European Association of Fish Pathologists

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Abstract Book

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Contents

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Workshop: EU Projects Cure4Aqua and IGNITION - 11 September 2023, 16:00 - 18:00

Animal health and welfare in aquaculture: novel approaches for disease management

Dr Ivona Mladineo[1], Dr Benjamin Costas[2]
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Farmed seafood is an important source of protein for food and feeds with a low-carbon footprint which has an important role to play in helping to build a sustainable food system. A strategic and long-term approach for the sustainable growth of a resilient EU aquaculture is, therefore, more relevant today than ever. For instance, improving animal welfare by reducing the use of veterinary drugs is necessary to 4inimize the industry's environmental impact. However, the efficient and cost-effective control of pathogens remains among the main challenges for the sector, particularly relevant for Europe, where there is a great variety of species and production systems, which hinders the implementation of good husbandry practices tailored to each aquatic species. Indeed, disease prevention and reduced disease impact are pivotal for producers, researchers and stakeholders.

Through active engagement with their key stakeholders, the two EU-funded projects, Cure4Aqua and IGNITION aim to jointly improve the resilience of EU aquaculture under environmental, biological, and socio-economic stress, by improving aquatic animal health and welfare and supporting the environmentally friendly, inclusive, safe, and healthy production of seafood. We will do so by developing cost-effective innovative vaccines to prevent disease caused by major pathogens of economic significance to EU aquaculture; identifying markers with diagnostic capacity to be integrated to selective breeding programs to improve stress and disease management; developing innovative, bio-based and sustainable solutions as an alternative to antibiotics for controlling fish pathogens at various life stages and alleviate the pressure of global antimicrobial resistance; developing new and non-invasive tools and technology (e.g., biosensors) to improve health and welfare monitoring at the fish farm level and diagnostics of fish pathogens both at the laboratory and the fish farm levels, including disease prediction IT tools; placing fish welfare at the foreground of aquaculture production, through the development of high welfare standards that consider different life-stages, production systems, and knowledge of welfare needs, and ensuring effective external communication, dissemination and exploitation of project activities and results to all relevant target groups.

Funded by the European Union under the Horizon Europe Programme, Grant Agreement No. 101084204 (Cure4Aqua). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

Open Workshop "One Health in fish and shellfish"

Dr Olga Haenen[1], **Dr David Bass**[2], **Dr Sandrine Baron**[3], **Dr Marie-Agnès Travers**[4], **Dr Matt Longshaw**[5]
[1]WBVR, Lelystad, the Netherlands, [2]CEFAS, Weymouth, UK, [3]ANSES, Ploufagran, France, [4]IFREMER, La Tremblade, France, [5]CALYSTA, Redcar, UK

In this EAFP workshop, a One Health approach regarding the interactions of cultured and ornamental fish, shellfish, their environment, pathogens, and human health is in focus, including a helicopter view, contact- and food-zoonoses by bacteria and parasites, and AMR issues.

PROGRAM:

- Olga Haenen: Welcome and intro.
- David Bass: "What is One Health?" The paper of Grant Stentiford (2022) will be used as basis, and given the background of David Bass as experienced researcher at CEFAS, on molecular biology and eDNA related to the aquatic environment, he will illustrate the One Health theme with examples from his field of work.
- Sandrine Baron: "AMR: One Health approach is an evidence". Because of the direct link of fish farming with the aquatic environment, including a key role in AMR dissemination into the environment, an approach on One Health is needed in aquaculture. Sandrine Baron (NRL for antimicrobial resistance of ANSES, France) is aquatic microbial ecologist, working on AMR test methods and aquaculture AMR to Aeromonas and Vibrio spp.
- Marie-Agnès Travers: "Circulating bacteria and diversity of genes in shellfish". Marie-Agnès Travers (IFREMER, France). She is an expert in shellfish infectious diseases on vibriosis and bacterial evolution, also on ARG genes in shellfish. She will present on beta-lactams and colistin resistant bacteria in marine environments used for shellfish farming.
- Matt Longshaw: "Zoonotic parasites". With increased globalisation, including travel and trying new and exotic foods, the risk of being infected with parasites through contact or ingestion increases. Matt presents the major parasite groups of concern, routes of infection to man, potential mitigation measures in the context of globalisation and environmental change. Matt is experienced fish parasitologist, working at Calysta UK.
- Olga Haenen: "Zoonotic bacterial infections in and from fish". As experienced allrounder in fish diseases at WBVR of WUR, NL, Olga presents most important contact- and food-zoonotic bacterial diseases in fish and man, including diagnosis, and prevention.
- Three more authors to present a pitch on One Health.
- Discussion: Notes are made to include into the publication on this workshop.

Ref: G. Stentiford, 2022, EAFP Bulletin 41(5): 188-191.

Open Workshop "New Challenges and Achievement in the Mediterranean Fish Health Management"

Dr Snježana Zrnčić[1], Dr Pantelis Katharios[2], Prof Francesc Padrós[3], Prof Marialetizia Fioravanti[4], Dr Andrea Gustinelli[4], **Dr Anna Toffan**[5]
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Mediterranean marine aquaculture efficiency is jeopardized by parasitic and other infectious diseases. To mitigate losses and increase the profitability of this aquaculture sector, both the industrial actors and researchers are focused on the timely implementation of the available preventive measures, accurate detection of the causative agents and research aiming to find appropriate treatments. The climate changes and foreseen increase of the sea temperature in the Mediterranean basin will cause a worsening of the fish health and welfare.

The workshop will encompass several topics covering the most relevant problems and possible solutions as a basis for discussion between researchers and industrial fish health managers:

1. Recently completed two Horizon 2020 research projects were focused on the improvement of performances of Mediterranean aquaculture and the workshop will evaluate their legacy and analyse the benefits of projects outputs on health management.
2. Considering the lack of available means of licensed therapeutic substances for treatments, the use of functional feeds and/or natural substances for disease prevention/mitigation is an alternative?
3. Limited production of authorised vaccines raises a need for autogenous vaccines production and use. What are the advantages and limitations of the legislative framework?
4. New genotypes of VER/VNN were detected to affect both seabream and seabass. The updates on the current situation and diagnostic capacity in the Mediterranean will be presented

The discussion will also consider some other key questions: Is the production model based on cages in jeopardy? How will we address the increase in health problems due to climate change? Fish disease surveillance is a utopian idea for such a complex system? Do we need more networking and face-to-face national and regional meetings besides EAFP?

Workshop: Getting your research published - 14 September 2023, 09:00 - 11:00

Workshop: Getting your research published – how to deal effectively with journal reviewers and editors

Assoc Prof Sarah Poynton[2], **Prof Barbara Nowak**[1]
[1] University of Tasmania, Launceston, Australia, [2]Johns Hopkins University, Baltimore, USA

In this workshop we will address a critical aspect of getting your research published, namely how to deal effectively with journal reviewers and editors. The focus will be on three topics: (i) crafting a compelling letter of submission, including stating the importance of your work and what it contributes to the field, (ii) how to recommend reviewers, and how to be diplomatic about asking that someone not be a reviewer, and (iii) understanding reviewer's comments, responding to the comments, and writing a clear letter of response.

The workshop will be taught be Sarah Poynton and Barbara Nowak, who have previously team taught successful EAFP writing and publication workshops, are the editors of the forthcoming textbook "Aquaculture Parasitology: A Global Synthesis for Finfish" to be published by Wiley.

Antimicrobial resistance surveillance in imported ornamental fish*

Dr Athina Papadopoulou[1,2], Dr Nicola Coyle[1], Miss Isobel Smith[1,3], Miss Niamh Langford[1,2], Dr Ben Maskrey[1], Dr Mickael Teixeira Alves[1], Dr Edel Light[2,4], **Dr David Verner-Jeffreys[1,2]**
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Introduction: Antimicrobials are used to control bacterial infections in ornamental fish. Previous studies shown the presence of antibiotic resistant bacteria (ARB), antibiotic resistant genes (ARG) and antibiotic residues in fish and water samples from ornamental fish imports. This project aimed to examine the extent to which this remains an issue and to assess the risk of antimicrobial resistance transfer between countries.

