Pordenone, 19 February 2020

ParaFishControl

Advancements and upcoming results

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Project Coordinator

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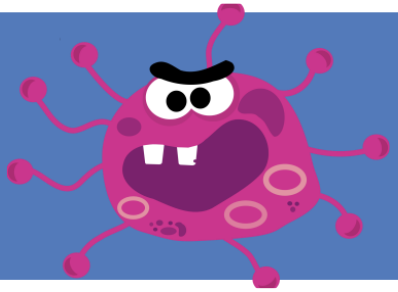
Advanced Tools and Research Strategies for Parasite Control in European farmed fish

5 years collaborative project:

- Total cost: 8 104 133.75 €
- EU contribution: 7 800 000 €



Parasites are constraining aquaculture industry

- Different parasites, culture conditions
- Different background knowledge

Global Objective

- To increase sustainability and competitiveness of European aquaculture
- Improving understanding of fish-parasite interactions
- Developing innovative solutions and tools for the prevention, control and mitigation of the major parasites

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WHY THIS PROJECT, WHY PARASITES?

Economic Impact of Parasites in Finfish Aquaculture

- Direct mortality
- Morbidity: Decreased FCR & growth, parasitic castration
- Increased susceptibility to other diseases (opportunistic)
- Reduced ability to cope with changes and handling
- Harvest downgrades, loss of market, reduced durability
- Costs of treatments, prevention strategies, mort disposal, etc.
- World (Shinn et al., Global Aquaculture Advocate, 2015):
  - Estimated losses due to parasitism: hatchery (20 %), grow out (1-10% harvest)
  - Parasites' annual cost : from $1.05 billion to $9.58 billion
- EU (+ Norway) (H. Rodgers, FishVetGroup):
  - The value of salmon aquaculture in 2016: €12.5 billion
  - Parasite impact: €525 to 725 million pa (direct & indirect)

Industry

Aquark

AQUATT
Science. Communication. Knowledge. Innovation.

Andromeda Group
YOUR NEARBY SEA FISHERMAN

VERTEBRATE ANTIBODIES

SKRETTING
AQUACULTURE RESEARCH CENTRE

INGENASA

Future Genomics Technologies

W42

Industrilaboratoriet

Research

CSIC
Consejo Superior de Investigaciones Científicas

IATS

IIM
Instituto de Investigaciones Marinas de Vigo

Real Jardín Botánico - CSIC

13 countries

28 partners

University of Stirling

NIOO

Wageningen UR
For quality of life

Institute for Veterinary Medical Research
Centre for Agricultural Research
Hungarian Academy of Sciences

DTU

Aarhus University
Science and Technology

INRA Transfert

USC
Universidade de Santiago de Compostela

University of Aberdeen

University of Copenhagen

hcmr
ΕΛΚΕΘΕ

Universitas Bergensis

Cefas

INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

Biology Centre CAS

Alma Mater Studiorum
Università di Bologna

azti tecnalia

Università degli Studi di Udine

WP leaders

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WP9		**Ariadna Sitjà-Bobadilla**	coordination.parafishcontrol@csic.es	**CSIC**

Parasite group	Parasite species	Fish
Crustaceans	***Lepeophtheirus salmonis***	AS
	Ceratothoa oestroides	ESB,GSB
Monogeneans	***Sparicotyle chrysophrii***	GSB
Myxozoans	***Tetracapsuloides bryosalmonae***	RBT
	Enteromyxum leei	GSB
	Enteromyxum scophthalmi	TB
	Sphaerospora molnari	CC
	Thelohanellus kitauei*	CC
Microsporidians	***Enterospora nucleophila****	GSB
Cilliates	***Ichthyophthirius multifiliis***	RBT, CC
	Philasterides dicentrarchi	TB
Dinoflagellates	***Amyloodinium ocellatum***	ESB
Amoebae	***Paramoeba perurans***	AS
Oomycetes	***Saprolegnia parasitica***	AS, RBT
Zoonotic helminths	**Anisakidae, Opisthorchidae, Diphyllobothriidae**	All

Abbreviations: AS = Atlantic salmon (*Salmo salar*); CC = common carp (*Cyprinus carpio*), ESB = European sea bass (*Dicentrarchus labrax*), GSB = gilthead sea bream (*Sparus aurata*). RBT= rainbow trout (*Onchorhynchus mykiss*), TB = turbot (*Psetta maxima*). ***** Emerging or exotic parasites.

