



ParaFishControl

Come in, WE'RE
OPEN



welcome!



ParaFishControl

 parafishcontrol.eu

 [@parafishcontrol](https://twitter.com/parafishcontrol)

 [ParaFishControl](https://www.linkedin.com/company/ParaFishControl)



ParaFishControl Workshop “North European Fish Parasite Management Strategies in Aquaculture Farms”

Organised at Aquaculture Europe 2019 – OUR FUTURE – GROWING FROM WATER

Date and Time: Wednesday, 9 October 2019; 10:30-15:30

Location: Estrel Berlin – Sonnenallee 225, Berlin, Germany

ParaFishControl Workshop
**“North European Fish Parasite Management Strategies
in Aquaculture Farms”**



ParaFishControl
Advancements and upcoming results
Dr. Ariadna Sitjà-Bobadilla
Project Coordinator



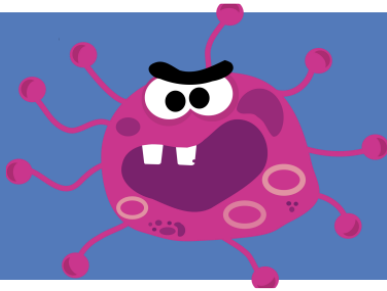


Advanced Tools and Research Strategies for Parasite Control in European farmed fish



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 (Shin et al., Global Aquaculture Advocate, 2015):
 - Estimated losses due to parasitism: hatchery (20 %), growout (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)



SOLUTIONS !!



WHO, HOW, WHAT?

Industry



Research



13 countries

28 partners



WP leaders



WP1		Geert Wiegertjes	Geert.Wiegertjes@wur.nl	WU
WP2		Ivona Mladineo	mladineo@izor.hr	IOR
WP3		James Bron	j.e.bron@stir.ac.uk	UoS
WP4		Oswaldo Palenzuela	oswaldo.palenzuela@csic.es	CSIC
WP5		Niels Lorenzen	nilo@vet.dtu.dk	DTU
WP6		Alastair Cook	alastair.cook@cefas.co.uk	Cefas
WP7		Miguel Angel Pardo	mpardo@azti.es	AZTI
WP8	 	Marieke Reuver Emma Bello (o.b.)	marieke.reuver@aquatt.ie	AquaTT
WP9		Ariadna Sitjà-Bobadilla	coordination.parafishcontrol@csic.es	CSIC

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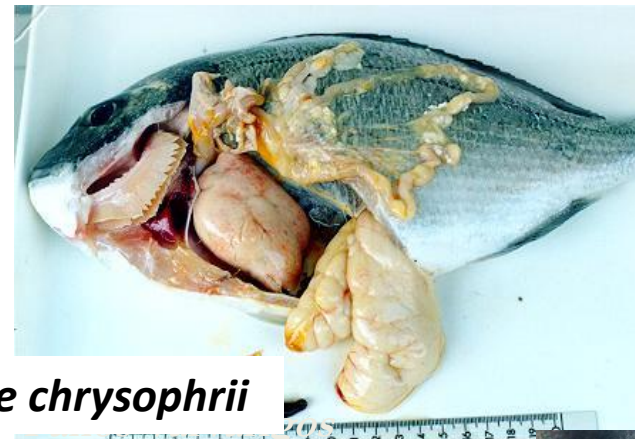
Enterospora nucleophila