Methodology: Seventeen samples were collected by the Cefas Fish Health Inspectorate, whilst undertaking routine import checks at Heathrow Airport, London UK from 2021 to 2022. In total, 315 isolates were isolated and were 8ptimized8zed using matrix assisted laser desorption ionization time of flight (MALDI-ToF) mass spectrometer, and primary identification testing. All isolates were whole genome sequenced (WGS), and they were also tested for antimicrobial sensitivity using broth microdilution and disc diffusion methods. In addition, a quantitative multiplex antibiotic residue assay was developed and 8ptimized to detect 35 compounds of common antibiotics and their metabolites in water used for fish transportation.

Results and Conclusions: Isolates from genera Aeromonas, Enterobacter, Klebsiella, Citrobacter and Shewanella were identified. As an example, for Citrobacter isolates tested, all had non-wild type phenotype to ampicillin, 36% to tetracycline, 28% to sulthamethoxazole, 24% to nalidixic acid and ciprofloxacin and 16 % to chloramphenicol. WGS identified ARGs in Citrobacter isolates related to quinolone (qnrB and qnrB97), cephalosporin (blaCMY-179 and blaCMY-183) and tetracycline (tetA) resistance among others. Further, 23 plasmids were detected in Citrobacter isolates. One plasmid contained 28 metal resistance genes, two ARGs and one biocide gene. ARGs, virulence and metal resistance genes were also detected in Aeromonas, Enterobacter, Klebsiella and Shewanella isolates. While some ARGs are likely intrinsic to these genera, the detection of many low and intermediate frequency ARGs suggests that horizontal transfer of ARGs is prevalent in these populations. Water samples tested (n=3) with the residue assay showed the presence of antimicrobials, or their metabolites, including enrofloxacin, florfenicol and oxytetracycline. Overall, results confirm bacteria associated with imported ornamental fish to the UK often have high levels of multi drug resistance and associated ARGs.

Funding: This work was funded by VMD. Project C7815 D.

**Supplementary Poster*

Implementation of a UK farmed trout passive antimicrobial resistance surveillance programme*

Dr Athina Papadopoulou[1,2], **Dr Nicola Coyle**[1], Niamh Langford[3], Eliot Stanton[1,4], Dr David Ryder[1], Andrew Joseph[1,2], Dr Mickael Teixeira Alves[1], Dr Edel Light[3], Dr David Verner-Jeffreys[1,2]
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Introduction: Antimicrobial Resistance (AMR) is a globally important issue threatening human and animal health and economic security. The role of fish farming in facilitating the spread of AMR is poorly documented. There is therefore an urgent need to build institutional capacity and an evidence base at a national level. This project reports the development of a passive surveillance system for three major pathogens in the UK trout sector (Aeromonas salmonicida, Yersinia ruckeri and Flavobacterium psycrophilum).

Methodology: Thirty-six isolates were received between December 2022 and March 2023. The isolates were identified using a combination of primary and secondary tests, including matrix assisted laser desorption ionization time of flight (MALDI-ToF) mass spectrometer. In addition, selected isolates were whole genome sequenced (WGS). Antimicrobial susceptibility testing (AST); broth microdilution was conducted alongside disc diffusion against a panel of antibiotics suspected of being used in aquaculture internationally. These included enrofloxacin, sulfamethoxazole-trimethoprim (SXT), oxolinic acid, gentamicin, chloramphenicol, oxytetracycline, ciprofloxacin, florfenicol, ampicillin, amoxicillin and sulfamethoxazole.

Results and Conclusions: AST has been initiated and of the 14/36 isolates tested to date, it was notable that two A. salmonicida isolates were multi-drug resistant. Parallel testing of more than 100 Y. ruckeri and A. salmonicida historical isolates recovered from diseased salmonids in the UK between 1967 and 2019 showed a similar pattern, with AMR more frequently observed in A salmonicida. Particularly to oxytetracycline (10.91%; non wild type (NWT) susceptibility)

and oxolinic acid (29.10%; NWT). Antimicrobial resistance genes (ARGs) have been identified in the 17 isolates sequenced to date. Although most ARGs were intrinsic to each species, two genes, mrc-3 and floR, conferring resistance to colistin and florfenicol respectively, were detected in plasmids in A. salmonicida isolates. Since previous studies have noted that Aeromonas may be potential reservoirs for mcr genes, further analysis will be conducted to determine the significance of the mcr-3 plasmid detected here. These, and the results of AST and WGS from historical and recently submitted isolates, will be presented to demonstrate the value of implementing similar passive and active AMR surveillance programmes for other farmed fish species.

Funding: This work was funded by VMD. Project C7815 C.

**Supplementary Poster*

Assessment of the diffusion of AMR in aquatic environments: specificity of freshwater fish farming*

Dr Sandrine Baron[1], Laëtitia Le Devendec[1], Eric Jouy[1], Dr Claire Chauvin[2], Dr Sophie Le Bouquin[2]
[1]Anses - Mycoplasmology-Bacteriology and Antimicrobial resistance Unit, Ploufragan, France, [2]Anses - Epidemiology, Health and Welfare Unit, Ploufragan, France

Antimicrobial resistance (AMR) is now recognized as among the top 10 threats to global health, with current trends in resistant infections in humans and livestock pointing toward a potential postantibiotic era (liguori 2022). The aquatic environment is considered as a hot spot for the dissemination of AMR, due to its direct link with the aquatic environment, the issue of monitoring AMR in aquaculture arises.

According to Liguori and collaborators (2022), a fundamental stumbling block to the advancement of AMR monitoring of water environments is a lack of agreed upon targets and standardized methods, including a lack of benchmarking and threshold data to inform evolutionary, epidemiological, and other risk modeling efforts.

AMR monitoring in Aquaculture is concerned by the same problematics. Using trout fish farming as a case study, we will provide some answers to the following questions:
Where? Fish farming depends on the quality of the water received. Sampling strategy should then include data collected upstream and downstream of the fish farming.
What? What kind of samples water or/and biofilm or/and sediment? A sample should be easy collected and always available.
Who? E. coli is a well-characterized bacterium used for the monitoring of AMR in humans and livestock (in national surveys, at the European level and elsewhere), as well as for the monitoring of water quality (as a fecal contamination indicator). Could E coli be used? What alternatives can be offered?
How? Currently amr monitoring plans are based on culture depend methods and phenotypic criteria. Nevertheless, the use of genetic indicator or genomic approach are under progress.

**Supplementary Poster*

One Health Approach applied to following up of antimicrobial resistance dissemination in freshwater fishfarms at the watersheed level*

Dr Sandrine Baron[1], Laëtitia Le Devendec[1], Emeline Larvor[1], Héloïse Duprey[1], Eric Jouy[1], Aurélien Tocqueville[2], Matthieu Gaumé[2], Pascal Gallot[2], Rodolphe Thomas[3], Dr Sophie Le Bouquin[3], Dr Claire Chauvin[3]
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A research project (Resist3A) aimed to investigate the AMR dissemination upstream and downstream of two trout fishfarms, located respectively at the source (FF1) and at the mouth (FF2) of a river impacted by agricultural, terrestrial farming and wastewater treatment plants.

During 18 months, every two weeks, water samples were collected upstream and downstream two fish farms and biofilm samples were collected in a same fishpond per farm. Abiotic environmental parameters (pH, conductivity…) were measured too. Enumeration of E. coli, Enterococcus spp., Aeromonas spp. and Pseudomonas aeruginosa was performed using culture methods on the 144 water samples and 72 biofilm samples collected.

Antimicrobial susceptibility testing of a selection of E. coli (n=142), Aeromonas (n=355), and Pseudomonas aeruginosa (n=123) isolates was performed using agar diffusion method.

