandwich ELISA
- High sequence identity with Yellow AJ

```
Meagre Z68: ITDKIYEIKTP
Yellow AJ:  INNKIYEIKTP
            *.:********

Meagre Z69: STYEFHTTNPV
Yellow AJ:  SKYEISTTNPV
            *.**: *****
```

Dates of conjugation & immunizations:

8th Feb '16 (peptides conjugation)
15th Feb '16 (1st immunisation)
1st March '16 (2nd immunisation)
15th March '16 (3rd immunisation)
25th March '16 (tail bleed to test antibody response)
3rd April '16 (final boost)
6th April '16 (fusion of splenocytes)
14th April '16 (screening positive clones)

15th April Provide testing material

GWP. FISH HEALTH (WP 24 & 25)

Enhancing the European aquaculture production by removing production bottlenecks of emerging species, producing new products and accessing new markets

FCPCT-GIA: Daniel Montero

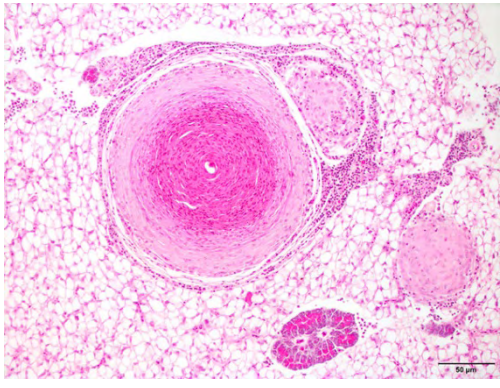
GWP Fish Health WP24 Meagre 2015

Task 24.1 Systemic Granulomatosis (led by HCMR).

Sub-Task 24.1.1. Feeding trials

Trial 4. Effects of vitamins. An experiment was carried out with different levels of vitamins E, C, K plus asthaxanthine.

- Fish growth and performance has been determined
- Macro and microscopical incidence of granulomas has been determined.
- Histological evaluation of the different organs has been conducted
- Health and oxidative status parameters determination are currently in progress



Granuloma in liver

GWP Fish Health WP24 Meagre 2015

Task 24.7 Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in meagre.

- Evaluation of bacterial incidence during 2015. Four sampling points during the period with no mortality associated. No pathogenic bacteria has been isolated.

- Nodavirus determination of Meagre population within FCPCTM facilities. Negative incidence of Nodavirus in the population.

- Incidence of damage in tails of early juveniles with no pathogenic bacteria associated.

GWP Fish Health WP24 Meagre

WORKING PLAN FOR 2016

Task 24.1.

Subtask 24.1.1.

Trial 4. To complete analysis of the different parameters and samples taken

Trial 5. To conduct Trial 5. The effect of Se, Mn and Fe will be examined in SG prevention.

Task 24.7.

- To evaluate different sampling points during the year to isolate other bacteria associated to this species.

- To conduct Nodavirus and Photobacterium challenge tests.

GWP Fish Health WP25. Greater Amberjack 2015

Task 25.2 Promoting resistance to parasitic incidence on greater amberjack (led by FCPCT).

Morphological study on the incidence of monogenean parasite in Greater Amberjack skin

Optical microscopy studies done.