Enteromyxum leei



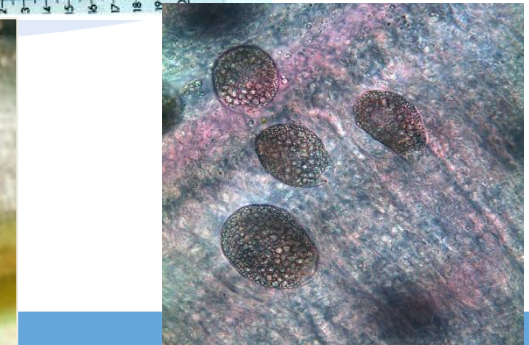
Photo by A. Silià-Bobadilla



Sparicotyle chrysophrii



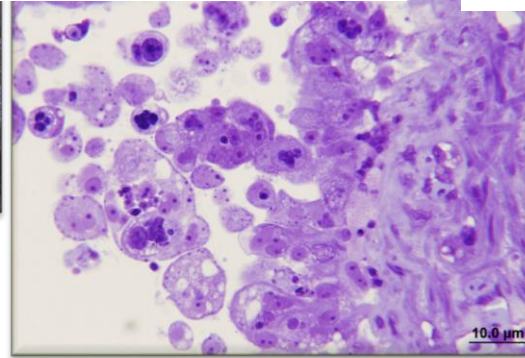
Ceratothoa oestroides



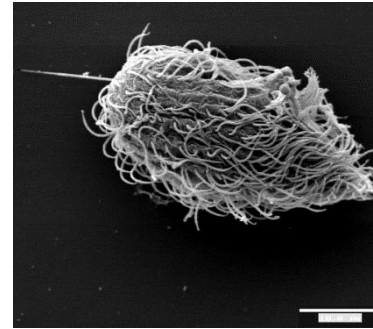
Amyloodinium ocellatum



Enteromyxum scophthalmi



Philasterides dicentrarchi



Lepeophtheirus salmonis



Paramoeba perurans



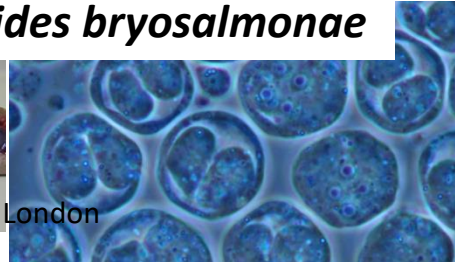
Saprolegnia parasitica



Tetracapsuloides bryosalmonae



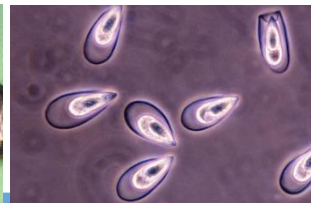
© NHM, London



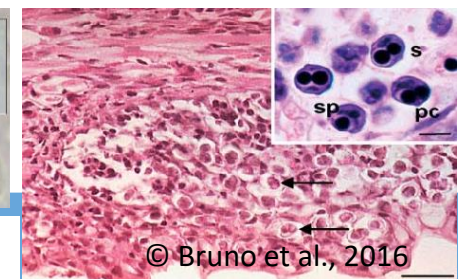
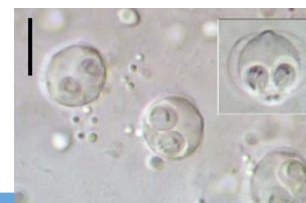
Ichthyophthirius multifiliis



