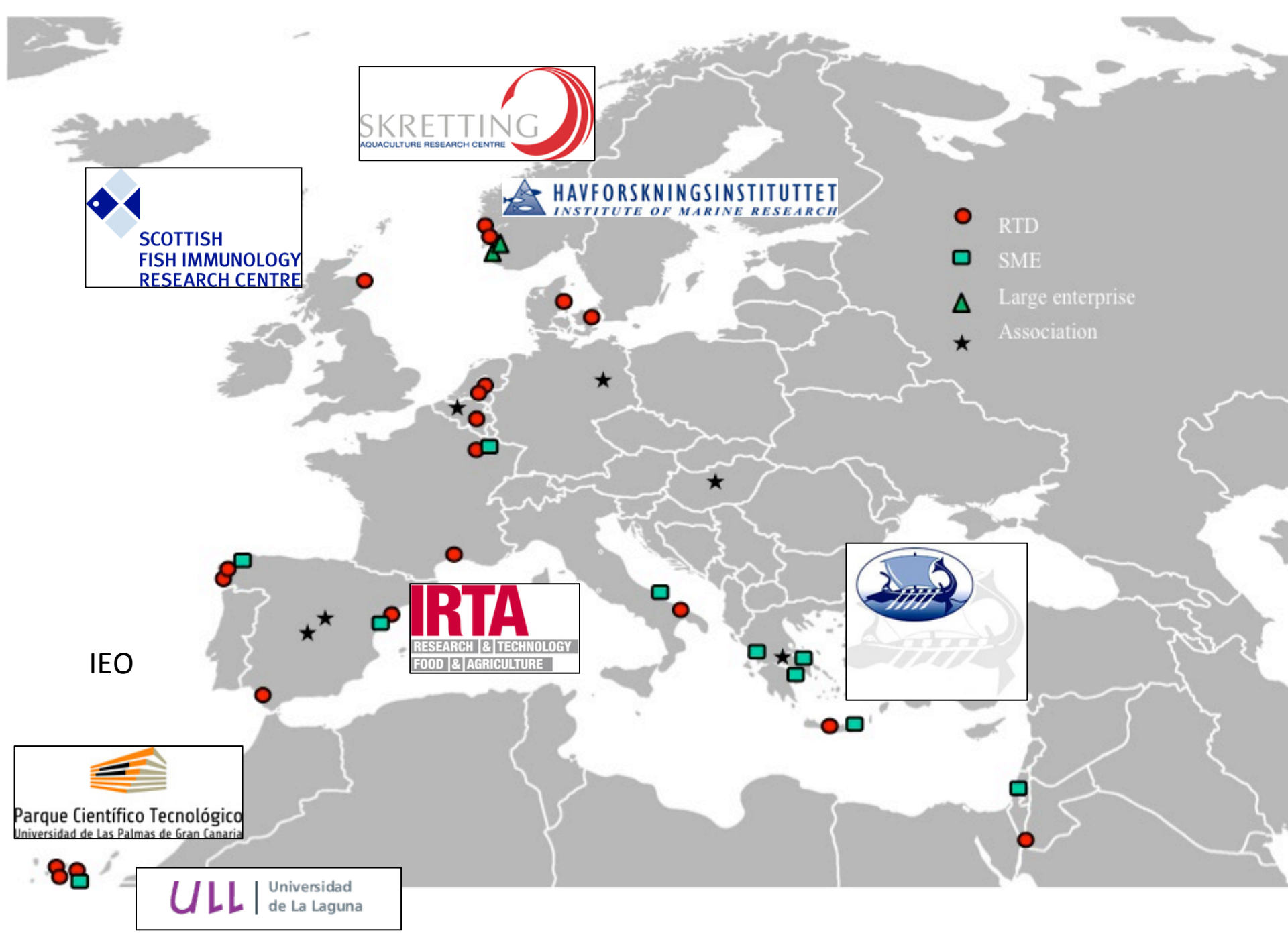
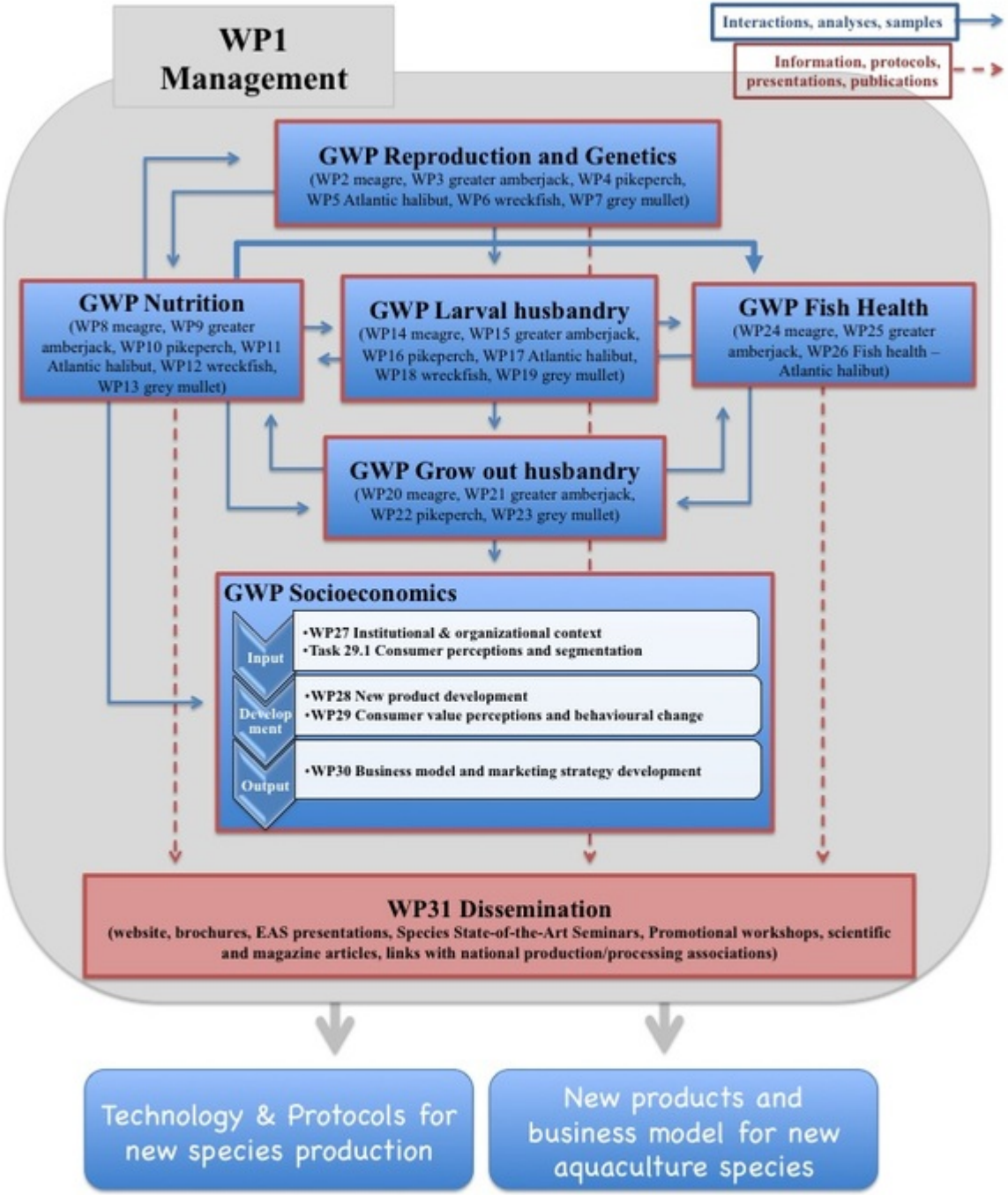
Fish Health