Electron microscopy currently in progress

Determination of other parameters currently in progress

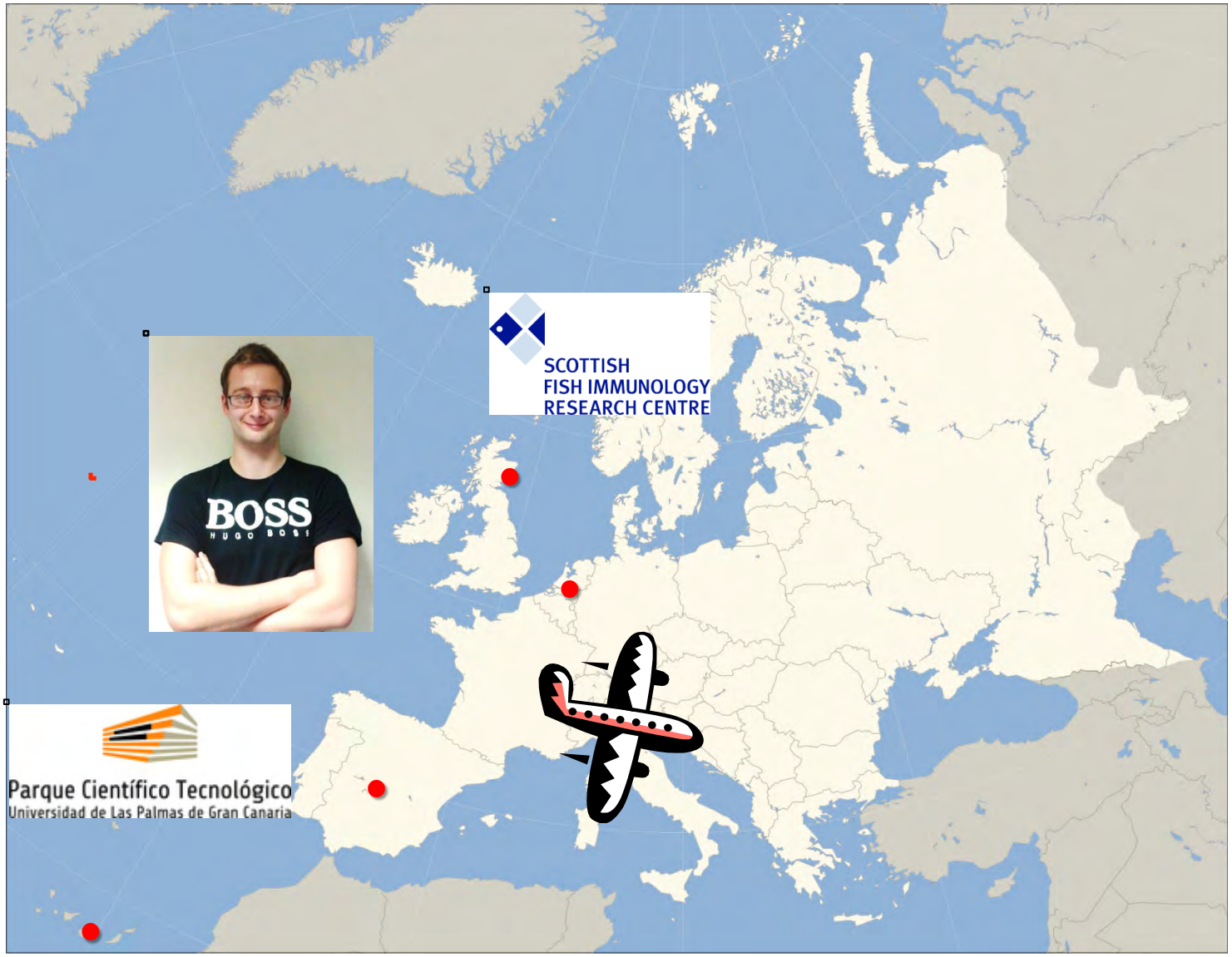
Determination of environmental conditions that can modulate Greater Amberjack resistance to parasitic infection

Determination of parasitic incidence

Studies of Greater Amberjack skin and immune system at different temperatures (in connection with Task 21.3 Development of appropriate husbandry practises (led by HCMR), Action 21.3.1.

Analysis of immune parameters and morphology - in progress

8th-14th November 2015



GWP Fish Health WP25. Greater Amberjack 2015

Task 25.3 Identification of immune markers (led by UNIABDN).

Samples from the different experiments provided to UNIABDN.
Samples of gill leucocytes from temperature experiment.
Other samples from different experiments and larval stages

Experiment conducted with different stimulations (PBS, Poly I:C, LPS, flagellin) on Greater Amberjack juveniles at FCPCT facilities at MBS, in vivo for 24h, and in vitro for 4h, 12h & 24h.

Samples transported to UNIABDN. Analysis in progress.

GWP Fish Health WP25 Greater Amberjack

Target Genes

Greater Amberjack – Target genes for this fish focus on the mucosal response to a variety of pathogens, through development of QPCR primers

GREATER AMBERJACK	
Gene type	**Target gene**
CYTOKINE	IL-10
	IL-17D
	IL-17A/F
	IL-22
IMMUNOGLOBULIN	IgM
	IgT
ANTI-VIRAL	iNOS
	Mx
ANTI MICROBIAL PEPTIDES	HEPCIDIN
	PISCIDIN
	DEFENCIN
HOUSEKEEPING	EF1α
	β-ACTIN

GWP Fish Health WP25. Greater Amberjack 2015

Task 25.5. Description, diagnosis and treatment of other bacterial/viral infectious diseases occurring in amberjack (led by FCPCT).