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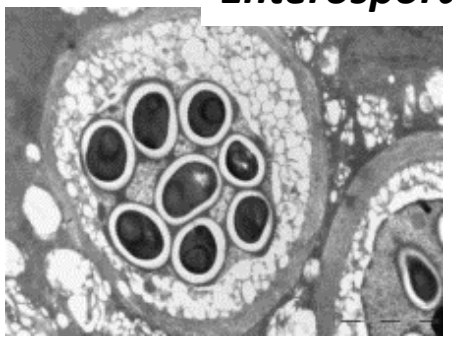
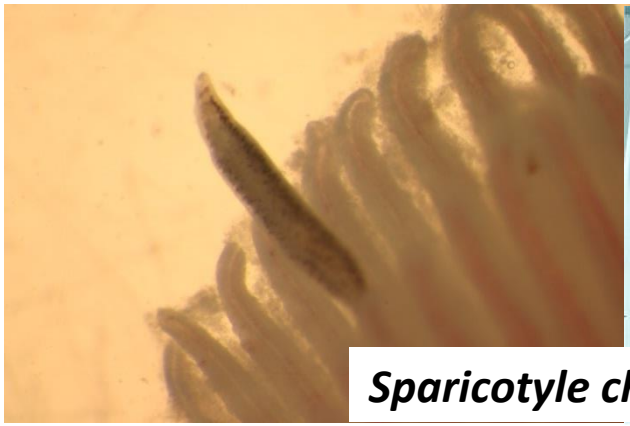
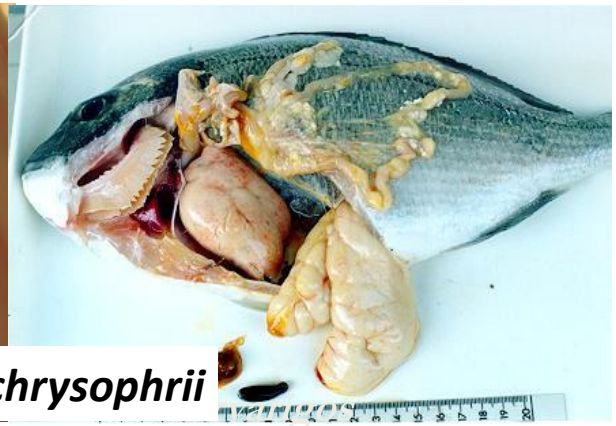
Enterospora nucleophila



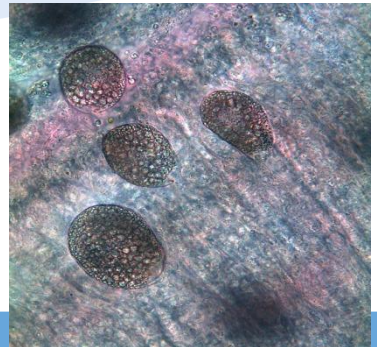
Enteromyxum leei





Sparicotyle chrysophrii

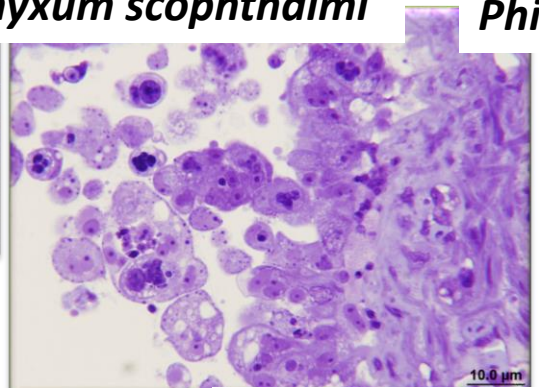
Anisakis sp.