SKRETTING
AQUACULTURE RESEARCH CENTRE
HAVFORSKNINGSINSTITUTTET
INSTITUTE OF MARINE RESEARCH
SCOTTISH
FISH IMMUNOLOGY
RESEARCH CENTRE
RTD
SME
Large enterprise
Association
IRTA
RESEARCH & TECHNOLOGY
FOOD & AGRICULTURE
IEO
Parque Científico Tecnológico
Universidad de Las Palmas de Gran Canaria
ULL
Universidad
de La Laguna


WP1
Management
Interactions, analyses, samples
Information, protocols, presentations, publications
GWP Reproduction and Genetics
(WP2 meagre, WP3 greater amberjack, WP4 pikeperch, WP5 Atlantic halibut, WP6 wreckfish, WP7 grey mullet)
GWP Nutrition
(WP8 meagre, WP9 greater amberjack, WP10 pikeperch, WP11 Atlantic halibut, WP12 wreckfish, WP13 grey mullet)
GWP Larval husbandry
(WP14 meagre, WP15 greater amberjack, WP16 pikeperch, WP17 Atlantic halibut, WP18 wreckfish, WP19 grey mullet)
GWP Fish Health
(WP24 meagre, WP25 greater amberjack, WP26 Fish health – Atlantic halibut)
GWP Grow out husbandry
(WP20 meagre, WP21 greater amberjack, WP22 pikeperch, WP23 grey mullet)
GWP Socioeconomics
Input
•WP27 Institutional & organizational context
•Task 29.1 Consumer perceptions and segmentation
Develop ment
•WP28 New product development
•WP29 Consumer value perceptions and behavioural change
Output
•WP30 Business model and marketing strategy development
WP31 Dissemination
(website, brochures, EAS presentations, Species State-of-the-Art Seminars, Promotional workshops, scientific and magazine articles, links with national production/processing associations)
Technology & Protocols for new species production
New products and business model for new aquaculture species