Telohanellus kitauei



Sphaerospora molnari



© Bruno et al., 2016



We are a mature project: ends 31 March 2020



2020

2015

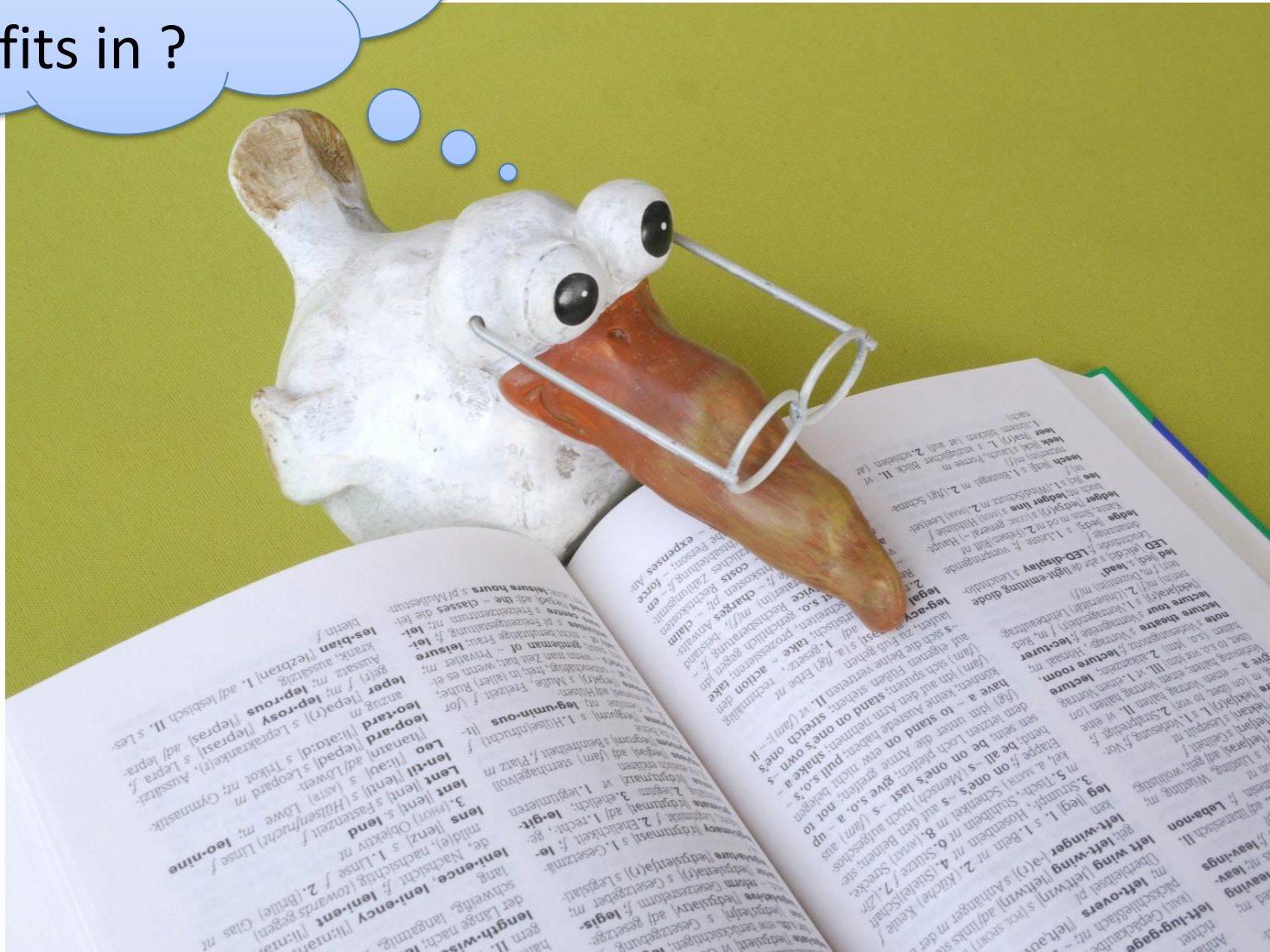
WHAT ARE WE DOING ?



Were
ParaFishControl
fits in ?



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EU aquaculture priorities

Aquaculture is a key component of both the **Common Fisheries Policy and the Blue Growth agenda**. In consultation with stakeholders, the Commission has identified the main priorities to facilitate its sustainable development:



Simplify administrative procedures



Ensure access to space through coordinated spatial planning



Enhance the competitiveness of EU aquaculture



Promote a level playing field for EU operators

Science f
FUTURE
Sustain



Scientif
Mechanis

Food from the Oceans

High Level Group of Scient
Scientific Opinio



Research and
Innovation



European Aquaculture
Technology and Innovation Platform

Thematic area: Aquatic Animal Health and Welfare

A Review of the Strategic Research and Innovation Agenda

Our Vision for the future of
European Aquaculture

2017

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 *Lepeophtheirus 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 *Ceratomyxa 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 *Neoparamoeba perurans* (AGD) (Owner: DTU)
- KO #38 - qPCR and ICH protocols for detection of *Tetracapsuloides bryosalmonae*s (PKD) (Owner: DTU)
- KO #39 - qPCR for *Enterosporea 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 scopthalmi* (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).



1-Generating a more positive public perception



Environmental impact



Animal welfare



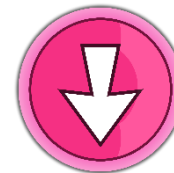
Food security

KO#6

**0% zoonotic helminths
in farmed fish**



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Zoonotic risks in:

- marine and freshwater farmed fish: 0 %
- fish products: 0%
- Feedback to EATiP and FEAP 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