Evaluation of bacterial incidence during 2015.

Four sampling points during the period two of them with mortality associated.

Isolation and identification of two different bacteria:

Bacillus oceanisediminis

Aeromonas spp (identification in progress)

Challenge test against *Listonella anguillarum*.

No mortalities seen in concentrations lower that the one causing death by anaphylactic shock

Challenge test against *Photobacterium damselae* sb. *pisicida*. Mortalities occurred. Isolation of a bacterial strain from Greater Amberjack.

GWP Fish Health WP25 Greater Amberjack

WORKING PLAN FOR 2016

Task 25.2.

To complete analysis of the different parameters and samples taken

To conduct an experiment with mucus stimulating products.

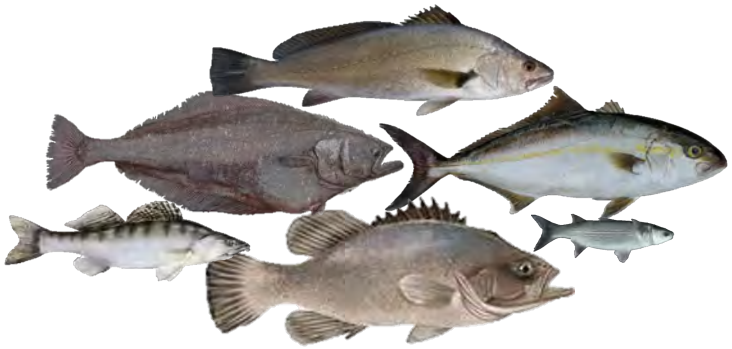
Experimental design in progress. Formulation of the diets in progress.

Task 24.7.

To evaluate different sampling points during the year to isolate other bacteria associated with this species.

To conduct *Nodavirus* and *Photobacterium* challenge tests.

GWP Fish Health
WP25



Salvador Jerez
IEO/ULL

ACM 2016
Nancy, 2-4 February 2016

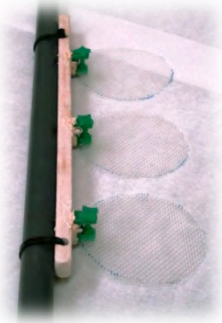
Task 25.4. Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites. **IEO**

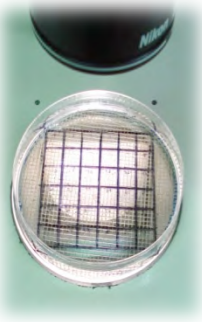
Monitoring of the infestation level by Zeuxapta seriolae in Seriola dumerili juveniles

A total of 180 juveniles of *Seriola dumerili* (262.1 ± 55.5 g)

Distributed in 12 indoor tanks (1-4 m^3) during 120 days

Weekly monitoring of the monogenean parasite eggs using the collector device previously designed, temperature and mortality in culture tanks