Fish Health

WP 24 Fish health - meagre Lead P1 148.4 MM

HCMR
FCPCT
IRTA
UNIABDN
SARC

WP 25 Fish health - greater amberjack Lead P5 95.1 MM

HCMR
FCPCT
UNIABDN
IEO
ULL

WP 26 Fish health - Atlantic halibut Lead P7 4.16 MM

IMR

DIVERSIFY

Fish Health

COOPERATION

WP24 Fish health– meagre (*Argyrosomus regius*)

- Participants: HCMR, FCPCT, IRTA, UNIABDN, SARC

Fish Health

This WP will address bottlenecks relating to meagre health. Tasks include:

(a) studies of key disease states
(b) development of appropriate treatments
(c) a first characterisation of the meagre immune system/ immune responses required for future immune intervention.

DIVERSIFY

Fish Health

COOPERATION

Specific objectives are:

1. Identify the causes of systemic granulomatosis and chronic ulcerative dermatopathy

2. Investigate anti-parasite treatments in juvenile meagre

3. Undertake preliminary characterisation of immune genes and study specific immune responses post-vaccination

4. Evaluate the occurrence of Nocardia infections in meagre and develop an autogenous vaccine

5. Develop diagnostic-prevention-treatment protocols for diseases in meagre.

DIVERSIFY

Fish Health

COOPERATION

HCMR:

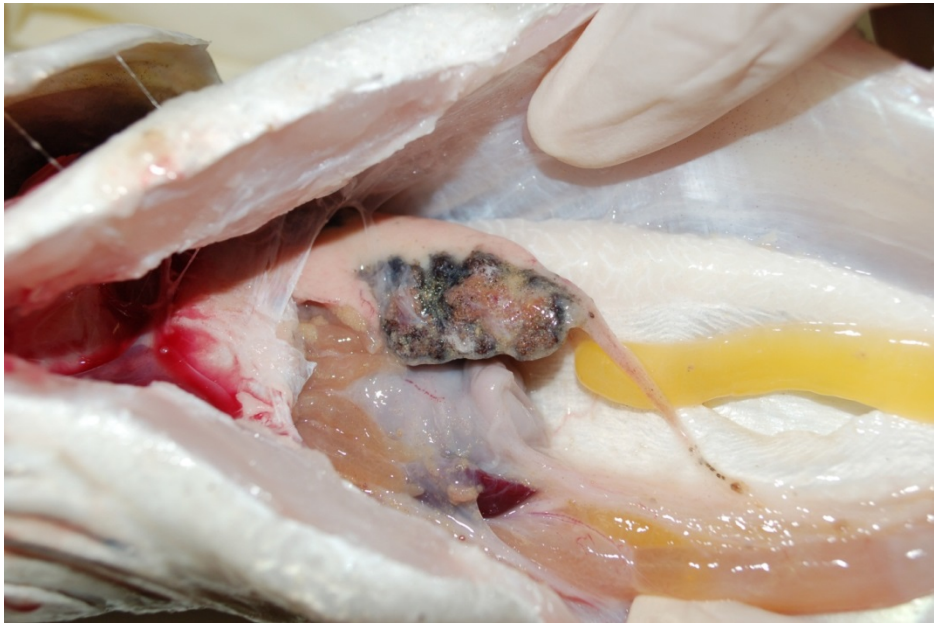
Fish Health

1. Identify the causes of systemic granulomatosis (SG), and chronic ulcerative dermatopathy

2. Evaluate the occurrence of Nocardia infections in meagre and develop an autogenous vaccine

3. Develop diagnostic-prevention-treatment protocols for diseases in meagre

Systemic granulomas



Chronic Ulcerative Dermatopathy

DIVERSIFY

Fish Health

COOPERATION

Systemic Granulomatosis

Feeding trials foreseen

trial #	Responsible	Factor to be assessed
1	HCMR	Vit D
2	HCMR	Ca/P
3	HCMR	Protein level
4	HCMR	Plant protein

Fish Health

Systemic Granulomatosis

Analysis to be conducted

- **Pathology**

1. Macro/microscopical assessment
2. Histology
3. SEM/TEM, TEM-X-Ray microanalysis
4. Blood biochemistry
5. Biomarkers, Antioxidant Enzyme activity