The proportion of E. coli isolates, which were susceptible to all tested antimicrobials, was higher in water than in biofilm (92.7% vs 89%). None isolate was resistant to carbapenems or extended-spectrum betalactams. No multidrug resistant isolate was detected. Only 13 isolates were resistant to one or two classes of antibiotic.

The Aeromonas isolates collected were collected from water samples (n=172) and from biofilm (n=183). From the source to the mouth in water sample, no decrease of susceptibility was observed for oxolinic acid, florfenicol, oxytetracycline, ceftazidime and meropenem. At the opposite, a slight reduction of susceptibility was observed for enrofloxacin. Isolates collected in the biofilm of the FF1, showed a reduced susceptibility to oxytetracycline and oxolinic acid. No reduction of susceptibility was observed among isolates collected in the biofilm of the FF2.

On the contrary, of the results observed for E. coli, the proportion of Pseudomonas aeruginosa susceptible to all the 13 antibiotics tested was higher in biofilm than in water (100% vs 74.5%).

These results confirm the importance of an approach including several compartments of the environment (water, biofilm), and three bacteria models allowed us to have a broader view of the AMR dissemination.

**Supplementary Poster*

Antimicrobial resistance in aquatic and fecal bacteria from a Recirculating Aquaculture System and comparison with human clinical isolates*

Joyshri Sarker[1], Aylar Saba Shirvan[1], Lionel Pineau[1], Céline David[1], Stéphane Corvec[2], Eric Batard[3], Maria Ugarte-Ruiz[4], Ségolène Calvez[1], **Dr Nora Navarro-Gonzalez**[1]
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Introduction: Aquaculture is considered a hotspot for the emergence and spread of antimicrobial resistance (AMR) because the coexistence of fish, bacteria and antibiotics in the aquatic environment provides the ideal conditions (Cabello et al, 2016). However, little is known on AMR in aquaculture. In particular, studies on the occurrence of AMR in recirculating aquaculture systems (RAS) are very scarce, in spite of being the fish farming system of the future. The objectives of this study are 1) to describe the sources and the persistence of selected indicator bacteria within a RAS, and 2) to compare their AMR profiles with those from clinically important isolates in human medicine.

Methodology: This longitudinal, observational study was performed at our experimental RAS (Nantes), which uses city water. All compartments of the system were sampled (water, sediment, rainbow trout feces, biofilm and feed) on five events. In total, 95 samples were processed for the isolation of Aeromonas spp, Pseudomonas spp., Enterococcus spp and Escherichia coli. Colonies were confirmed with MALDI-TOF and tested for their AMR phenotype with broth microdilution. Twenty Aeromonas and 20 Enterococcus faecium isolates from human infections were obtained from the Nantes University Hospital collection and tested with the same procedure.

Results: All samples were negative for E. coli. Only one sample (water) was positive for Pseudomonas spp., whereas Aeromonas spp. was frequently isolated (77/95). Enterococcus faecium was isolated from feed (2/4). Oppositely, uncommon Enterococcus species were found in the RAS environment. City water was negative for all the tested bacteria. For most antibiotics, Minimum Inhibitory Concentrations from RAS Aeromonas and those from human infections were similarly distributed. Enterococcus MIC results are in progress.

Conclusions: The absence of the selected bacteria in city water indicates that purchased fry and feed are their main source or vehicle into our RAS. Feed in a particular is a source of Enterococcus of human clinical importance; however, they do not seem to persist in this environment. Lastly, the Aeromonas MIC distributions do not suggest the emergence of an AMR-strain that is more successfully infecting humans.

References: Cabello et al, 2016. Lancet Infect Dis 16: e127–33

**Supplementary Poster*

AMR in Flavobacterium psychrophilum: new resistance patterns in recent isolates from Denmark*

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Introduction: Infections caused by the fish bacterium Flavobacterium psychrophilum [Rainbow Trout Fry Syndrome (RTFS) and Bacterial Coldwater Disease (BCWD)] are treated with antibiotics worldwide. In Denmark, amoxicillin, oxytetracycline and oxolinic acid (OXO) have been used. However, florfenicol (FLOR) is the only antibiotic that has been used for the treatment of this disease since 1996 due to the emergence of resistance to the other antibiotics.

Methodology: In this study, we evaluated the resistance patterns of seven recent isolates of F. psychrophilum isolated from diseased fish in the period 2019-2022. Following the bacterial identification by MALDI-TOF, the AMR profile was characterized by disc diffusion and by the analysis of the sequenced genome (WGS, Oxford Nanopore R10.4.1 pore chemistry).

Results: The analyses showed the emergence of FLOR resistance (acquired resistance - MFS transporter) in two isolates from 2021 and 2022 from the same farm (farm A). One of these two isolates was sensitive to OXO while the other was characterized by a reduced susceptibility to OXO. From the same fish harboring the FLOR-resistant bacterium, OXO-resistant F. psychrophilum was also isolated, creating concerns in the possibility of the development of bacterial populations resistant to both antibiotics (this has been now confirmed, as we recently isolated F. psychrophilum resistant to both FLOR and OXO from the same farm).
The other sequenced isolates presented OXO resistance on disc diffusion and missense mutations (nucleic acid mutations causing amino acid substitutions) in the Quinolone Resistance Determining Region (QRDR) of the DNA gyrase subunit A (gyrA).

Conclusions: The study of antimicrobial resistance is of primary importance for efficient and effective control measures.

**Supplementary Poster*

Addressing Antibiotic Usage and AMR Data Gaps in West African Aquaculture through the Fleming Fund Fellowship Programme*

Andrew Joseph[1], Dr David Verner-Jeffreys[2], Eric Boyce Sawyerr[3], Suliat Adeleke
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Information on the use of antibiotics and antimicrobial resistance in finfish aquaculture in West Africa, as with many other significant aquaculture producing countries, is very limited. The UK FAO Reference Centre for AMR has partnered with Government departments in Ghana and Nigeria to develop expertise to support antibiotic usage (AMU) and AMR surveillance in aquaculture. Through the Fleming Fund Fellowship programme, an AMU fellow from the Fisheries Commission (Ghana) and an AMR laboratory fellow from the Federal Department of Veterinary and Pest Control Services (Nigeria) were both provided with a tailored programme of mentorship and training.

As part of their training, both fellows developed and implemented a survey questionnaire to gather information on the motives for use, farmer perception and different types of antimicrobials currently used in the Tilapia and Catfish industry in Ghana and Nigeria respectively. In total a combined total of 100 questionnaires were answered over 3 months using google forms, telephone and in-person interviews during fish farm visits. The survey targeted participation from small scale progressive to large scale commercial farmers.

Results:

- Over half of farmers reported using antibiotics within the last year to treat and prevent disease (Ghana/Nigeria).
- Use and administration was largely without professional supervision.
- Only 16% (Ghana) and 24% (Nigeria) of farmers surveyed reported consulting a veterinarian (or para-veterinarian) when sourcing and administering antibiotics.
- Antibiotics used were typically supplied by agricultural stores and human pharmacies, rather than via prescription.

Conclusion:
The Fleming Fund fellowship is building a network of highly motivated and trained ambassadors for AMR in partner countries to tackle this shared threat. Through the programme, baseline data on AMU and AMR in West African aquaculture has also been generated. This will be used to inform the development of national scale One Health integrated AMR surveillance programmes in both Ghana and Nigeria to safeguard animal health, food security and safety, the environment and public health.

**Supplementary Poster*

Understanding AMR dynamics and opportunities for interventions in freshwater aquaculture systems in northern Vietnam through the application of systems thinking*

Maria Garza[1], Tai Mai Van[2], Prof Van Phan Thi[2], Dr Andrew P. Desbois[3], Dr Chadag V Mohan[4], Dr Mehroosh Tak[1], Prof Barbara Häsler[1], Dr Lucy Brunton[1]

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Introduction: Vietnam is one of the top users of antimicrobials in aquaculture and despite a wide variety of interventions being described, their effect and interaction in the system as well as the role of socio-economic and policy drivers is poorly understood. The aim of this study was to investigate the multidimensional impacts and causal links of AMR drivers and interventions in freshwater aquaculture systems in Northern Vietnam.