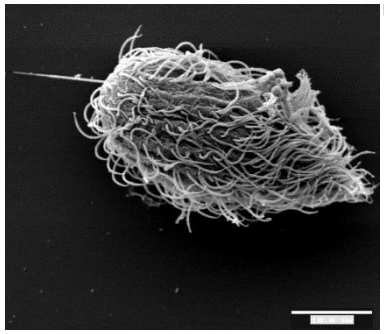
Ceratothoa oestroides

Amyloodinium ocellatum

Enteromyxum scophthalmi



Philasterides dicentrarchi

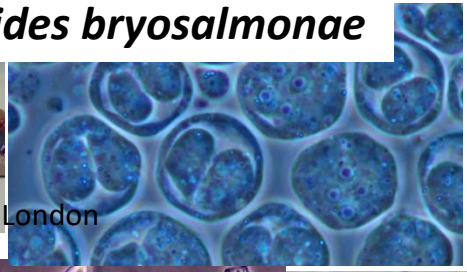


Lepeophtheirus salmonis

Paramoeba perurans

Saprolegnia parasitica

Tetracapsuloides bryosalmonae



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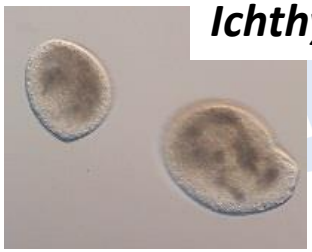
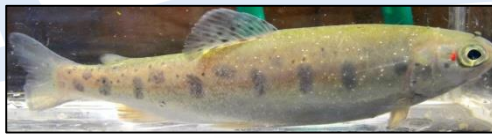
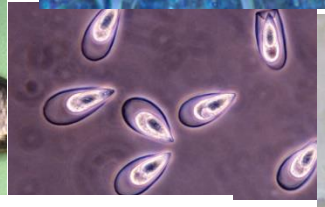
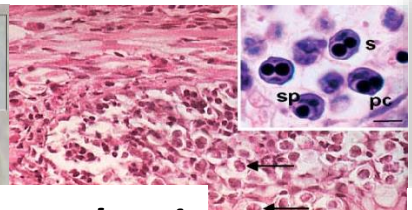
Ichthyophthirius multifiliis





