ParaFishControl

ParaFishControl New Diagnostic Methods and Tools for Parasitic Diseases

ParaFishControl Final Conference

"Innovative Strategies to Control Parasites in Aquaculture Farms"

~~Brussels,~~ 11th March 2020

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Diagnostics Approach in PFC

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WP4 covering "Diagnostics"

- **General objective**
  - To provide an essential diagnostics toolbox and a collection of diagnostic protocols for the main aetiologies involved in parasitic outbreaks in European aquaculture production.
  - Different aetiologies, different needs and approaches:
    - Harmonization and validation of reference methods, for selected parasites (T4.1).
    - Generation of quick point-of care tools for diagnosis of parasites whose management can improve with such (T4.2).
    - Development of assorted tests and markers for environmental monitoring, risk assessment, epidemiology & life cycle research, strain identification, virulence markers, vaccination assessment, etc. (T4.3 & 4.4)
    - Collection of parasite diagnostics protocols repository (T4.5)

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Tetracapsuloides bryosalmonae

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- **Reviewed methods & compared reproducibility of procedures and tests on reference (blinded) samples in different laboratories – qPCR and IHC.**

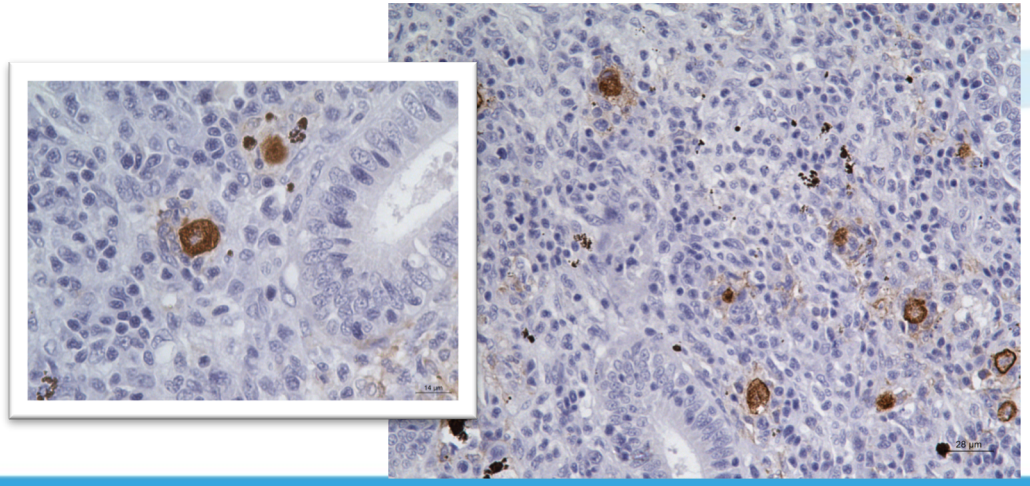
Slide no	A	B	C	D	**Original**
1	Pos/low	Pos/++	Pos/+	Pos/+	**Pos/+**
2	Neg	Neg	Neg	Neg	**Neg**
3	Pos/high	Pos/+++	Pos/+++	Pos/+++	**Pos/++**
4	Pos/high	Pos/+++	Pos/+++	Pos/++(+)	**Pos/++**
5	Neg	Neg	Neg	Neg	**Neg**
6	Pos/high	Pos/++	Pos/++	Pos/+++	**Pos/+++**
7	Pos/Low	Pos/+	Pos/(+)	Pos/++	**Pos/++**

Sample	**Laboratory**				
	A	**B**	**C**	**D**	**E**
I	26.93 ± 0.14	29.62	27.84	26.4	28.7
II	35.65 ± 0.13*	-	-	37.8**	0
III	21.4 ± 0.13	23.68	20.72	21.6	20.1
IV	23.08 ± 0.27	31.79	24.99	23.9	25.1
V	34.21 ± 0.3*	-	37.88**	0.0	0
VI	21.37 ± 0.32	23.56	18.95	18.9	22.7
VII	26.22 ± 0.11	25.21	23.45	26.2	24.2