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

Interaction wild-farmed fish

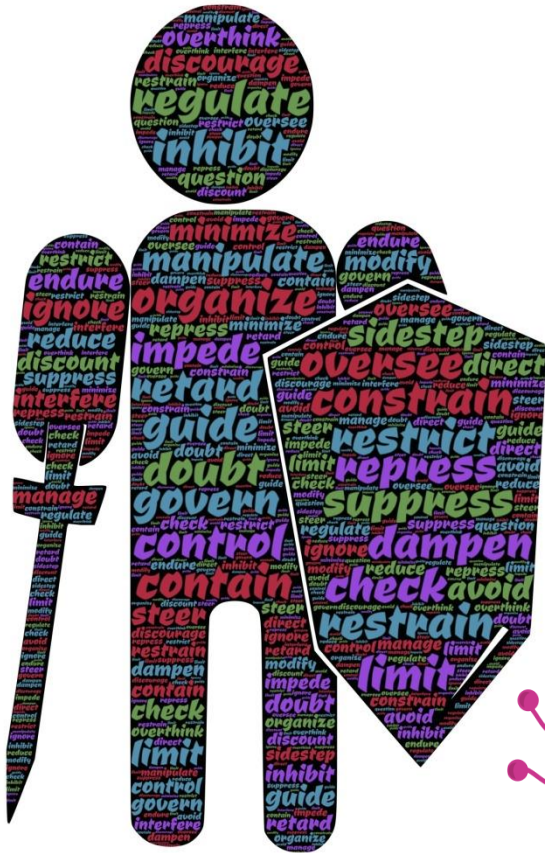


1. ***S. chrysophrii*** and ***C. oestroides*** between wild and farmed GSB and ESB
2. Characterisation of possible transfer of ***S. parasitica*** from farmed to wild salmonids and viceversa.
3. Epidemiological model to determine the transfer of ***I. multifiliis*** between farmed and wild salmonids.

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 *Ceratomyxa* (aggressive natatory infective stages), at a lower degree for *Sparicotyle*, while *Saprolegnia* and *Ich* were evaluated using different approach, but even so.



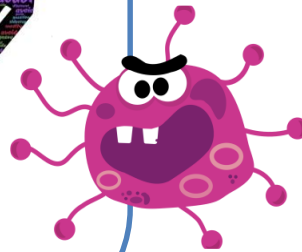
2 - Develop appropriate governance models