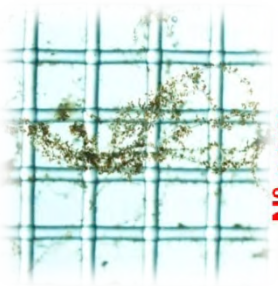


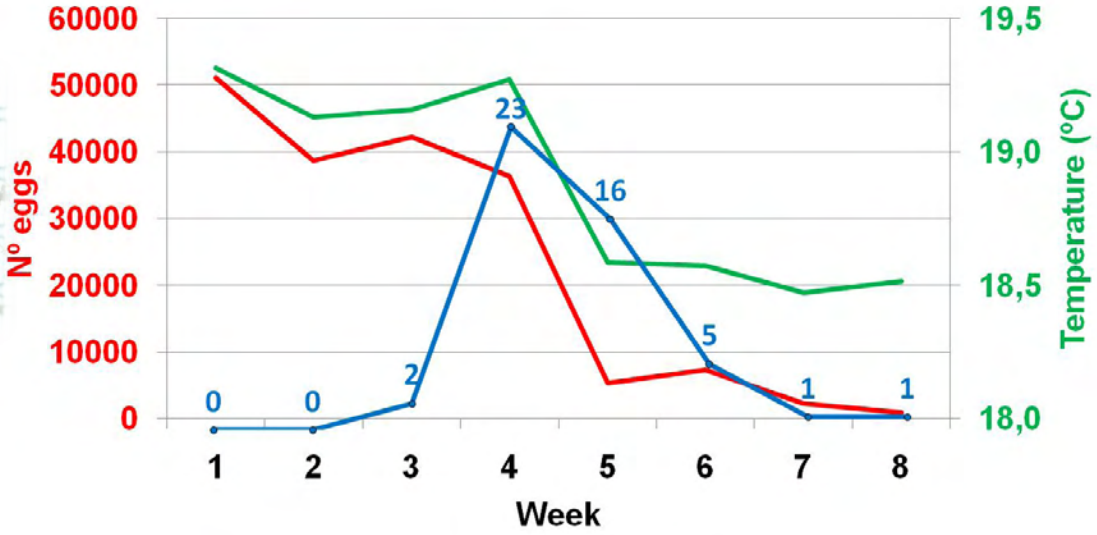
Task 25.4. Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites. **IEO**

Monitoring of the infestation level by Zeuxapta seriolae in Seriola dumerili juveniles

The total mortality seen was 27%

The highest number of dead fish coincided with the peak of egg number





Mean (weekly) number of *Z. seriolae* eggs collected (**red**), number of dead fish (**blue**), and temperature (**green**)

Task 25.4. Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites. **ULL**

Identification of monogenean parasites

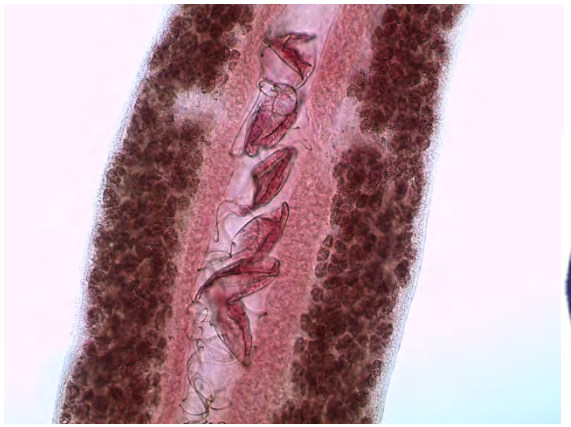
Zeuxapta seriolae

Morphological identification (Akmirza 2013).

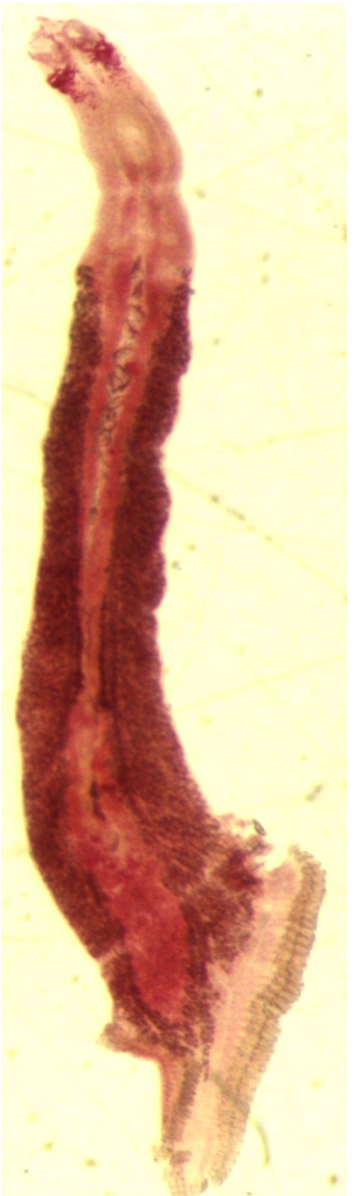
Molecular identification by sequencing the 28S gene

(Sepúlveda and González, 2014).

First sequencing of the Cytochrome Oxidase I (COI) region.