- **Zootechnics**

- Growth
- Survival
- Feed efficiency
- Body composition

DIVERSIFY

Fish Health

COOPERATION

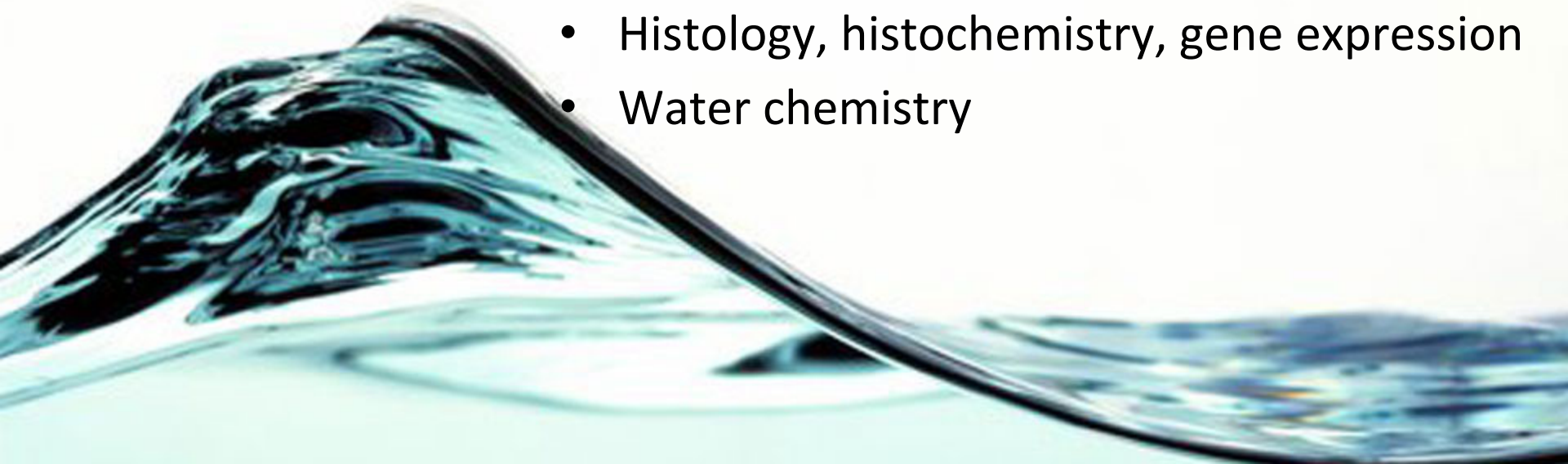
Chronic Ulcerative Dermatopathy

Rearing

- Rearing in natural sea water vs borehole
- Sampling of fish every 5-10 days (until visible lesions)
- Transfer in sea water (resolution of lesions)

Analysis will include

- Histology, histochemistry, gene expression
- Water chemistry



Fish Health

Nocardia infection (led by HCMR)

- Screen fish from various locations in Greece
- Isolate as many strains as possible
- Strain characterization and grouping (through metabolic profile, genetic analysis)
- Preparation of autogenous vaccine (inactivated bacterin)
- Toxicity testing in meagre

DIVERSIFY

Fish Health

COOPERATION

Diagnostic-recommendation manual for meager health (led by HCMR)

Participants HCMR, FCPCT, IRTA, UNIABDN

Results/findings from all tasks of WP24

Compilation in a pdf, uploaded in project's site

DIVERSIFY

Fish Health

COOPERATION

IRTA:

Fish Health

Task 24.3. Anti-parasitic treatments

D24.9 Determination of effective treatments for common monogenean parasites in meagre. Del date: month 48

Participants: IRTA

Use of monogenean parasites as a model

Try 4 to 6 products

In close collaboration with Culmarex to provide parasitized fish

This task should be started in 2015 but it can be moved according to the availability of juveniles.

DIVERSIFY

Fish Health

COOPERATION

Task 24.4. Nocardia infection in meagre

Subtask 24.4.1 Isolation and characterization of the pathogen

D24.4 Isolation and characterization of Nocardia from infected meagre.

Del date month 36

D24.6 Experimental vaccine for Nocardia for meagre.

Del date month 42

Participants HCMR, IRTA

Isolation of the parasite in collaboration with Culmarex (years 1 to 3)

Physiological characterization of the bacteria, for future vaccine design (years 1 to 4)

DIVERSIFY

Fish Health

COOPERATION

Task 24.4. Nocardia infection in meagre

Sub-task 24.4.2. Preparation of an autogenous vaccine

D24.10 Kinetics of antibody and cytokine production established post-pathogen exposure or stimulation with PAMPs. Del date month 48

Participants: IRTA, UNIABDN, HCMR

HCMR will develop the vaccine (year 4-5)

IRTA will carry out the challenge (year 4 for preparing challenge conditions and model and year 5 for the challenge itself)

Exchange of visits between IRTA and UNIABDN for using the PAMPs

DIVERSIFY

Fish Health

COOPERATION

Task 24.5. First characterisation of the immune system

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

Participants: UNIABDN, IRTA

IRTA will take samples of larvae and juveniles along years 1 and 2 and send them to UNIABDN

Exchange of visits for training in the use of PAMPs

Fish Health

DIVERSIFY

COOPERATION

(Note - Dotted lines are optional methodological approaches.)