* Contamination reported by the lab

**interpreted as negative by the laboratory

- **Developed new Mab against P14G8, a trout stage-specific antigen. Good reference test (IHC)**



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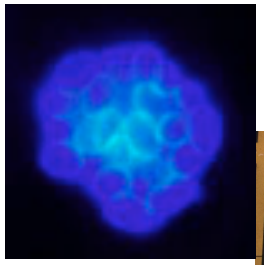
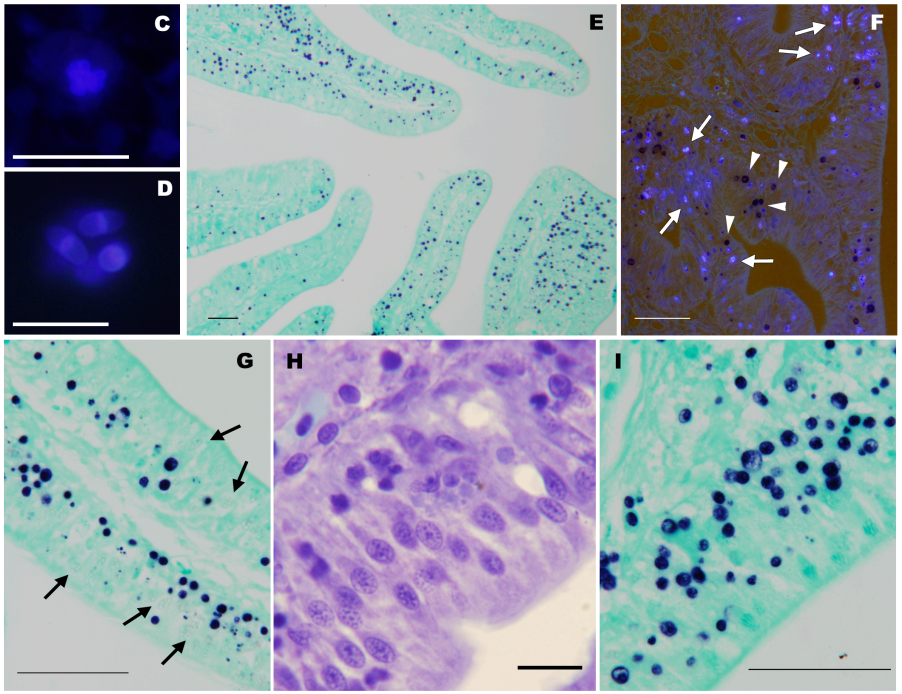
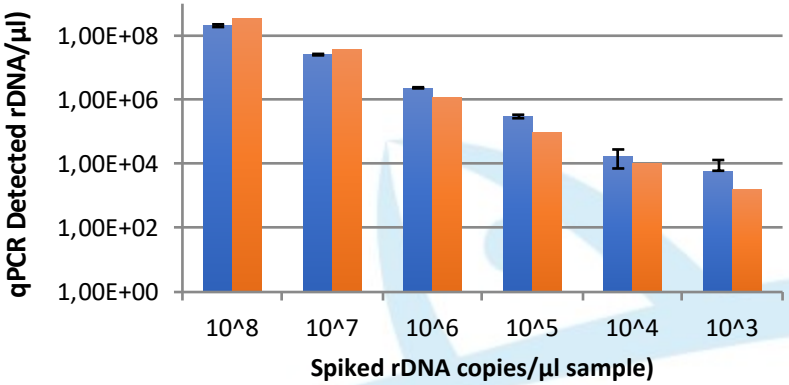
Enterospora nucleophila



- **Developed new tests (qPCR and ISH)**



***E. Nucleophila* rDNA copies in 1g intestine (EtOH)**



- **Compared reproducibility of qPCR test on reference (blinded) samples in different laboratories (6 participants)**
- **Attempted PoC tests (LFD devices): failure to obtain NGS –omic data and identify diagnostic candidate targets**

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Enteromyxum spp.