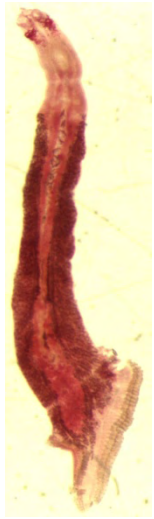


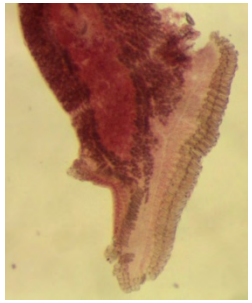
Eggs inside a parasite uterus



Task 25.4 Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites. **ULL**

Testing the effect of different substances (sucrose, glucose and mannose), as pro- or anti-Zeuxapta seriolae-attaching substances, based on the parasite potential lectin-substrate affinities.





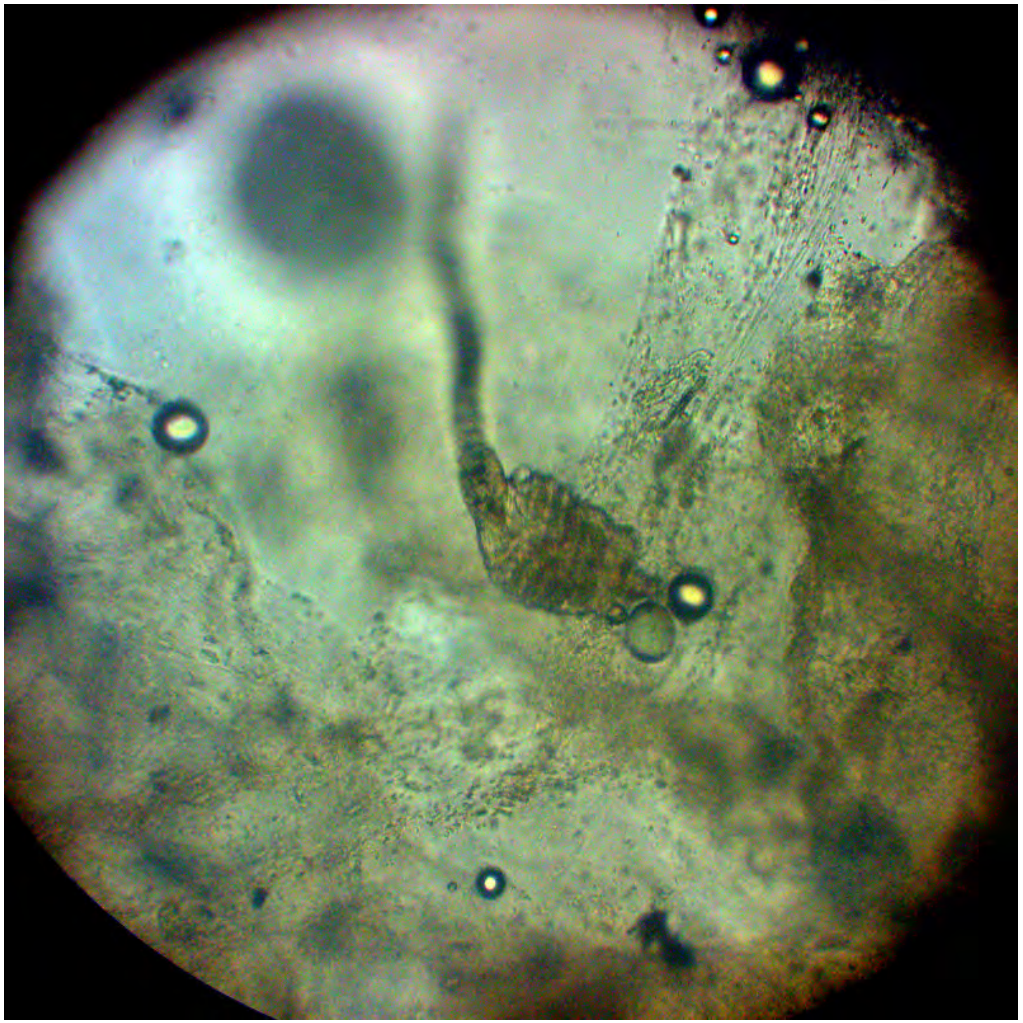
- 4-6 ind./external arch; 2-4 ind./2nd-3rd arch

-One nylon mesh filtered parasite and two gill-attached parasites 5-6h observation with glucose-seawater

-Very active under glucose and sucrose, inactivity and tissue release after 2-5 min in 0.25M mannose-seawater

-High gill cell viability after 18h

Parasite attachment to a gill arch



The figure shows a fragment in which the parasite is disengaged from the gill arch after 2 min in presence of 0.25 M mannose in sea water.