Intraperitonial injection of adult animals with stimulants

Isolate spleen and head kidney from Mature Meagre.

↓

Propagate primary cell cultures from tissues for exposure to stimulants

B1-3 glucan

Poly IC nucleic acid

Bacterial LPS

Isolate RNA from immune stimulated cells

↓

cDNA synthesis

↓

PCR amplification and Sequencing of target and endogeneous control genes

Targeted Approach

Choose target genes for evaluation of immune system

↓

Locate gene homologs in databases (GenBank)

↓

Align gene homologs to locate consensus regions

↓

Design primers for PCR amplification from Meagre cDNA

Non-Targeted Approach

Whole Transcriptome of stimulated and non-stimulated target tissues to select immune genes for analysis

Collect time-series samples for gene expression analysis and histology (ISH?)

↓

Extract RNA, make cDNA, qPCR

Begin larval culture for evaluation of ontogeny immune system

Design of Taqman qPCR assays.

DIVERSIFY

Fish Health

COOPERATION

Task 24.6. Monitor specific immune responses

D 24.10 Kinetics of antibody and cytokine production

Participants: IRTA, UNIABDN

Exchange of visits between partners after vaccination (years 3-4)

Use of a vaccine against *V. anguillarum* (commercial vaccine?)

DIVERSIFY

Fish Health

COOPERATION

UNIABDN:

Lead for Tasks 24.5 and 24.6

Jun Zou

Fish Health

Immune gene characterisation:

Clone RAG, Ig and TcR genes as markers of adaptive immunity

Clone IL-1, TNF, AMPs, IFN/Mx as markers of innate immunity

Clone cytokines of adaptive immunity as markers of Th responses

Antisera:

Develop anti-IgM and anti-IgT Abs to allow measurement of antibody responses

Monitor immune responses:

After vaccination with a bacterin (IRTA) in blood/mucus and in gill/kidney

DIVERSIFY

Fish Health

COOPERATION

FCPCT:

Fish Health

- Task 24.1. Systemic Granulomatosis.
  - Subtask 24.1.1. Trial 4 .(FCPCT). Combined effect of vit. E, C and carotenoids
  - Subtask 24.1.1. Trial 5. (FCPCT). Effect of Se, Mn and Fe
  - Subtask 24.1.2. Health and pathological assesments. Histological studies from samples of trials 4&5, subtask 24.1.1

Fish Health

- Task 24.7. Description, diagnosis and treatment of other bacterial/viral infectious diseases (Led by FCPCT).
  - Monitoring the outbreak of diseases within the project
  - Susceptibility against Vibrio, Photobacterium and betanodavirus will be described
  - Establishment of treatment protocols with antibiotics (depending on the limitation of their use) for the pathogen isolated from natural outbreaks of diseases.

DIVERSIFY

Fish Health

COOPERATION

WP25 Fish Health-Greater Amberjack (*Seriola dumerili*)

- Participants: HCMR, FCPCT, UNIABDN, IEO, ULL

Fish Health

This WP will address bottlenecks relating to amberjack disease control. Tasks include:

(a) dietary regimes that improve larval and adult disease resistance
(b) diagnostic tests for several major pathogens, and
(c) immune markers to aid selection of resistance, with a focus on mucosal defences.

DIVERSIFY

Fish Health

COOPERATION

Specific objectives are:

1. Provide early diagnosis tools for Epitheliocystis

2. Develop “antiparasite diets” to be used prior to sea cage culture

3. Begin characterisation of the immune system, with a focus on mucosal (skin/gill) defences

4. Develop anti-monogenean parasites infection rearing protocols

5. Develop diagnostic-prevention-treatment methods for diseases in greater amberjack

DIVERSIFY

Fish Health

COOPERATION

HCMR:

Task: Epitheliocystis in mesocosm reared amberjack

- Screening for epitheliocystis agents
- Diversity of “chlamydia” agents through phylogenetic analysis
- Develop specific molecular probes for early detection
- Validation of tool in larval rearing trials

DIVERSIFY

Fish Health

COOPERATION

ABDN:

Identify immune markers of mucosal defences

Clone IL-17, IL-22, iNOS, AMPs and IgT

Study their modulation at mucosal sites during:

1) In vitro using gill cultures
2) In vivo following different diet regimes and during development

DIVERSIFY

Fish Health

COOPERATION

FCPCT:

Fish Health

- Task 25.2. Promoting resistance to parasitic incidence (led by FCPCT).
  - Use of mucus stimulation products (MOS, beta-glucans or phytobiotics) during early on-growing.
  - Providing samples for Task 25.3
  - Resistance to monogean infection by cohabitation
    - Morphometric and ultrastructure studies of target tissues (i.e. gills and skin)

Fish Health

- Task 25.5. Description, diagnosis and treatment of other bacterial/viral infectious diseases (Led by FCPCT).
  - Monitoring the outbreak of diseases within the project
  - Susceptibility against Vibrio, Photobacterium and betanodavirus will be described
  - Establishment of treatment protocols with antibiotics (depending on the limitation of their use) for the pathogen isolated from natural outbreaks of diseases.