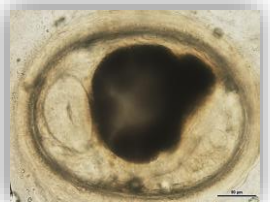


Thelohanellus kitauei



Sphaerospora molnari

© Bruno et al., 20



Opisthorchid metacercariae


We are a mature project: ends 31 March 2020

47 KOs collected in P3

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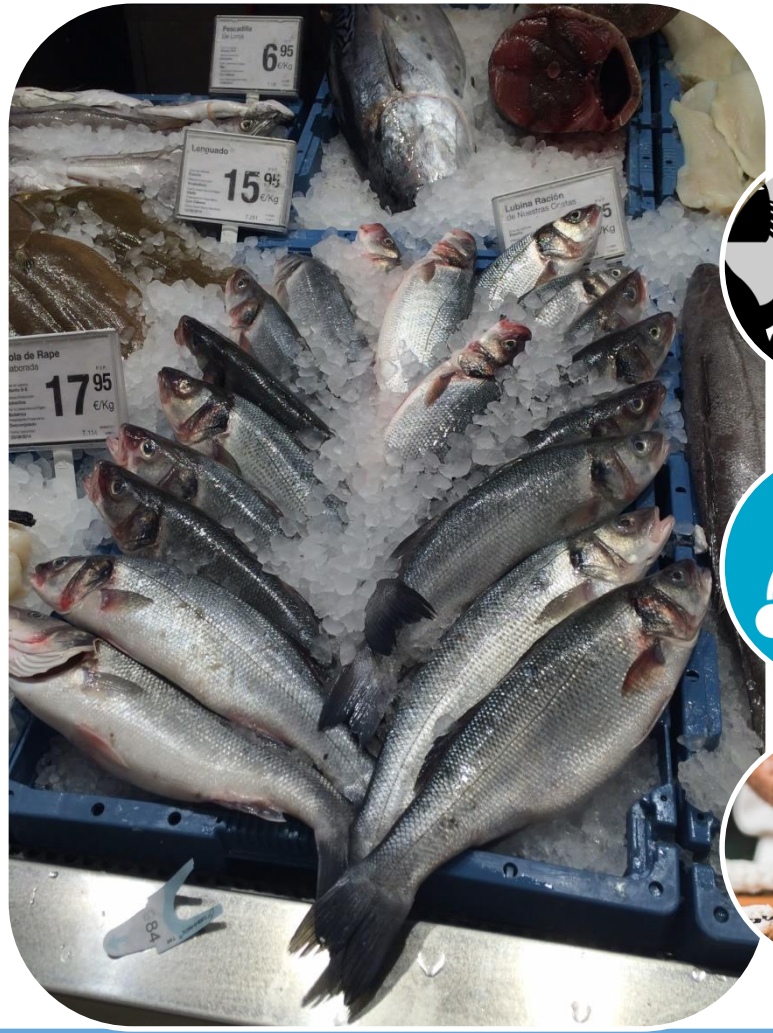
- KO #1 - Risk Factors for parasite infections (Owner: Cefas, ANDROMEDA, AQUARK, CSIC, IOR, SKRET, USC, UNIBO, UNIUD)
- KO #2 - Infection model for *Saprolegnia* (both *diclina* and *parasitica*) (WU)
- KO #3 - White spot disease control with bacterial surfactant (Owner: KNAW, KU - Patent number EP17202669)
- KO #4 - Sparicotylosis alternative treatments (Owner: IOR)
- KO #5 - Anisakis detection portable kit (Owner: AZTI)
- KO #6 - Best Practices of farm management to avoid presence of Anisakis in farmed fish (Owner: AZTI et al.)
- KO #7 - Alternative treatment strategies for *Saprolegnia* (Owner: UNIBO)
- KO #8 - Ich recombinant protein and homogenate vaccines (Owner: KU, MTA)
- KO #9 - Expression of two proteins of *Ich* in *Pichia* (Owner: KU, W42)
- KO #10 - PKD Vaccine (Owner: UNAB)
- KO #11 - In vivo infection model for AGD in salmon (Owner: Cefas)
- KO #12 - In vitro infection model for AGD in salmon (Owner: UoS)
- KO #13 - Compound that works against *Paramoeba perurans* (AGD) (Owner: UoS)
- KO #14 - Salivary gland proteins from *Lepeohtheirus salmonis* (Owner: UiB and UoS)
- KO #15 - Vaccines against *Sphaerospora molnari* in carp (Owner: BCAS)
- KO #16 - Immunostimulants against *Sphaerospora molnari* in carp (Owner: BCAS, SKRET)
- KO #17 - qPCR for *Sphaerospora molnari* and *T. kitauei* (Owner: BCAS)
- KO #18 - Immunostimulants against *Amyloodinium ocellatum* (Owner: UNIUD)
- KO #19 - Alternative treatments for *Ceratothoa oestroides* (Owner: IOR)
- KO #20 - Point of care for *E. leei* sea bream (Owner: CSIC, INGENASA)
- KO #21 - Infection models for *E. leei* (Owner: CSIC)
- KO #22 - Immunostimulants for *Ich* (Owner: KU)
- KO #23 - Alternative treatment strategies for Ich (Owner: MTA)
- KO #24 - Alternative treatment strategies for Ich (Owner: KU)
- KO #25 - Vaccines for *Saprolegnia* (Owner: UNAB)
- KO #26 - New monoclonal antibodies for PKD (Owner: VAL)
- KO #27 - Point of care tests for *Enteromyxum* spp. (Owner: CSIC, INGENASA)
- KO #28 - Alternative treatments for *Philasterides dicentrarchi* (Owner: USC)
- KO #29 - Vaccines for *Philasterides dicentrarchi* (Owner: USC, CSIC, INIA)
- KO #30 - Point of care test for *P. perurans* (Owner: Cefas)
- KO #31 - Repository of diagnostic methods (Owner: CSIC, DTU, UNIBO, IOR, UoS, UNAB, Cefas, BCAS, MTA, USC, KU)
- KO #32 - SHIELD new diet for sea bream (Owner: SKRET)
- KO #33 - Strategy to block the interaction of BAFF with its receptor as a treatment against proliferative kidney disease (PKD) (Owner: INIA-UNAB - Patent in progress)
- KO #34 - Challenge model for *Amyloodinium ocellatum* (Owner: UNIUD)
- KO #35 - Sequencing of *Amyloodinium ocellatum* (genome) (Owner: UNIUD)
- KO #36 - Vaccine for *Amyloodinium ocellatum* (Owner: UNIUD)
- KO #37 - Two different qPCR protocols for detection of *Neoparamoeaba perurans* (AGD) (Owner: DTU)
- KO #38 - qPCR and ICH protocols for detection of *Tetracapsuloides bryosalmonaeis* (PKD) (Owner: DTU)
- KO #39 - qPCR for *Enterospora nucleophila* (Owner: CSIC)
- KO #40 - Methods for detection, quantification and discrimination of genotypes/serotypes of *P. dicentrarchi* (Owner: USC).
- KO #41 - Tools for detection and identification of zoonotic metacercariae (Owner: UNIBO)
- KO #42 - Sequencing of *Enteromyxum leei* (genome) (Owner: CSIC)
- KO #43 - Sequencing of *Enteromyxum scophthalmi* (genome) (Owner: CSIC)
- KO #44 - Sequencing of *P. dicentrarchi* (genome/ transcriptome) (Owner: USC).
- KO #45 - Sequencing of *Sphaerospora molnari* (genome/transcriptome) (Owner: BCAS).
- KO #46 - Sequencing of *Paramoeba perurans* (genome/transcriptome) (Owner: Cefas, UoS).
- KO #47 - Sequencing of *Sparicotyle chrysophrii* (genome/transcriptome) (Owner: IOR, HCMR, CSIC).