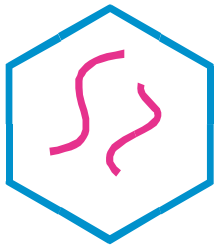
WP26 Fish Health

Sonal Patel
IMR

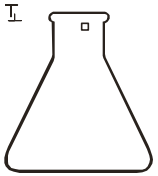
Task 26.1 Production of VNN capsid protein

- Expression in
  - *E. coli*
  - *Leishmania tarentolae*
  - *Tobacco plant*

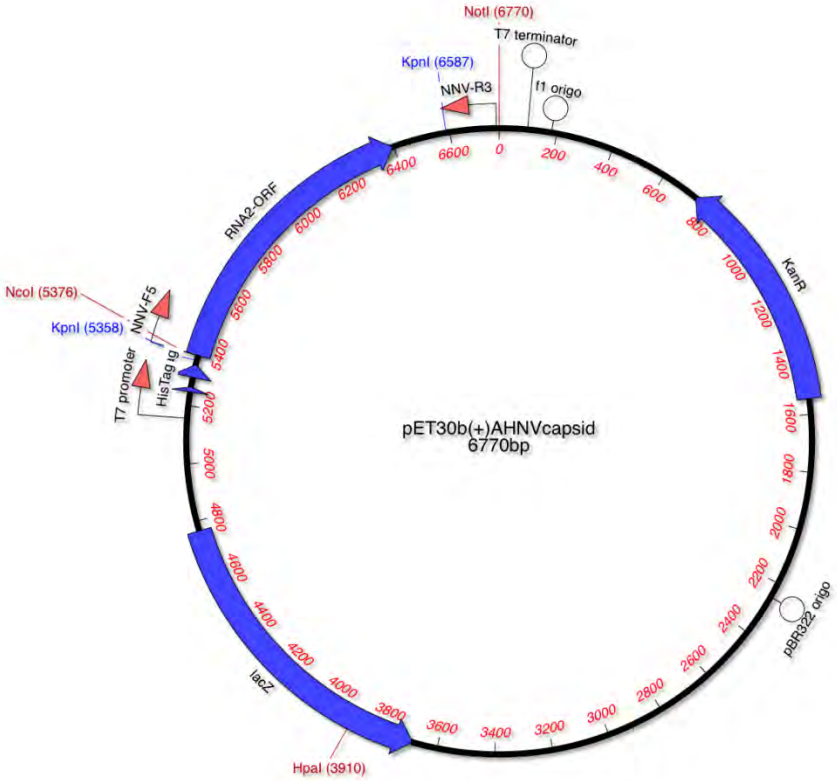
Expression in *E. coli*

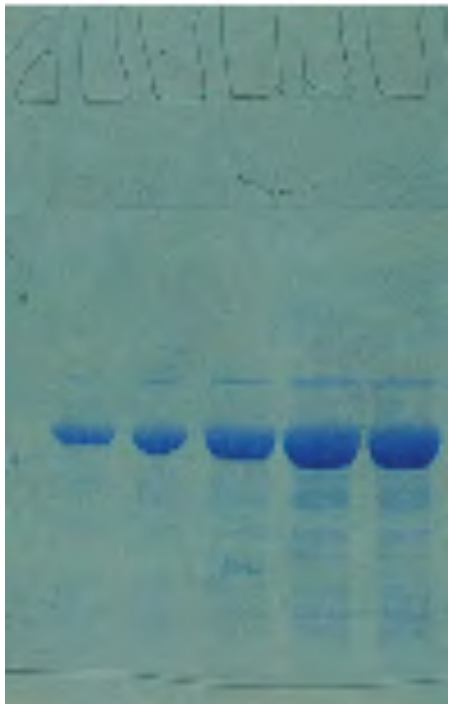


capsid gene



Transformation and expression in *E. coli* as inclusion bodies





Serial dilutions of purified recombinant protein

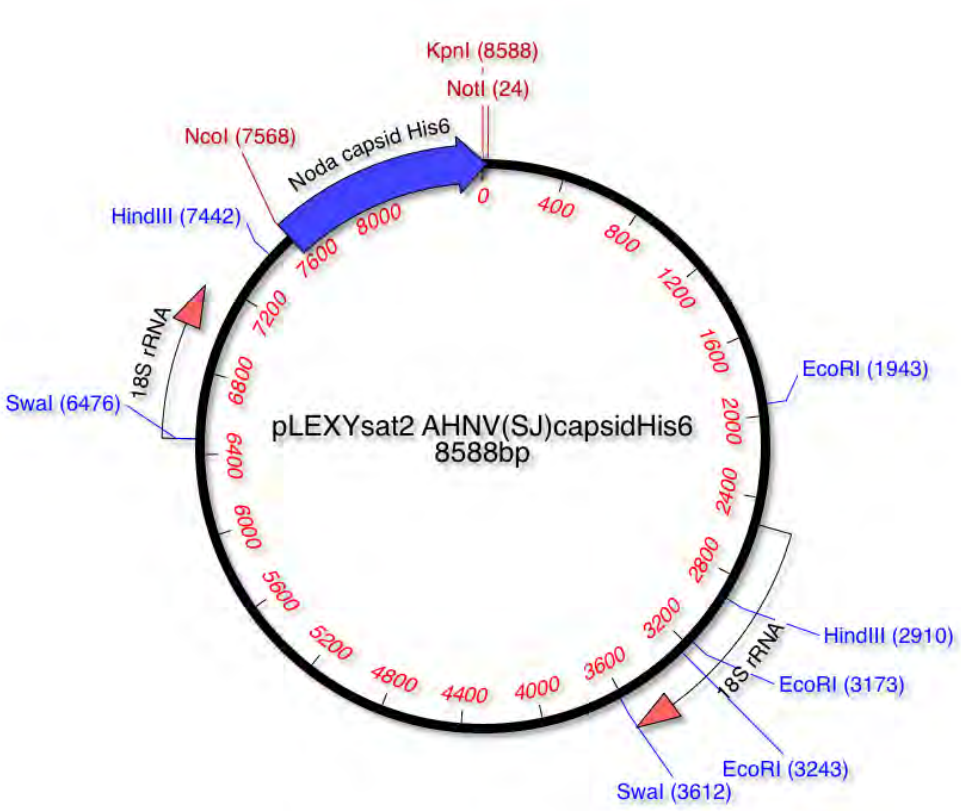