DIVERSIFY

Fish Health

COOPERATION

IEO/ ULL:

Fish Health

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

Deliverables:

D25.6 Rearing protocol against monogenean parasites. The efficacy of the bath lectin treatment will be assessed at two densities for survival, growth and fish health (plasma analysis of triglycerides, cholesterol, protein, enzymes, cortisol, glucose, lactate, osmolality, electrolytes) and physiology (viability and integrity of skin cells and osmorregulatory epithelia: gills and gut ATPase activity).

Results will be related with the presence and density of parasites in experimental tanks, that will be estimated throughout periodical monitoring of collectors devices for eggs and fish sampling for adult parasites. Month 57 (starting from Yr 2)

Leader: IEO; **participants**, ULL

Exchange of samples and techniques

IEO will provide samples of skin, gills and gut to ULL

Exchange of visits for training

No visit scheduled

DIVERSIFY

Fish Health

COOPERATION

WP26 Fish health– Atlantic halibut (*Hippoglossus hippoglossus*)

- Participants: IMR

Researchers: Sonal Patel and Audun H. Nerland

Fish Health

This WP will address a key bottleneck relating to Atlantic halibut larval health, namely nodavirus (Viral Neural Necrosis, VNN) outbreaks in larval and juvenile stages. Tasks include:

(a) production of the VNN capsid protein in *E. coli*, tobacco plants and possibly microalgae
(b) oral delivery of the recombinant capsid protein in Artemia to late larval stages
(c) assessment of the degree of protection obtained with the different formulations, assessed by histology and immunohistochemistry with antibodies to NVV.

DIVERSIFY

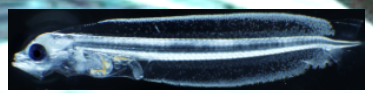
Fish Health

COOPERATION

Specific objective is:

To determine the effect of delivering recombinant capsid protein during late larval stages on protection to nodavirus (VNN)

Will liaise closely with the TargetFish programme (EU 7th FP) – details to be discussed with co-ordinator of Targetfish programme



DIVERSIFY

Fish Health

COOPERATION

WP26 – Atlantic halibut: Tasks

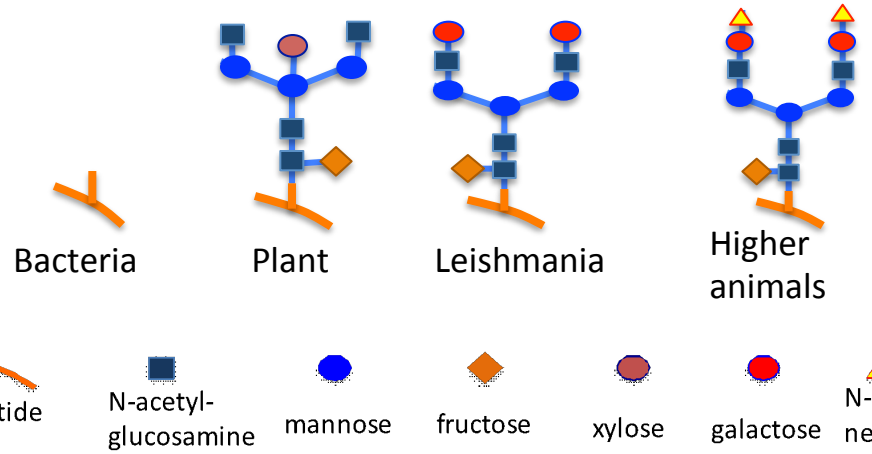
Plan:

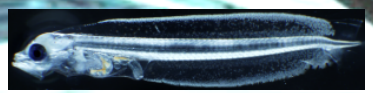
1. Capsid protein expression → 2. Delivery → 3. Challenge with Nodavirus

Expression of nodavirus capsid protein (E. coli, tobacco plant and protozoa)