Lets have a look at some of them…

1-Generating a more positive public perception



Environmental impact

Animal welfare

Food security

KO#6
0% zoonotic helminths in farmed fish

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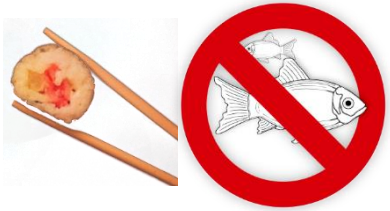
efsa
European Food Safety Authority

Zoonotic risks in:

- **marine and freshwater farmed fish: 0 %**
- **fish products: 0%**
- **Feedback to EATiP, FEAP and AAC done**
- **Feedback to ESFA to be done**

- **Allergenicity of zoonotic helminths: expanded to *Contracaecum***
- **Recommendation to determine the presence and transfer of allergens from aquafeeds to fish**



- **Smart solution to ensure safety of fish products**
- **Good practice handbook for Minimum Parasite Infection**

UNE
NormalizaciónEspañola

We have recently presented a Spanish Standardization Proposal to the committee CTN 173/SC2 based on WP7 results

…and launched a dialogue with European authorities for an emendment to current legilsation

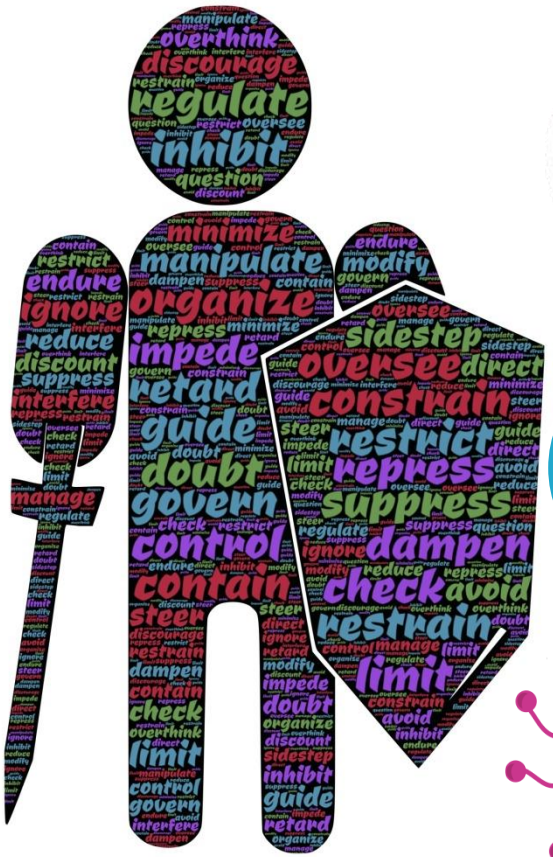
Interaction wild-farmed fish




1. ***S. chrysophrii*** and ***C. oestroides*** between wild and farmed GSB and ESB
2. Characterisation of possible transfer of ***S. parasitica*** from farmed to wild salmonids and viceversa.
3. Epidemiological model to determine the transfer of ***I. multifiliis*** between farmed and wild salmonids.