Expression in *Leishmania tarentolae*

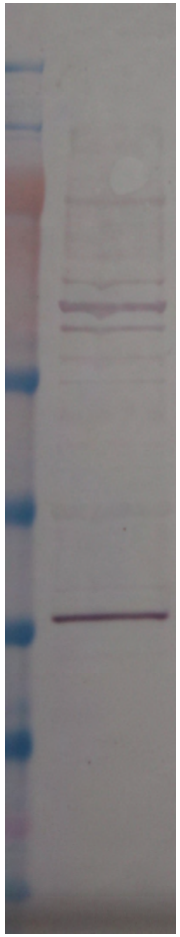
capsid gene

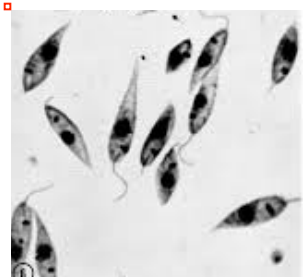
Transformation and amplification of plasmid in *E. coli*

Linearization of plasmid by SwaI digestion

Electroporation into *L.tarentolae* for integration in the 18S rRNA gene and expression of rekombinant capsid protein





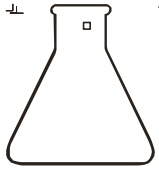


Western blot using rabbit anti-AHNVcapsid antibodies

Transient expression in *Nicotiana benthamiana* (performed at the John Innes Centre, UK)