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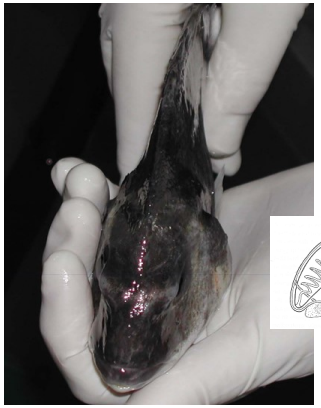
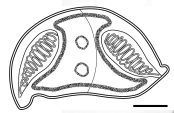
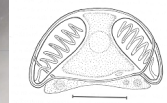
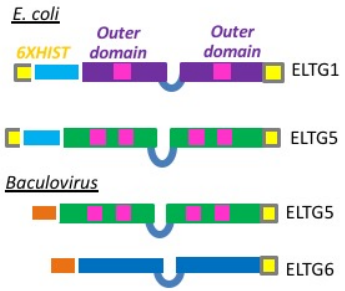
- Attempted PoC tests (LFD devices)

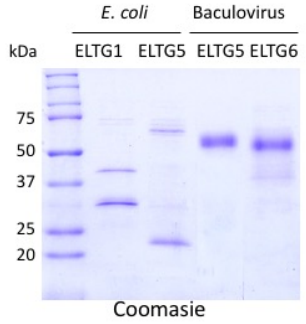






- Selected candidate targets from NGS data. >14,000 transcripts shortlisted to 6 proteins expressed in both spp (unknown role)
- Genes cloned and sequences verified, 3 targets selected
- Immunogenic peptides designed (synthetic 22-29 aa). Pabs and Mabs screened - ⊗
- Recombinant antigens designed and produced in different expression systems. Pabs and Mabs screened- ⊗
- One recombinant antigen redesigned, Pabs screened, Mabs just finished, being checked for specificity





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(Neo)Paramoeba perurans

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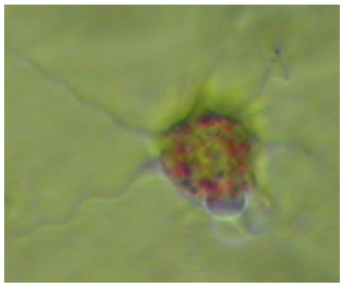
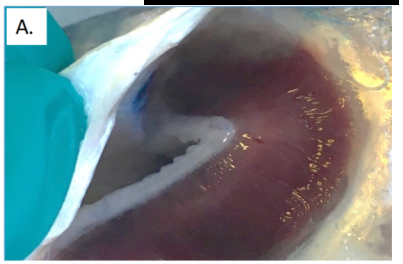
- Reviewed methods (qPCR) & compared reproducibility of tests on reference (blinded) samples in two laboratories.



- Point-of-Care test based on LAMP assay developed
  - Dirty-cheap sampling methods (Non-Lethal). 15 minutes pre-processing from swabbing to LAMP,
  - Sensitivity roughly 1 amoeba per sample
  - Internal control for sample quality prevents false negatives due to quick sample preparation
  - Clinical validation: 100% positives when gill score >3, correlation between score and amplification
  - Cost about 2.8 Eur/sample (roughly 37% of Taqman test)



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New tests developed

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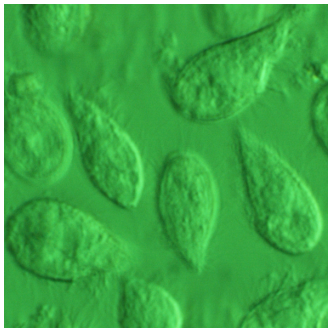
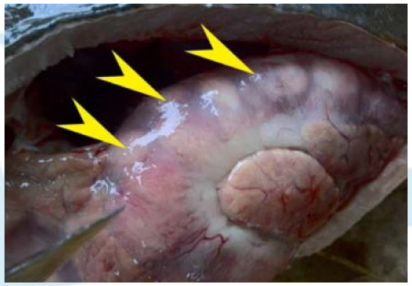
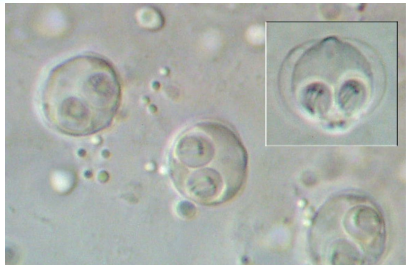