Expression in tobacco plants

Glycosylation



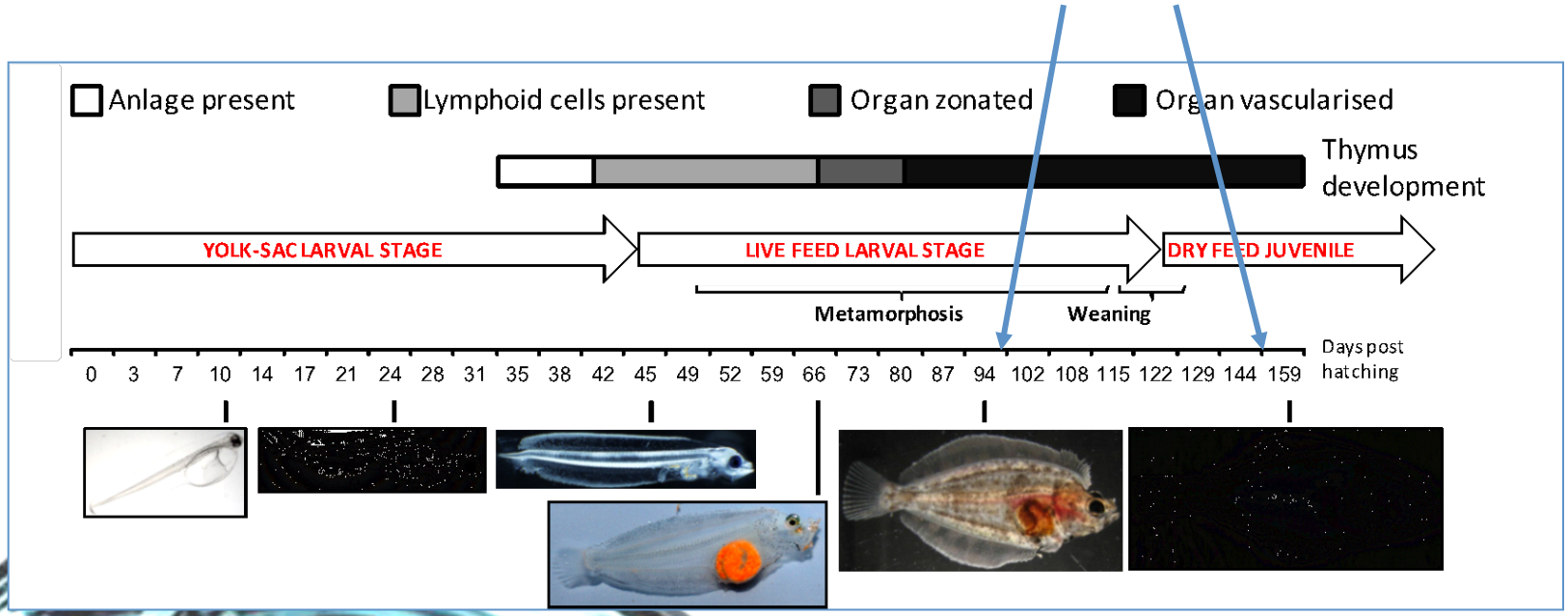


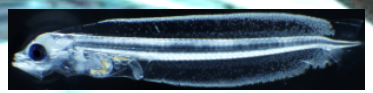
WP26 – Atlantic halibut: Tasks

Plan:

1. Capsid protein expression → **2. Delivery** → 3. Challenge with Nodavirus

Delivery to late larval stages and juveniles





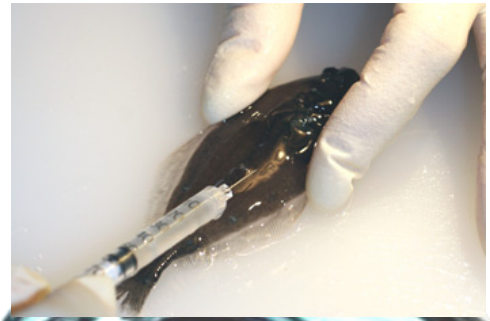
WP26 – Atlantic halibut: Tasks

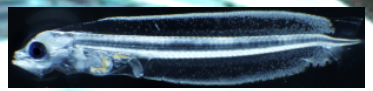
Plan:

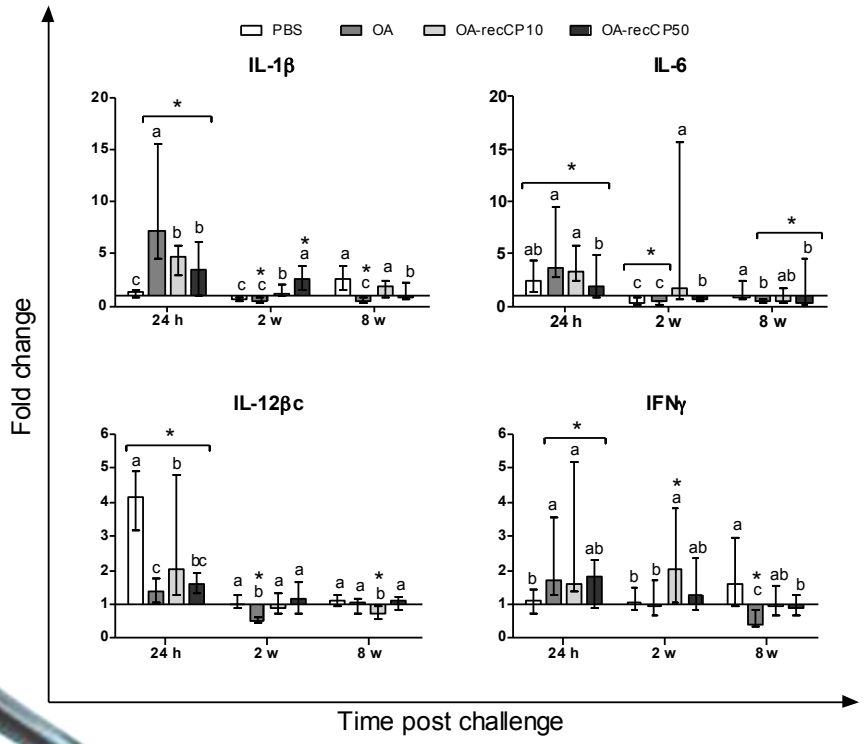
1. Capsid protein expression ⟶ 2. Delivery ⟶ **3. Challenge with Nodavirus**

Challenge

1. Mortality
2. Immune response







DIVERSIFY

Fish Health

COOPERATION

WP 24 deliverables

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

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

D24.3 Cloning of key marker genes of innate & adaptive immune responses in meagre (ABDN)

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

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

D24.6 Experimental vaccine for Nocardia for meagre (HCMR)

D24.7 Diagnostics protocol for Chronic Ulcerative Dermatopathy in meagre (HCMR)

D24.8 Report on the prevention/treatment of Chronic Ulcerative Dermatopathy in meagre (HCMR)

DIVERSIFY

Fish Health

COOPERATION

WP 24 deliverables

D24.9 Determination of effective treatments for common monogenean parasites in meagre (IRTA)
D24.10 Kinetics of antibody and cytokine production established post-pathogen exposure or stimulation with PAMPs (ABDN)
D24.11 Recommended levels of pro- and anti-oxidant nutrients to prevent Systemic Granulomatosis in meagre (FCPCT)
D24.12 Determination of efficacy of vaccination of meagre against Nocardia (IRTA)
D24.13 Description of immune gene expression pre- and post-immunization of meagre with Nocardia (IRTA)
D24.14 Diagnostics protocol for Systemic Granulomatosis, causes and solutions in meagre (HCMR)
D24.15 Report on the prevention/treatment of Systemic Granulomatosis in meagre (HCMR)
D24.16 Report of the major bacterial and viral diseases found in meagre, and where useful treatments have been developed, complete protocols for their implementation by the industry will be provided (FCPCT)
D24.17 Diagnostic-recommendation manual for meagre fish health (HCMR)

DIVERSIFY

Fish Health

COOPERATION

WP 25 deliverables

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

D25.2 Mucus defences of greater amberjack analysed and immune potential characterised **(not HCMR !!!!)**

D25.3 Impact of dietary regime on parasite resistance and mucosal defences of greater amberjack juveniles (ABDN)

D25.4 Protocol for early diagnosis of epitheliocystis during early stages of greater amberjack culture (HCMR)

D25.5 Impact of oral administration of greater amberjack with mucus stimulation products on immune resistance to parasitic infections and development of molecular markers for its evaluation (FCPCT)

D25.6 Rearing protocol against monogenean parasites (IEO)

D25.7 Report on the major bacterial and viral diseases found in greater amberjack, and where useful treatments have been developed, complete protocols for their implementation by the industry will be provided (FCPCT)

D25.8 Diagnostic-recommendation manual for greater amberjack fish health (HCMR)

DIVERSIFY

Fish Health

COOPERATION

WP 26 deliverables

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

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

D26.3 Determine immune response and effectiveness of orally delivered VNN capsid protein on protection of Atlantic halibut larvae (IMR)

Fish Health

DIVERSIFY

Fish Health

COOPERATION

Milestones

MS Number	Description	Delivery date
51	Design of primers for amplification of meagre target gene DNA sequences	12
52	Grow-out of larvae and collection of samples from immune ontogeny time-line	24
53	Amplification and sequencing of target gene sequences from stimulated tissues	30
54	Completion of challenge and collection of samples for study of immune gene modulation	36
55	Complete preparation of cDNA synthesis from all meagre samples	40
56	Complete gene expression analysis for immune ontogeny	42
57	Complete gene expression analysis for immune stimulus /response	45