Advancing impact of diseases

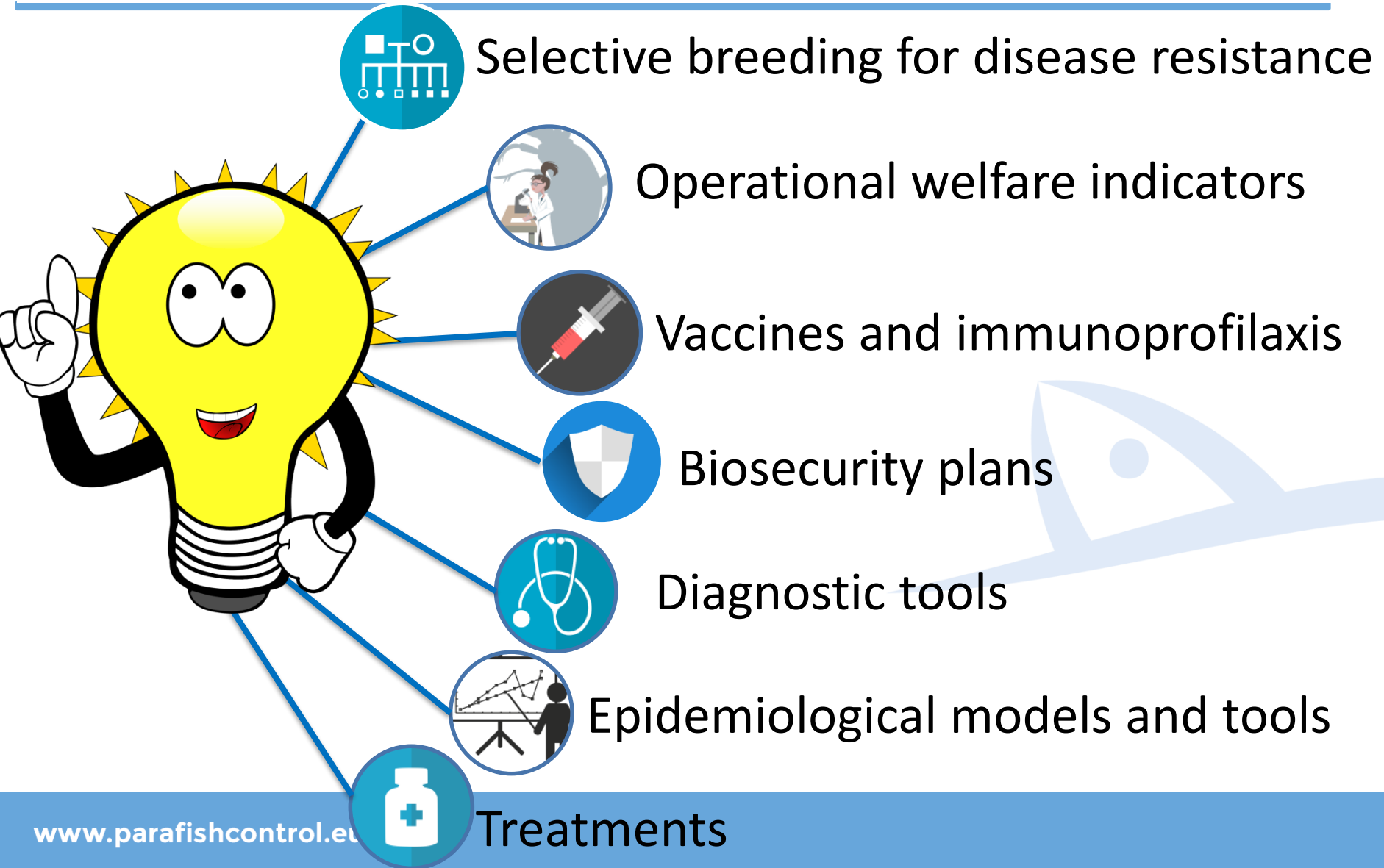


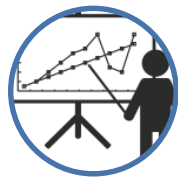
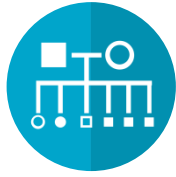
Minimizing impact of treatments



Avoiding spreading of pathogens

3 - Promote preventive measures to diseases and adverse health conditions





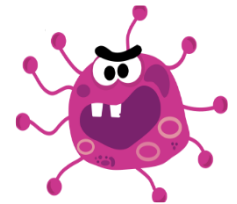

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Information essential for diagnostics, treatments, vaccines, biosecurity, breeding, welfare indicators, etc.

Knowing the parasite:

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



Knowing the host:

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



- 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



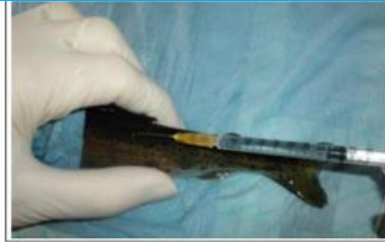


KO #36 - *Amyloodinium ocellatum*
Vaccine trials in lab

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



Field-based
vaccination trials

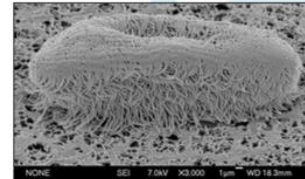


Enteromyxum leei
functional feeds

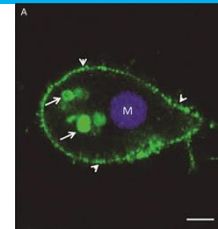
And others "in the oven"...



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



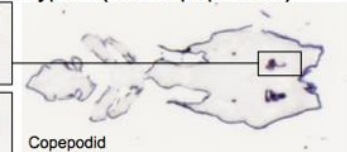
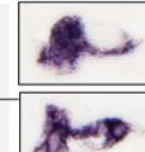
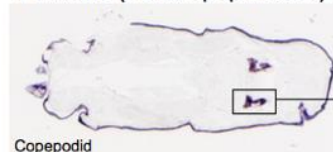
KO#29 *Philasterides dicentrarchi*
Variable Surface Protein (VSP)
expressed in *Pichia pastoris* as vaccine targets



KO#14 Salmon louse
Salivary gland proteins - vaccine targets

Astacins (metallopeptidases)

Trypsin (serine peptidase)