Methods: This study focused on two provinces of northern Vietnam, which are key fish production areas for domestic consumption and undergoing transformations. We used the development of causal loop diagrams as an applied systems thinking method. First, a conceptual model was developed based on available literature and key informant interviews. Next, a question guide was developed to enquire about dynamics in the system influencing health management and use of antimicrobials, and to understand who benefits from current dynamics and policies, how and why. This complemented participatory group model building workshops with four groups of aquaculture stakeholders, including producers, officers, drug sellers and retailers, to understand drivers of AMR and potential actions. Data were analysed using thematic analysis and cause-effect analysis and two additional workshops were used to validate the causal-loop diagrams findings.

Results: The development of causal loop diagrams showed that factors influencing antimicrobial use, as the main driver of AMR, included restructuring of public services, vulnerability to land use change, and financial instability due to input and market dependencies. Causal relationships, loops and actions to improve surveillance and mitigate AMR were identified, including the participation in home-grown sustainability governance, engagement in cooperatives, access to aquaculture and extension services support, and demand for food safety by consumers. Current interventions in Vietnam were found to address only limited parts of the system and stakeholders, mainly producers.

Conclusions: Findings of this study contribute to a policy analysis of current AMR interventions in aquaculture and demonstrate areas of possible action. Participatory approaches to modelling bring together the knowledge of system stakeholders and engage them in the research process, to understand the impact of interventions and challenges, and ensure effective, sustainable, and more equitable interventions.

**Supplementary Poster*

Oral Presentations

1.1 Viral Diseases I – 11 September 2023, 11:45 - 13:00

Abstracts listed in presenting order

Nervous necrosis virus (NNV) reassortant strains isolated from wild fish: in vitro and in vivo replication and virulence for sole

Dr Lucía Vázquez-Salgado[1], Dr José G. Olveira[1], Dr Carlos P. Dopazo[1], **Dr Isabel Bandín**[1]
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Introduction: Nervous Necrosis Virus (NNV) is a major pathogen affecting farmed and wild fish in Southern Europe. NNV is a small non-enveloped virus with a genome organized in two molecules of ssRNA (+), namely RNA1 and RNA2, coding for the viral polymerase and capsid protein, respectively. The RNA2 molecule allows NNV classification into 4 genotypes: barfin flounder-, red spotted grouper-, striped jack- and tiger puffer nervous necrosis virus (BFNNV, RGNNV, SJNNV, and TPNNV). Reassortment between RGNNV and SJNNV genotypes has been also reported in Southern Europe. Reassortant strains contain mutations in both RNA molecules that can affect the proper function of viral proteins, impacting NNV replication, pathogenicity, and virulence. The aim of this study was to assess in vitro and in vivo replication, of four NNV reassortant strains obtained from wild fish in the North-western coast of Spain as well as to analyze their virulence for Senegalese sole.

Methodology: In vitro replication (genomic copies and infective viral particles) was assessed by growth curves performed in E-11 monolayers at 20 and 25 ºC. Experimental infections were performed in juvenile Senegalese sole by bath and intramuscular injection at 18 and 22 ºC. Viral replication as well as cumulative mortality and fish immune response were assessed.

Results: The growth kinetics in cell culture of the three NNV reassortant strains obtained from pilchard were equivalent to those of the strain set as reference (isolated from farmed sole), whereas lower replication values were provided by the strain isolated from mackerel. In vivo assays showed a better replication of one pilchard reassortant at 22 ºC, whereas the other strains displayed similar values at both temperatures. At 22ºC disease signs were observed in fish challenged with the four strains. Low (≃17%) to moderate mortalities (≃40%) were recorded, but lower than that caused by the reference strain (≃70%). At 18 ºC low mortality levels and no clinical signs were observed. Ongoing studies will provide results about the sole immune response against the four reassortants.

Conclusion: This study provides evidence that NNV reassortant strains isolated from wild fish can replicate and cause disease in sole.

Role of rotifers in Betanodavirus transmission to European sea bass larvae

Dr Lorena Biasini[1], Dr Lucia Vázquez-Salgado[2], Dr Francesco Pascoli[1], Dr Andrea Marsella[1], Dr Alessandra Buratin[1], Dr Tobia Pretto[1], Dr Miriam Abbadi[1], Dr Erica Melchiotti[3], Dr Isabel Bandín[2], Dr Anna Toffan[1]
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Introduction: In marine hatcheries, invertebrates such as rotifers or artemia, frequently used for fish larvae feeding, may act as possible source of pathogens. It has been demonstrated that Atlantic sole larvae can manifest viral encephalophaty and rethinophaty (VER) if fed with artemia that had previously internalized the viral etiological agent of the pathology (Betanodavirus; NNV). The aim of this work was to investigate whether rotifers, used as first food for sea bass (Dicentrarchus labrax) larvae, could also play a role in the transmission of VER.

Methodology: In a preliminary experiment, a batch of rotifers was exposed to the virus (RGNNV-type strain) for 24 hours and sampled at different time points. The rotifers were then rinsed to remove non-internalized virus and subsequently introduced into a virus-free environment. The sampled rotifers were analysed to evaluate the viral presence using immunofluorescence techniques and immunohistochemistry, whereas the viral load was monitored by quantitative real-time PCR and viral titration (TCID50) using SSN-1 cell lines. In the following in vivo experimental test, the sea bass larvae were fed with a meal of NNV-exposed rotifers. A positive control group with NNV-infected larvae by bath immersion and a mock-challenged fish group were set up as well. All groups were sampled daily and evaluated with the techniques previously described.

Results: The preliminary assay highlighted that the virus does not replicate within the rotifers; however, NNV is certainly internalized in large quantities. Results from the in vivo experiment established that one single oral administration of NNV-exposed rotifers to sea bass is sufficient to induce the appearance of clinical signs, 100% cumulative mortality and

viral replication kinetics similar to those observed in the positive control group. However, the course of the infection lagged by 10 days if compared to the bath-infected larvae.

Conclusions: This research demonstrated that rotifers could assume the role of mechanical vectors of NNV towards sea bass larvae. Hence, due to the hazardous nature of Betanodavirus in aquaculture, such horizontal transmission should be prevented with the introduction of stringent routine controls on the live feed to avoid the entry of NNV into the hatcheries.

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A new epitheliotropic herpesvirus from Lake Sturgeon Acipenser fulvescens

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Introduction: Juvenile Lake Sturgeon propagated by a conservation stocking program in Manitoba, Canada displayed epithelial lesions in two consecutive year classes reared at the hatchery. Mortality was not observed and lesions eventually regressed.

Methodology: A combination of methods including microscopy, virology, molecular diagnostic testing, next generation sequencing and phylogeny were used to diagnose the putative causative agent.

Results: Cellular changes in the area of the lesions were reminiscent of other aquatic herpesvirus infections. Skin tissue homogenates from sturgeon displaying lesions produced putative cytopathic effect on sturgeon cell lines and tested negative for other sturgeon viruses. DNA samples from infected and naïve cell monolayers were submitted for next generation sequence analyses as a non-biased diagnostic approach. DNA contigs that were assembled from PacBio HiFi reads containing core genes conserved across members of the Alloherpesviridae family ranged in size from 72,000 to 3 million base pairs. The largest contig consisted of sequence that was homologous to sturgeon chromosomes and contiguous with alloherpesvirus sequence. Bayesian inference of phylogeny reconstructed with the virus major capsid protein (mcp) sequence revealed a new evolutionary lineage within the Alloherpesviridae family. Investigation into the ecology of the virus using a new qPCR test provided evidence of an ancient host-pathogen relationship as high virus titers were found in 100% of the 1,167 wild Lake Sturgeon adults tested in the Hudson Bay drainage basin. Similar virus titers were detected in 100% of broodstock somatic and germ cell tissues and 97% of sampled larval offspring. Homologous sequence was found in six other sturgeon species through a new genotyping PCR assay and the topology of the corresponding phylogenetic tree strongly supported co-evolution of the virus and its host.