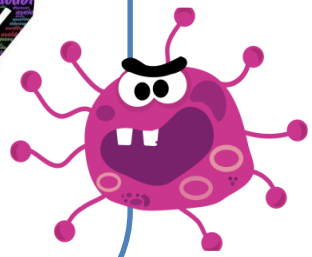
The transfer depends on parasite species and microlocation (we still do not precisely which parameters, e.g. currents, bottom type, salinity...). Thus, there is a strong evidence that it does exist, but at different "success rate". It is most prominent in *Ceratothoa* (aggressive natatory infective stages), at a lower degree for *Sparicotyle*, while *Saprolegnia* and *Ich* were evaluated using different approach, but even so.

2 - Develop appropriate governance models



Advancing impact of diseases

Minimizing impact of treatments



Avoiding spreading of pathogens

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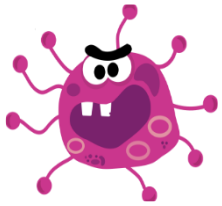
Information essential for diagnostics, treatments, vaccines, biosecurity, breeding, welfare indicators, etc.

Knowing the parasite:



In vitro culture, life cycle, experimental transmission, genome, transcriptome, proteome

Knowing the host:

Immune response, histopathology, transcriptomics (RNA seq, qPCR-array)

Some basic KOs

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KO #35 - Sequencing of *Amyloodinium ocellatum* (genome)
KO #42 - Sequencing of *Enteromyxum leei* (genome)
KO #43 - Sequencing of *Enteromyxum scophthalmi* (genome)
KO #44 - Sequencing of *P. dicentrarchi* (genome/ transcriptome)
KO #45 - Sequencing of *Sphaerospora molnari* (genome/transcriptome)
KO #46 - Sequencing of *Paramoeba perurans* (genome/transcriptome)
KO #47 - Sequencing of *Sparicotyle chrysophrii* (genome/transcriptome)

KO#12 Neoparamoeba perurans
RTgill-W1 In vitro challenge model

Book for standard Operating protocols for parasite transmission and isolation

KO#49 Philasterides dicentrarchi
nkl gene good candidate for genetic breeding

KOs Vaccines and immunoprophylaxis

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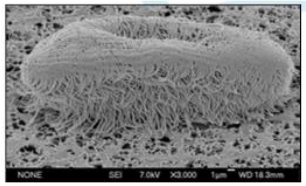
KO #36 - Amyloodinium ocellatum
Vaccine trials in lab

KO#9 Ichthyophthirius multifiliis
Recombinant vaccine in *Pichia pastoris*

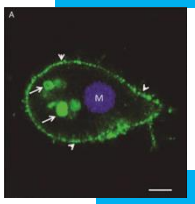


KO#10 **Proliferative Kidney Disease** (PKD)
DNA Vaccine field trials

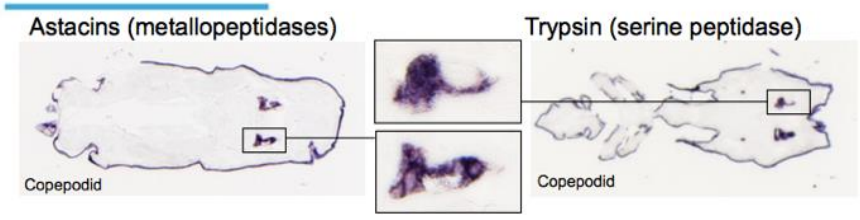
Field-based

vaccination trials

KO#29 Philasterides dicentrarchi
Variable Surface Protein (VSP)
expressed in Pichia pastoris as vaccine targets