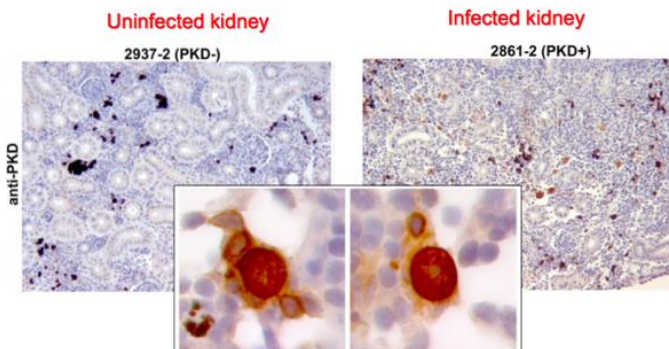
Copepod

Copepod

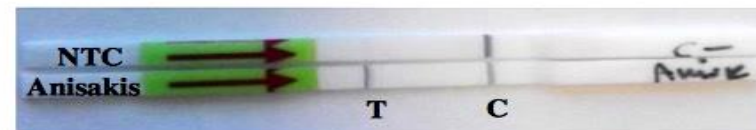
KOs diagnostic tools



KO#26 Proliferative Kidney Disease (PKD) P14G8 Mab



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#41 Zoonotic metacercaria Tools for detection and identification

KO#39 *Enterospora nucleophila* qPCR & ISH protocols

POC: *Enteromyxum* spp.



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

Repository of parasite diagnostic methods



KOs treatments



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KO#7 *Saprolegnia* Treatments validation

Benzoic acid
Acetic acid
Iodoacetic acid
Copper sulphate
Virkon™S
Actidrox®
Detarox® AP



KO#4 Sparicotylosis Alternative Treatments

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



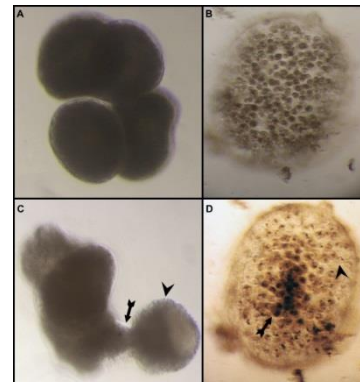
KO#28 *Philasterides dicentrarchi* Nk-lysin (Nkl) AMP

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

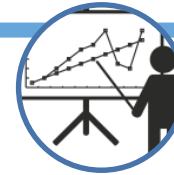


KO#3 *Ichthyophthirius multifiliis* Bacterial Bio-surfactant (*Pseudomonas*)



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

START-UP: SUNDEW



Risk
Factor
Analysis

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






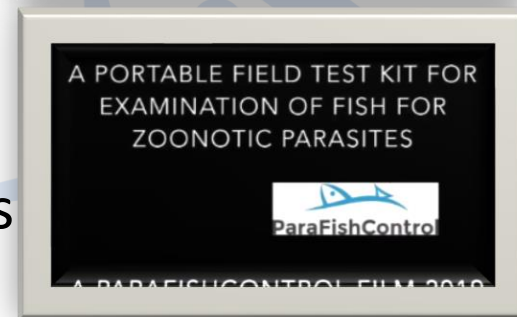
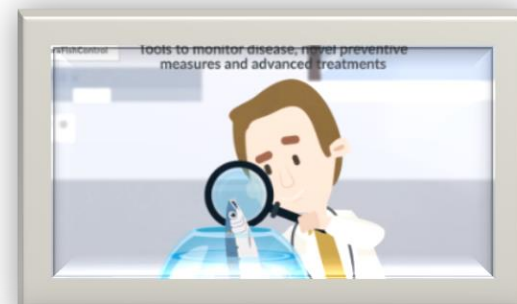
Manuals describing IPMS for parasite management and handbooks describing good practices

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



**Integrated
Parasite
Management
Strategies
(IPMS)**

- Newsletters (3 issues - [Link](#))
- LinkedIn: 132 members 
- Twitter: 711 followers 
- Videos (3 – click on the pictures!)
- Press releases and popularised publications: 12
- Participation in scientific conferences: 121
- Participation in other workshops & events: 51
- Ph D thesis: 3
- Organisation of workshops and training courses
13 
- Patents: 1 (521-049/16-7000)