Conclusions: A new virus, sturgeon herpesvirus 3 (AciHV-3), is endemic in wild Lake Sturgeon of the Hudson Bay drainage basin in Canada. The results support our hypothesis that the virus has established a life-long chromosomally integrated latent infection in Lake Sturgeon germ cells and is inherited via germline transmission of the AciHV-3 genome. Virus homologues found in other sturgeon species suggest a global distribution of AciHV-3 with evolutionary divergence driven by host-virus co-speciation.

Whole genome sequencing reveals low genetic diversity and no continuous reintroduction of the Piscine Myocarditis Virus in Faroese farmed salmon

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Introduction: Piscine Myocarditis Virus (PMCV) is a dsRNA virus with 3 open reading frames and responsible for Cardiomyopathy syndrome (CMS) in Atlantic salmon which has proven to be of increasing concern to the farming industry. PMCV has yet to be successfully cultivated, which has limited the full characterization of it and to date there is only one full genome publicly available. For this study we have developed a method to whole genome sequence directly from field samples that can assist in understanding virology, pathology, and mapping of transmission pathways.

Methodology: Samples originating from 23 salmon farming production sites in the Faroe Islands and from returning wild salmon were collected for disease surveillance purposes by the authorities and the farming companies over a period of 12 years. The samples underwent multiplex PCR with 18 overlapping primer pairs designed with amplicon lengths around 500 bp targeting the whole genome before being sequenced on an Illumina or Nanopore platform. For comparative purposes the phylogenetic analyses also comprise Norwegian and Irish genome sequences.

Results: Whole genome sequences were obtained from both sequencing platforms and yielded 48 new PMCV genomes. Phylogenetic analyses revealed a monophyletic Faroese cluster comprising samples originating from farmed salmon. ORF1 and ORF2 both show highly conserved regions with dN/dS ratios of 0.38 and 0.19, respectively, whilst the smaller ORF3 has no such region and a dN/dS ratio of 0.64. The genome obtained from a returning wild salmon differs significantly from samples from the Faroe Islands, Norway, and Ireland. When comparing amino acid residues in assigned CMS samples to the rest there is no apparent association.

Conclusion: This study based on a broad spatio-temporal representation of samples from Faroese salmon farming found PMCV to be highly homogeneous. From the first detected outbreak in 2013 to present day there is no sign of continuous reintroduction of new PMCV variants to Faroese salmon farming. Furthermore, the results show no apparent correlation between assigned CMS cases and potential virulence markers.

The infectious salmon anaemia virus sialic acid 4-O-acetyl esterase extensively prunes target cell surfaces in infected hosts

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Introduction: Many sialic acid-binding viruses express a receptor-destroying enzyme (RDE) that removes the virus-targeted receptor and limits viral interactions with the host cell. The viral RDE supports several steps of the infectious cycle, but we know little about its direct effects on the host. Infectious salmon anaemia virus (ISAV) binds 4-O-acetylated sialic acids on vascular and erythrocyte surfaces and causes severe disease in farmed Atlantic salmon.

Methods: We have investigated the distribution of the ISAV receptor in tissues and red blood cells of ISAV infected fish by virus binding assays over the cause of an experimental infection.

Results: We discovered that the infection causes a progressive, global loss of the vascular and red blood cell ISAV receptor. The cell surface modulation is specific, as it did not occur on infection with another virus of similar tropism. In erythrocytes, the loss of sialic acid O-acetylation was associated with increased lectin binding, suggesting a potential to influence cellular signalling and interactions. Plasma from infected fish caused the same cell surface modulation, and antibodies that limited ISAV attachment inhibited the effect. Furthermore, the viral haemagglutinin esterase was sufficient to induce the loss of receptor, but only when its hydrolytic activity was preserved. This suggests that the ISAV RDE/esterase is responsible for the loss of cell surface 4-O-acetylated sialic acids in infected fish.

Conclusions: To our knowledge, we are first to describe the extent to which a viral esterase modulates cell surfaces in infected individuals. Our findings raise the questions if the observed loss of 4-O-acetylated sialic acids affects immune functions in infected fish by modulating interactions with sialic acid immunoglobulin-like lectins or in other ways contributes to the typical disturbance of vascular function and anaemia in infected fish.

DNA-layered Salmonid alphavirus-based replicon vaccine induced significant protection in common carp (Cyprinus carpio) against spring viremia of carp virus

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Introduction: The development of new vaccines can greatly assist in addressing the current health challenges in aquaculture. Spring viremia of carp virus (SVCV) of the family Rhabdoviridae has a considerable economic impact in Europe, as it infects cyprinids such as common carp (Cyprinus carpio). In previous studies, DNA vaccines containing the glycoprotein (G) gene of the virus induced moderate to full protection against SVCV even in a single low dose in juvenile carp. However, the backbone of those vaccines was the pcDNA3 plasmid. Another promising nucleic acid vaccine construction against viral diseases was the Salmonid alphavirus-based replicon (SAV), which was an efficacious vaccine against infectious salmon anemia (ISA) in Atlantic salmon (Salmo salar). However, the SAV replicon has been tested in vivo in Atlantic salmon only.

Methodology: In our study, an SAV replicon expressing gene G of SVCV was designed, and its efficacy was compared with the previously described pcDNA3-SVCV-G construct in common carp. The SAV replicon was used as a naked RNA (pSAV-RNA-SVCV-G) and also as a DNA-layered vector (pSAV-DNA-SVCV-G). The three different vaccine prototypes were injected i.m. in a 0.1 µg/g of fish dosage (n=25 per group) at water temperature 20±1ºC. Fish were kept at this temperature for two weeks, then the temperature was gradually decreased to 13ºC at a rate of 1ºC/day. The three vaccinated groups and the control group (injected with empty pcDNA3 plasmid) were challenged by immersion with SVCV, three weeks after vaccination.

Results: 30 days after virus challenge, the cumulative mortalities were: 44% in both control and pSAV-RNA-SVCV-G vaccinated groups, 52% in pcDNA3-SVCV-G group, while in pSAV-DNA-SVCV-G group, the mortality was 8% only. According to these results, the SAV replicon applied as naked RNA and the pcDNA3-based vaccine did not induce any protection against SVCV under these conditions, however, the SAV applied as DNA raised significant protection after a single low dose of i.m. injection.

Conclusion: Our results show that the SAV-based replicon may serve as a potential vaccine candidate for non-salmonid fish aquaculture as well in the future if further clinical and field trials confirm its efficiency.

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Effect of an attenuated live vaccine against Salmonid Rickettsial Septicaemia is dependent on temperature the first days after vaccination

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The main cause of antibiotics usage in the Chilean aquaculture industry is Salmonid Rickettsial Septicaemia (SRS) caused by the facultative intracellular bacterium Piscirickettsia salmonis. A live attenuated vaccine against the disease (ALPHA JECT Livac SRS, PHARMAQ AS) been used extensively in Chilean farmed salmonids later years. Here we demonstrate that the vaccine is efficient in protecting against P.salmonis-induced mortality in Atlantic salmon (Salmo salar) for at least 15 months in experimental injection and cohabitation laboratory challenge models. The protection was however highly sensitive to temperature during immunization. While fish vaccinated and immunized at 10°C and above were found to be well protected, a significant increase in mortality was found in groups immunized at 7°C and 8°C, which represents the lower end of the temperature range commonly found in Chile. This temperature-dependent loss of effect was found to correlate in a linear regression with the amount of vaccine-strain RNA that was detected by real-time RT-PCR in the liver the first week after vaccination. Good vaccine efficacy was restored when fish were exposed to 15°C the first 5 days after vaccination, before lowering the temperature to 7°C for the remaining immunization period. This suggests that correct temperature the first days after vaccination is pivotal to obtain a protective immune response with ALPHA JECT Livac SRS. The results underscore the importance of temperature when vaccinating poikilothermic animals with live vaccines.