plasmid

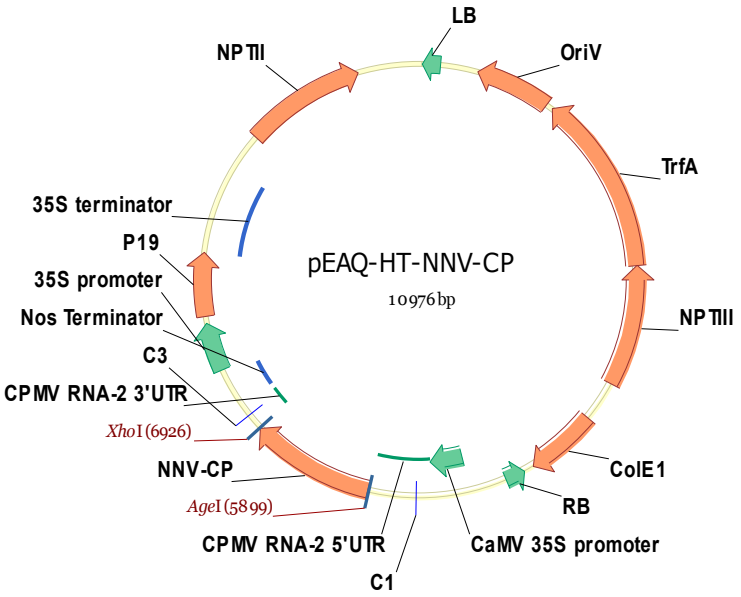
Capsid gene

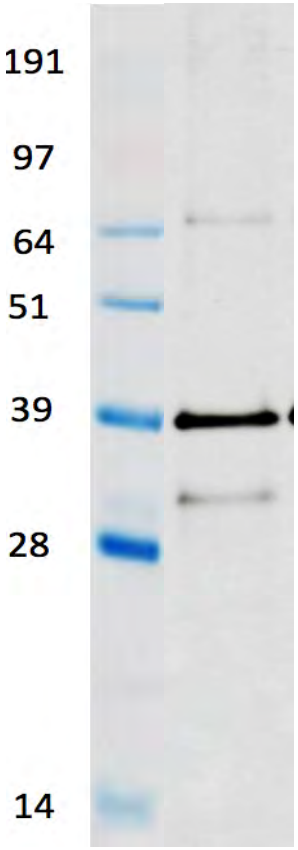


Amplification of plasmid in *E. coli*

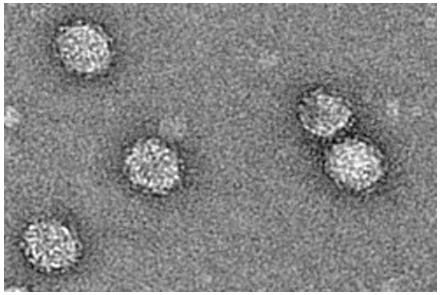
Transformation of *Agrobacterium tumefaciens*

Agroinfiltration





Western blot using anti-NNV antibodies



TEM of virus like particles formed by recombinant proteins

Conclusions

Expression of the nodavirus capsid protein was achieved in all three systems.

However, it was only in the *E. coli* system that sufficient and high expression for further use of the protein as antigen for vaccination purposes was possible.

The initial testing of possible expression of VNN capsid protein was carried out using non-inducible systems, and the next step is to use an inducible expression kit to increase production.

GWP deliverables in next 12 months

D24.4 Isolation and characterization of Nocardia from infected meagre (HCMR). Month 36

D24.5 The effect of high plant protein diets in the development of Systemic Granulomatosis in meagre (HCMR). Month 36

D25.1 Marker genes of mucosal immunity in greater amberjack cloned and ways to increase their expression level determined (ABDN). Month 39

D25.2 Mucus defences of greater amberjack analysed and immune potential characterised. Month 39

D26.2 Testing of the delivery of vaccine candidates through Artemia to Atlantic halibut larvae (IMR). Month 36

Dissemination activities

ORAL PRESENTATION:

Histological effects and impact of juvenile specimens of *Sparicotyle chrysophrii* and *Zeuxapta seriolae* on cultured gilthead seabream and greater amberjack.

Repullés-Albelda, Padrós, Raga & Montero.

17th International Conference on Diseases of Fish and Shellfish, Las Palmas, September 2015.

POSTER:

Progress in understanding the ontogeny of the immune system in meagre (*Argyrosomus regius*). Results of the EU DIVERSIFY project in 2014 and 2015.

Campoverde, Milne, Andree, Gisbert, Estevez & Secombes.

Aquaculture Europe 2015, Rotterdam, Netherlands, October 2015.

DIVERSIFY

COOPERATION

Thank you, Merci, Gracias, Grazie, ευχαριστίες, Tak, Takk, Dank, תודה

1 4 9 5 UNIVERSITY OF ABERDEEN

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