- *Philasterides dicentrarchi* - quantitative detection in fish & seawater (qPCR). Sensitivity 2 cell/L.
- Tools for genotyping & serotyping of different isolates
- *Carp Myxozoans:*
  - *Telohanellus kitauei* - intestinal giant-cystic disease of carp (new in Europe from Koi, uncertain distribution) – New highly specific Taqman qPCR for detection in fish, invertebrates, and environmental samples
  - *Sphaerospora molnari* – Gill & skin sphaerosporosis of carp. New Taqman qPCR and optimization for environmental samples and *in vivo* / *in vitro* proliferation studies.





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New tests developed

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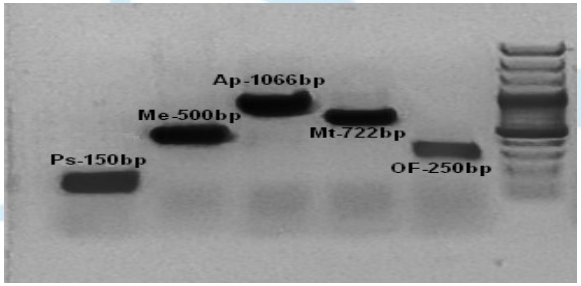
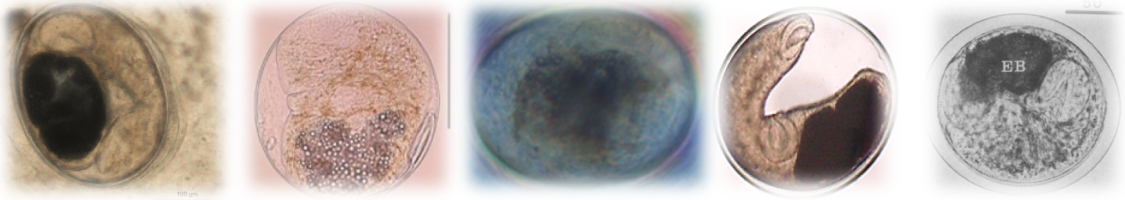
- Whole-genome screening for discriminating pathogenic *Saprolegnia parasitica* and *Saprolegnia diclina* from non-pathogenic genotypes. New highly specific tests for both species in fish & environmental samples (qPCR). Sensitivity roughly 2 spores/L

- New multiplex PCR to detect and discriminate fish-borne zoonotic trematodes (Opisthorchiids and Heterophiyds) in fish or fish products



Ps=*Pseudamphistomum truncatum*, Me=*Metorchis* spp., Ap=*Apophallus* spp., Mt=*Metagonimus*, OF=*Opisthorchis felineus*. Molecular marker 100 bp.



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Diagnostic Procedures and Protocols for Parasitic Diseases

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D4.5: Collection & repository of recommended protocols for parasite diagnosis in European aquaculture

- Diagnostic methods & tools reviewed and developed in the project
- Additional procedures for relevant parasites not covered in PFC

Output to be published as ebook: "Parasites in European Aquaculture: A Diagnostics Approach" – Springer

- Open Access ebook
- 250 pp
- Targeting Q1, 2021

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Main Contributors of WP

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European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

- EURL-DTU (*T. bryosalmonae* tests & Interproficiency validation tests)
- IATS-CSIC (*Enterospora* & *Enteromyxum* tests, WP coordination)
- UNIBO (Zoonotic metacercariae tests)
- Institute of Parasitology-BCAS (*Thelohanellus kitauei* & *Sphaerospora molnari* tests)
- USC (*Philasterides dicentrarchi* tests)
- UNAB (*T. bryosalmonae* tools, *Saprolegnia* tests)
- CEFAS (*Neoparamoeba perurans* tests)
- UoS (*Neoparamoeba perurans* tests)
- INGENASA (LFD devices & Interproficiency tests)
- Vertebrate Antibodies Ltd (Mab production & characterisation)
- Futuregenomics (NGS)

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Thank You

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