Scotland's fish vaccine priorities and the Farmed Fish Health Framework (FFHF)

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In 2020, aquaculture generated £362 million (Gross Value Added) to the Scottish Economy, with Atlantic Salmon making up 96% of the value. The highest level of salmon production recorded in Scotland took place in 2021, with 205,000 tonnes of salmon produced alongside 8000 tonnes of trout. However farmed fish face a number of health challenges which contribute to significant mortality, including those associated with or amplified by a changing marine environment.

Improving the health and welfare of farmed fish is priority and both salmon and trout producers are working collaboratively with the Scottish Government, fish vets, regulators and the Sustainable Aquaculture Innovation Centre under the umbrella of a Farmed Fish Health Framework to improve fish health. Improving access to health treatments, including medicines and vaccines is an important area of focus for collaboration.

This session highlights the top five health conditions which Scottish trout and salmon producers consider could be significantly be reduced by vaccine development. We will discuss the market appetite for vaccine availability and promote a multi-disciplinary approach to help enable vaccine development in this challenging area.

Bacterial coinfection dynamics in channel catfish culture

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Introduction: Catfish production represents the largest component of the U.S. aquaculture sector. Within this industry, bacterial pathogens such as Edwardsiella ictaluri, Flavobacterium covae, and virulent Aeromonas hydrophila (vAh) are the primary species responsible for catfish diseases. In many diagnostic cases, polymicrobial infections have been found, making it difficult to properly assess these pathogens' primary and secondary nature, along with issues pertaining to treatment avenues. As such, several recent trials have evaluated the infection dynamics of these three pathogens using coinfection disease models.

Methodology: Single and mixed combinations of F. covae (ALG-530-00), vAh (ML09-119), and E. ictaluri (S97-773) were used for in vivo pathogen challenges in juvenile channel catfish. Three separate trials were conducted: 1) F. covae and vAh, and 2) E. ictaluri and vAh. The challenges were performed with full and half-doses standard challenge doses of the single pathogen and full and half-doses of the mixed pathogens.

Results and Conclusions: Concerning experimental coinfection with vAh and F. covae, at 96 h post-challenge, the single vAh infection (immersed in 2.3 x 107 CFU mL-1) resulted in final cumulative percent mortality (CPM) of 28.3 ± 9.5 %. The full-dose F. covae group (immersed in 5.2 x 106 CFU mL-1) was 23.3 ± 12.9 %. A coinfection full-dose combination (98.3 ± 1.4 %) and a half-dose administration (76.7 ± 17.1 %) significantly increased mortality (P< 0.001). Regarding the experimental coinfection with vAh and E. ictaluri, the full-dose, single vAh infection (immersed in 1.9 x 107 CFU mL-1) resulted in a final CPM of 25.0 ± 2.9 % at ten days post-challenge. The CPM for the full-dose E. ictaluri group (immersed in 4.0 x 105 CFU mL-1) was 11.7 ± 4.4 %. When both pathogens were co-administered, the full-dose combination (41.7 ± 7.3 %) and half-dose combination (40.0 ± 10.4 %) demonstrated pronounced mortality. Laboratory-based exploration of these bacterial coinfections and their inherent interactions using in vivo models and evaluations at the bench will allow fish health diagnosticians to better understand these complex infections. This preliminary work will also pave the way for more targeted treatment plans for catfish culture in the southern U.S.

'Pasteurellosis', an important disease in Norwegian salmon farming

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Until recently, outbreaks of 'pasteurellosis', in Norwegian farmed salmon have occurred only very occasionally since being first diagnosed in Northern Norway in 1989. This situation has now changed however, with approximately 50 farming localities affected annually between 2018 and 2022. While the bacterial species associated with 'pasteurellosis' in Scottish salmon, Pasteurella skyensis, has been identified in Norwegian aquaculture, the situation in Norwegian salmon is almost completely dominated by a bacterium (as yet officially undescribed) generally referred to as 'Pasteurella atlantica genomovar salmonicida'. This presentation will provide an update on the current clinical situation and molecular epidemiology of pasteurellosis in Norwegian aquaculture over the last thirty years.

Characterisation of the main Aeromonas strains associated with recent mortality events in pangasius catfish culture in Vietnam

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Introduction: Pangasius catfish (Pangasianodon hypophthalmus) is cultured intensively across Asia, however bacterial pathogens can cause diseases such as motile Aeromonas septicaemia (MAS), which is caused by several Aeromonas spp., most notably A. hydrophila. A commercial vaccine is available in Vietnam for P. hypophthalmus to protect against MAS but outbreaks still occur and these require the application of antibiotics, a practice that risks the emergence and selection of resistance. Our previous investigation characterised the most prevalent circulating strains of A. hydrophila associated with recent mortality losses in the Mekong Delta and the aim of this present study was to explore further the genetic basis for the success of these strains.

Methodology: In total, 345 isolates presumed to be A. hydrophila were collected from individuals exhibiting signs of MAS at farms experiencing mortalities in eight provinces across the Mekong Delta. The isolate collection (2013–2019) was characterised by a polyphasic genotyping approach that included rep-PCR, multi-locus sequence typing (MLST)

and whole-genome sequencing (WGS). Our genomes were supplemented with available WGS data to characterise genome properties of the outbreak strains, including core and accessory genomes and the genes found exclusively in these strains.

Results: Initial characterisation and genotyping by a species-specific PCR suggested that most outbreak isolates (202/345) were A. hydrophila. However, further investigation by MLST and WGS revealed the majority of these (151/202) were Aeromonas dhakensis ST656. The remaining isolates (51/202) were A. hydrophila ST251, a hypervirulent lineage (vAh) already causing concern in aquaculture worldwide. Multiple antibiotic resistance genes were detected, with resistance phenotypes confirmed for several agents by minimum inhibitory concentration assays, including oxytetracycline and sulfamethoxazole. Outbreak strains contained unique sets of genes not found in closely related strains, including some associated with O-antigen synthesis.

Conclusion: This study highlights A. dhakensis and vAh to be associated with recent outbreaks of MAS in P. hypophthalmus across the Mekong Delta, Vietnam, with several genetic adaptations providing an alluring explanation for their success. Vaccine formulations should contain antigens for these major strains to confer appropriate protection, which will reduce the need for antibiotic treatments and support the pangasius catfish sector in the region.

The two-component system RstAB positively regulates the expression of secreted virulence factors in Photobacterium damselae subsp. Piscicida

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Photobacterium damselae subsp. *piscicida*(*Phdp*) is a Gram-negative bacterium that infects several warmwater marine fish species, leading to huge economic losses in aquaculture. *Phdp* extracellular products, including secreted proteins and outer membrane vesicles (OMVs), have been shown to display important pathobiological activities. In particular, *Phdp* secretes two toxins – apoptosis-inducing protein of 56 kDa (AIP56) and *Photobacterium* binary toxin (PBT) – that kill host macrophages and are key for virulence, and a peptidoglycan hydrolase (PnpA) that degrades the peptidoglycan of potential *Phdp* bacterial competitors. Additionally, we recently found that field isolates of *Phdp* secrete two trimeric autotransporter adhesins – PadA and PadB (for *Photobacterium* adhesin A and B, respectively) – that may also be involved in *Phdp* virulence. Despite the importance of these *Phdp* secreted factors, nothing is known about the molecular mechanism that regulate their expression.

In this work, the regulatory role of RstAB for expression of *Phdp* secreted proteins was investigated. An *rstB* deletion mutant (Δ*rstB*) and correspondent complemented strain (Δ*rstB*+p*rstB*) were generated using MT1415 as background, and used to determine the impact of *rstB* deletion on the expression of secreted proteins by performing RT-qPCR and SDS-PAGE of culture supernatants. In vivo virulence assays in European sea bass (*Dicentrachus labrax*) were also performed, to determine the *rstB* virulence role. Additionally, the ability of recombinant RstA to bind to the promoters of *aip56*, *pbt*, *pnpA*, *padA* and *padB* was investigated by Electrophoretic Mobility Shift Assays (EMSAs) and RstA residues important for DNA interaction were identified by combining site-directed mutagenesis with EMSAs.