KO#14 Salmon louse
Salivary gland proteins - vaccine targets



Enteromyxum leei
functional feeds
And others "in the oven"…

KOs diagnostic tools

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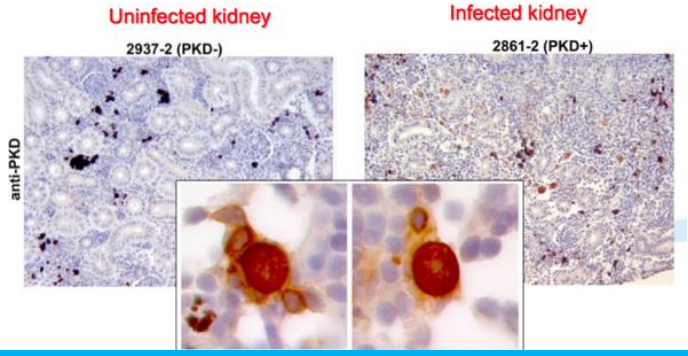
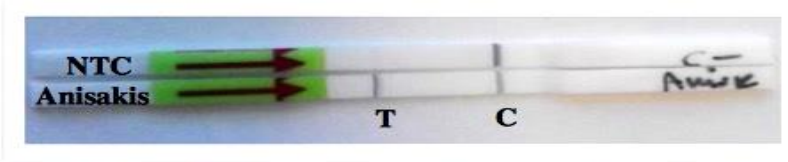
KO#26 Proliferative Kidney Disease (PKD) P14G8 Mab



KO#38 PKD: qPCR & ICH protocols

KO#5 *Anisakis*
Portable detection kit



LF RPA Assay for the detection of Anisakis simplex: Test line (T); Internal Control line (C). Negative Template control (NTC)

KO #40 *P. dicentrarchi*
Methods for detection, quantification and discrimination of genotypes/serotypes

KO#39 *Enterospora nucleophila*
qPCR & ISH protocols

KO#41 Zoonotic metacercaria
Tools for detection and identification

POC: ***Enteromyxum*** **spp.**

Reference diagnostic methods for: *P. perurans, E. nucleophila T. bryosalmonae*

Repository of parasite diagnostic methods

OPEN ACCESS

And others "in the oven"…

KOs treatments

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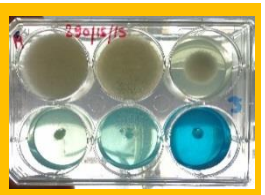
KO#7 *Saprolegnia*
Treatments validation

Benzoic acid (MIC:100 ppm)
Acetic acid (MIC:250 ppm)
Iodoacetic acid (MIC:250 ppm)
copper sulphate (MIC:250 ppm)
Virkon™S (MIC = MLC = 1,000 ppm)
Actidrox® (MIC = 5,000 / MLC= 500)
Detarox® AP (MIC = 1,000 ppm / MLC= 100)

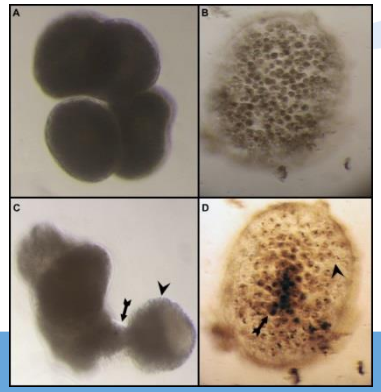


KO#4 Sparicotylosis
Alternative Treatments

Bithionate sodium
Cedrol
Curcumin
Eucalyptol
Garlicin
(+)-trans-chrysanthemic acid
Camphor

KO#28 Philasterides dicentrarchi
Nk-lysin (Nkl) AMP

KO#3 Ichthyophthirius multifiliis
Bacterial Bio-surfactant (*Pseudomonas*)



PATENT
EDP ref: P1600045PC00 – International PCT Application No. PCT/EP2018/081923

START-UP: SUNDEW

KO #33 PKD Strategy to block the interaction of BAFF with its receptor as a treatment (patent in progress)

Optimal strategy for the use of salmon lice cleaner fish

www.parafishcontrol.eu

And others "in the oven"…

KOs epidemiological models and tools

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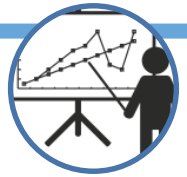

Risk Factor Analysis

KO#7 Identification of risk factors for parasite introduction into and amplification within fish farms: *Sparicotyle chrysophrii, Enteromyxum leei, Enterospora nucleophila, Ceratothoa oestroides*

Alpha version of the economic model

- Farm level tool developed in R Shiny
- Provides a user-friendly web-based interface through which partners and fish farmers can investigate some of the key findings
- Provides information on the relative likelihood of infection and intensity based on factors chosen by an end user.