16 peer-viewed articles in the last year



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- 1) Cano I, et al. (2019). *In vitro* gill cell monolayer successfully reproduces in vivo Atlantic salmon host responses to *Neoparamoeba perurans* infection. *Fish & Shellfish Immunology* 86: 287-300. doi: <https://doi.org/10.1016/j.fsi.2018.11.029>.
- 2) Fernández C, et al. (2019). Methacarn preserves mucus integrity and improves visualization of amoebae in gills of Atlantic salmon (*Salmo salar* L.). *Journal of Fish Diseases* 42:6. doi: <https://doi.org/10.1111/jfd.12988>.
- 3) Hossameldin A N., et al. (2019). Detection of the intranuclear microsporidian *Enterospora nucleophila* in gilthead sea bream by *in situ* hybridization. *Journal of Fish Diseases* 42:6. doi: <https://doi.org/10.1111/jfd.12993>.
- 4) Byadgi O, et al. (2019). Expression of infection-related immune response in European sea bass (*Dicentrarchus labrax*) during a natural outbreak from a unique dinoflagellate *Amyloodinium ocellatum*. *Fish & Shellfish Immunology* 84:62-72. doi: <https://doi.org/10.1016/j.fsi.2018.09.069>.
- 5) Syahputra, K., et al. (2019). Transcriptomic analysis of immunity in rainbow trout (*Oncorhynchus mykiss*) gills infected by *Ichthyophthirius multifiliis*. *Fish & Shellfish Immunology* 86: 486-496. doi: <https://doi.org/10.1016/j.fsi.2018.11.075>.
- 6) Korytar T, et al. (2019). The kinetics of cellular and humoral immune responses of common carp to presporogonic development of the myxozoan *Sphaerospora molnari*. *Parasites & Vectors* 12:208. doi: <https://doi.org/10.1186/s13071-019-3462-3>.
- 7) Muñoz-Atienza E, et al. (2019). CK11, a Teleost Chemokine with a Potent Antimicrobial Activity. *Journal of Immunology* 202 (3) 857-870. doi: <https://doi.org/10.4049/jimmunol.1800568>.
- 8) Piazzon MC, et al. (2019). Acting locally - affecting globally: RNA sequencing of gilthead sea bream with a mild *Sparicotyle chrysophrii* infection reveals effects on apoptosis, immune and hypoxia related genes. *BMC Genomics*; 20:200. doi: <https://doi.org/10.1186/s12864-019-5581-9>.
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- 10) Øvergård AC, et al. (2018). Salmon louse rhabdoviruses: Impact on louse development and transcription of selected Atlantic salmon immune genes. *Developmental & Comparative Immunology* 86:86-95. doi: [10.1016/j.dci.2018.04.023](https://doi.org/10.1016/j.dci.2018.04.023).
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Session 1 - Setting the Scene

10:30 - 10:50	<i>Economic Impact of parasitic diseases in Rainbow Trout</i>	Niels Henriksen, Dansk Akvakultur
10:50 - 11:10	<i>Economic Impact of parasitic diseases in Atlantic Salmon</i>	Audun Iversen, NOFIMA
11:10 - 11:30	<i>Economic Impact of Parasitic Diseases in Carp</i>	Béla Urbányi, Szent István University
11:30 - 11:50	<i>ParaFishControl Project and Results</i>	Ariadna Sitjà-Bobadilla, CSIC

Session 2 -Alternative Treatments

11:50 - 12:00	<i>Alternative treatments for Saprolegnia</i>	Perla Tedesco, University of Bologna
12:00 - 12:10	<i>Biological control of ichthyophthiriasis</i>	Louise von Gersdorff Jørgensen, University of Copenhagen
12:10 - 12:20	<i>Health management strategies for cleaner fish</i>	Panos Christofilogiannis, AQUARK
12:20 - 13:00	Open discussion and feedback from participants	
13:00 - 14:30	<i>Lunch Break</i>	

Session 3 - Diagnostics and Prevention

14:30 - 14:40	<i>New tools for the diagnosis of the amoebic gill disease (AGD)</i>	Ariadna Sitjà-Bobadilla, CSIC
14:40 - 15:00	<i>Towards an improved image of aquaculture products regarding food safety</i>	Ariadna Sitjà-Bobadilla, CSIC
15:00 - 15:10	<i>Vaccine for Ichthyophthirius multifiliis</i>	Louise von Gersdorff Jørgensen, University of Copenhagen
15:10 - 15:20	<i>Vaccine for proliferative kidney disease (PKD)</i>	Carla Piazzon, CSIC
15:20 - 15:30	<i>Vaccine for Saprolegnia</i>	Javier Dieguez, CSIC
15:30 - 15:40	<i>Vaccine for Sphaerospora molnari</i>	Tomas Korytar, BCAS
15:40 - 16:00	Open discussion and feedback from participants	