The results of the virulence assays performed showed that Δ*rstB* is strongly impaired in virulence, when compared to the WT and *ΔrstB*+p*rstB* strains. RT-qPCR and SDS-PAGE analysis revealed that deletion of *rstB* decreased the expression of AIP56, PBT, PnpA and PadA, but not PadB. However, EMSAs showed that recombinant RstA directly interacts with the promoter regions of all genes tested, including *padB*. Altogether, these data indicate that the canonical RstAB pathway positively regulates expression of *aip56*, *pbt*, *pnpA* and *padA* and point to the involvement of a cross-talk mechanism in the regulation of *padB* expression.

Tenacibaculum maritimum can boost an inflammatory response in European seabass (Dicentrarchus labrax) upon peritoneal injection but cannot trigger tenacibaculosis disease

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Introduction: Despite being a bacterial pathogen with devastating consequences, Tenacibaculum maritimum's transmission, infection route and pathogenesis are not fully disclosed. The present study aimed to evaluate the short-term innate immune response of European seabass (Dicentrarchus labrax) after intraperitoneal-challenge with T. maritimum's extracellular products (ECPs), whole cells without and with ECPs, as well as their ability to induce tenacibaculosis.

Methodology: A time-course trial was performed in which groups of seabass (35.61±6.5 g) were intraperitoneally-challenged with 5.5x105 CFU mL-1 T. maritimum with and without ECPs, ECPs alone or marine broth (sham control). A group of fish were also bath challenged with the same inoculum. Undisturbed fish were randomly selected just before infection as controls (time 0). Twelve fish per treatment were randomly selected, euthanized, and sampled at 0, 3, 6, 24 and 48 h post-challenge. Blood, liver and head-kidney samples were collected for assessing immune parameters, oxidative stress and gene expression, respectively. To determine the severity of both bath and intraperitoneal-challenges, cumulative mortality was followed for one week.

Results: Tenacibaculosis symptoms, such as skin/fin abrasions and mortality were only observed in the bath-challenged fish, in which 100% mortality was recorded. It was observed an increase of immune cells in the peritoneal cavity for the fish challenged with bacteria plus ECPs, when compared with the other treatments. Blood circulating leukocytes, lymphocytes and thrombocytes had a significant decrease immediately after the challenge, mainly seen in fish challenged with bacteria plus ECPs. At 48 h post-challenge, bactericidal activity increased for the treatments with bacteria (with and without ECPs). The same tendency was seen for some of the oxidative stress parameters.

Conclusions: The results of mortality trial and the observed immune responses suggests that the infection route is a determinant factor regarding T. maritimum-induced pathogenesis. The intraperitoneal challenge may result in a fast recruitment of immune cells which can undermine the invasion of bacteria. The head-kidney samples are currently under analysis to attempt to disclose the systemic response triggered by T. maritimum.

Alternative control measures to antibiotics: Flavobacterium psychrophilum in rainbow trout fry and the effect of salt and warm temperatures

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Introduction: Flavobacterium psychrophilum is a worldwide bacterial pathogen affecting salmonid aquaculture [Rainbow Trout Fry Syndrome (RTFS) and Bacterial Coldwater Disease (BCWD)]. Due to the unavailability of a commercial vaccine and the rise of AMR, alternative measures are of ecological and economic interest.

Methodology: In this study, we investigated the effect of salt water (1%) and warm temperature (18±1°C) in the control of F. psychrophilum in rainbow trout fry. Fish experimental trials were set up. In the first part of the experiment, fish (0.7 g) were at first infected by bath challenge and then exposed to either salt water (1 dpi – 12°C) or to increased water temperature (1 dpi or at onset of mortalities). Water parameters were changed gradually over 24 hours. Negative infection controls were included (freshwater and salt water at 12°C; warm freshwater at 18°C) and fish survival was followed over time. In the second part of the experiment, a cohabitation challenge was established and the effect of salt water (1%) on disease transmission evaluated (1.5-2 g/fish). Fish survival was followed over time.

Results: Following bath challenge, the salt treatment delayed the appearance of clinical disease while in the warm temperature groups fish survival decreased more rapidly than the positive control. In the second part of the experiment, we performed a co-habitation challenge. Ip injected fish reached 0% survival within two weeks in both groups. Cohabitant fish swimming in salt water had a significant increase in survival (42.6%) compared to the positive controls (17.9%). Infected dead and moribund fish were confirmed positive for F. psychrophilum.

Conclusions: Increasing water salinity delayed and partly prevented RTFS. The delay can be an advantage, as it gives time to achieve AMR test results and initiate treatment before reaching heavy mortalities in a fish batch. Further studies should evaluate the robustness of the preventive effect of this approach, its effect on the microbial communities (fish and farm environment), and whether it could be combined with other measures like e.g. phage therapy. The effect of the warm water was surprising, as F. psychrophilum favours colder temperatures and grow slower at higher temperatures.

Virulence and in vitro genomic variations of Cyprinid herpesvirus 3

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Cyprinid herpesvirus 3 (CyHV-3) is one of the most pathogenic viruses infecting common carp and koi. The rapid spread of this virus has impacted many countries worldwide. Knowledge on the evolution of CyHV-3 is essential to design preventive strategies and control its spread. Viral adaptation to cell culture of a CyHV-3 strain produced a less virulent form, representing a promising vaccine candidate. This loss of virulence was associated with a 1363-bp deletion in the open reading frame (ORF) 150.

To understand the mechanisms leading to this deletion, we combined in vitro cell cultures, qPCR and long-read ultra-deep sequencing to analyse the genome composition of large series of viral passages on CCB cells. A first series of 100 successive passages was 21ealized, and a second series of 50 successive passages was carried out at 23°C on 10 sub-cultures of CCB cells. Here, a thermal stress was applied at passage 25 (15°C or 28°C). Finally, the presence of the ORF150 deletion was searched in vivo, by analysing the viruses from common carp raised in Indonesian farms.

Genomic analyses of the 100 successive passages revealed that CyHV-3 produces a complex assemblage of haplotypes, which composition changes very quickly. These haplotypes carry a great number of structural variations (SVs) that accumulate in the genome, such as insertions, deletions… Of note, the ORF150 deletion could almost completely disappear after as little as ten cell passages. Comparison of genome compositions of the 10 sub-cultures revealed that, while a minority of SVs were common to all sub-cultures, the majority of them accumulated randomly. The thermal stress at passage 25 had a purifying effect on many – yet not all – SVs that had been generated over the successive passages. Finally, the in vivo survey conducted on nearly 250 carp did not allow to detect the ORF150 deletion.

Altogether, these observations indicate that SVs constitute a significant component in the in vitro evolution of CyHV-3, under conditions devoid of environmental pressures. Though still technically challenging, the same analyses need now to be conducted in vitro, on a wide number of animals originating from different environments.

Experimental Susceptibility of UK cyprinid species to spring viraemia of carp virus (SVCV)

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Introduction: Spring viraemia of carp virus (SVCV) can cause acute haemorrhagic and contagious viraemia which can result in high mortality in cultured and wild fish worldwide. The disease is listed by the World Organisation for Animal Health and the United Kingdom is currently recognised as free from SVCV. With an increased interest to import various species for recreational angling, there is an increased risk of introduction of SVCV, necessitating an understanding of their susceptibility.

Methodology: Duplicate tanks with combinations of 30 fish intraperitoneally injected with SVCV, genogroup 1d, or cell culture medium as negative controls, were mixed with 30 cohabitant fish. Species consisted of: (1) barbel and chub, (2) rudd and (3) golden tench and golden orfe, each with equal numbers of common carp as susceptible shedders. The fish were held at 15°C and sampled mid-challenge and at 31 or 42 days. Samples were analysed by cell culture isolation, histopathology, RT-PCR and sequencing.