Manuals describing IPMS for parasite management and handbooks describing good practices

- **Manual 1 - Salmonids**
- **Manual 2 - European sea bass & gilthead sea bream**
- **Manual 3 - Turbot**
- **Manual 4 - Common Carp**

Next event

ParaFishControl

FINAL
CONFERENCE
BRUSSELS

11th March 2020

CSIC Headquarters

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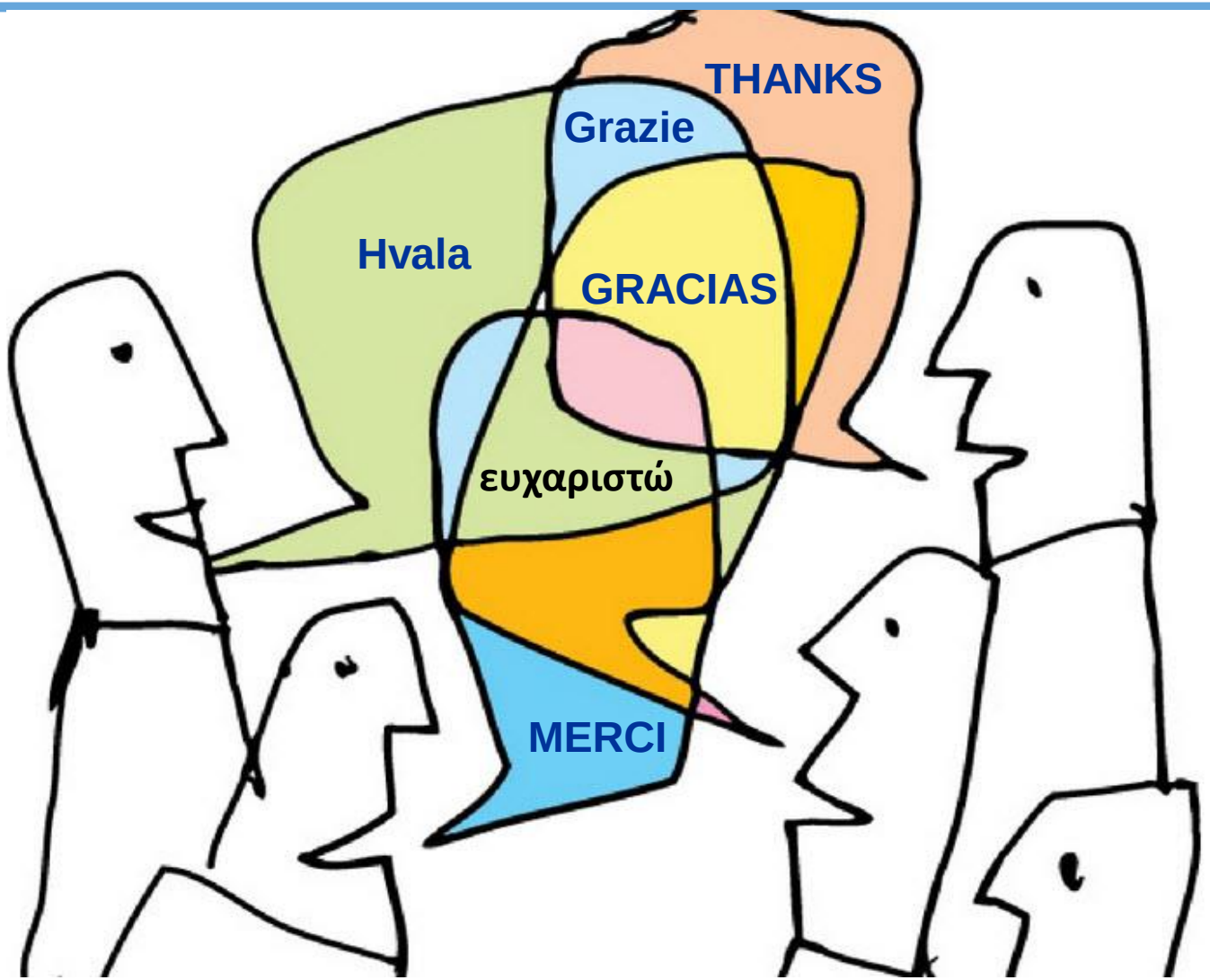
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