Results & Conclusions: Common carp were susceptible with 83.3 to 100% mortality when injected and 7.5 to 36.7% cohabitant mortality. Rudd were not susceptible. Disease was observed in barbel, chub, orfe and tench with mortalities (≤16.7%) and transmission to cohabitant fish (≤3.3% mortalities) indicating low, but not negligible susceptibility. Although the virus is not highly pathogenic to the species, they may still be carriers of the virus and spread the disease to other fish species. The data will be used to provide evidence to help prevent and control future disease incursions in the cyprinid fish sectors.

Beating primary heart cell cultures as a tool for measuring the effect of viruses and environmental pollutants in vitro

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Introduction: Circulatory disorders in the heart are an increasing challenge to the sustainable development of salmonid aquaculture worldwide. The main pathogens causing these problems are piscine orthoreoviruses (PRVs), which often cause Heart and Skeletal Muscle Inflammation (HSMI). In addition to pathogens, acute or chronic toxic exposures to nano- and micrometre-sized plastics and oil spills are a significant risk to aquatic life. The aim of the present study was to develop novel cardiac cell cultures from salmonids to investigate the effects of viral and environmental stressors on cardiac cells. In addition, gene expression signatures in these cardiac primary cultures of salmonids (SCPCs) after infection with PRV-1, PRV-3, piscine myocarditis virus (PMCV) and salmonid alphavirus 3 (SAV-3) were investigated to determine whether innate immune responses could be used as markers to confirm virus replication.

Methods: A novel method was used to culture SCPCs, which are constantly beating for up to eight weeks. Three-week-old SCPCs from Atlantic salmon, brown trout and rainbow trout were infected with the viruses and then cultured at 8°C and 15°C for a further four weeks. The virus-infected cultures were sampled immediately after infection and at 3, 7, 14, 21 and 28 days post infection. In addition, SCPCs and salmonid larvae were exposed to nano- and microplastics or crude oil. The number of contractions of the cultures was counted and samples of the cells were taken. Cell and media samples were used for virus detection, and cell samples were used to measure 10 genes involved in antiviral and pro-inflammatory responses by RT-qPCR.

Results: The heart cell cultures were more sensitive to the effects of environmental stressors than heart in vivo. While the in vivo studies did not show any change in heart rate, the in vitro studies showed an increase in the number of contractions after exposure. Preliminary results from the virus exposures show that antiviral genes were upregulated when cardiac cells were actively replicating viruses and that these genes are likely to play an important role in the cardiac immune response.

Conclusion: SCPCs may therefore be a valuable tool for monitoring host-pathogen or host-environment interactions involving cardiac cells.

Piscine myocarditis virus (PMCV) p33 protein is processed into a sub-product with characteristics of fusion-associated small transmembrane (FAST) proteins

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Introduction: Piscine myocarditis virus (PMCV) is well known for its ability to cause myocarditis in Atlantic salmon aquaculture, a disease known as cardiomyopathy syndrome (CMS). The disease relates to health and welfare issues for the fish and large economic losses to the industry as it mainly affects marked sized fish. In 2011, we described PMCV as the first vertebrate virus with similarities to Totiviridae, a family of viruses infecting single-celled organisms and spread from cell to cell following cell division or fusion, without leaving the cells.

All toti-like viruses found in multicellular organisms are described with additional protein-encoding sequences in either ends of their genome, when compared to Totiviridae. Increasing evidence suggests that these additional proteins or peptides have functional properties related to infection and their spread in a more advanced host. PMCV was the first toti-like virus described with additional sequences, included as a separate third gene. In recent years, toti-like viruses with similar protein coding additions have also been found in lumpfish, golden shiner and common carp. The third gene of PMCV encodes a 33.4 kDa protein, p33, that is further processed into smaller sub-products. Here we present functional characteristics of the C-terminal sub-product of PMCV p33.

Methodology: We have performed computer-based analyses of the amino acid sequence, including analyses of pairwise amino acid identities with known and predicted proteins/peptides. Functional properties of the p33 C-terminal sub-product have been characterized through recombinant expression in vitro using plasmid expression vectors, combined with phase contrast and fluorescence microscopy observations and western blot analyses.

Results and conclusion: Strong evidence from both computer-based and experimental analyses shows that the C-terminal sub-product of p33 has both amino acid sequence and functional characteristics of fusion-associated small transmembrane (FAST) proteins. FAST proteins are the smallest viral membrane fusion proteins and are nonstructural proteins that induce cell-to-cell membrane fusion and have previously only been described for certain non-enveloped viruses within Spinareoviridae. We suggest that the PMCV p33 FAST protein has a role in facilitating virus replication and/or cell-cell fusion that may assist spread of virus progeny from infected cells.

Nervous Necrosis Virus sense and antisense genomic RNA detection through a novel duplex fluorescent in situ hybridization (FISH) assay

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Introduction: The Mediterranean aquaculture has suffered significant economic losses due to viral nervous necrosis (VNN) caused by the Nervous Necrosis Virus (NNV), belonging to Betanodavirus genus, family Nodaviridae. The VNN mortality outbreaks mainly involve European sea bass (Dicentrarchus labrax) and gilthead sea bream (Sparus aurata), but NNVs have been also detected in several wild finfish and aquatic animals such as bivalve molluscs. At present, the epidemiological role of bivalve molluscs is not fully understood as the replication of NNV within their tissues has not been reported yet.

Methodology: In order to understand the localization within aquatic animal tissues and the potential replication of the NNV, a duplex fluorescent in situ hybridization (FISH) assay was developed for the detection of sense and antisense NNV genomic RNA. The developed FISH assay specificity and sensitivity has been tested using all betanodavirus genotypes (BFNNV, RGNNV, SJNNV, TPNNV), the reassortant strains SJNNV/RGNNV and RGNNV/SJNNV, and serial dilutions of a viral isolate belonging to RGNNV genotype, respectively. Moreover, the assay has been applied to European sea bass and clam (Ruditapes philippinarum) samples with the purpose of localize NNV in the tissues.

Results: The designed method resulted to be specific for the detection of the sense and antisense genomic RNA of the two NNV strains most widespread in the Mediterranean basin, the RGNNV genotype and its reassortant strain, the RGNNV/SJNNV. FISH was also applied to the European sea bass brain samples collected from an NNV-positive batch, where it was able to show the localization at cellular level of the NNV genome and its complementary RNA (cRNA), produced during viral replication. Furthermore, specific signals were detected in the oocyte's cytoplasm of clam suggesting the intra-cellular localization of NNV in invertebrates and its active replication in these cells.

Conclusions: The developed method could be considered an important tool able to investigate specifically the pathogenetic mechanism of the two NNV strains circulating in the Mediterranean basin. The obtained results demonstrated the detection and the localization of the RGNNV or RGNNV/SJNNV genome in the aquatic animal tissues.

Characterization of piscine orthoreovirus-1 attachment to salmonid erythrocytes

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Introduction: Piscine orthoreovirus-1 (PRV-1) is the causative agent of heart and skeletal muscle inflammation (HSMI), an important disease in Atlantic salmon aquaculture. The main target cells for PRV-1 are erythrocytes, which in fish are nucleated cells that support viral replication. In an ongoing study, we are working to characterize the attachment of PRV to salmonid erythrocytes.

Methodology: We have developed an ex vivo assay in which high-titer PRV from plasma samples of fish is adsorbed to erythrocytes isolated from Atlantic salmon blood samples. Viral attachment to the cell surface is monitored by flow cytometric detection of PRV sigma 1 protein.

Results and conclusion: The virus binds to all erythrocytes and is present on the cell surface after only 5 minutes of incubation time. Binding is significantly reduced by preincubation of the virus with antibodies specific for sigma 1, indicating that sigma 1 is required for binding. Pretreatment of cells with wheat germ agglutinin (WGA) does not interfere with virus adsorption, suggesting that sialic acid does not contribute to attachment. Binding also appears to be specific to Atlantic salmon erythrocytes, as the virus does not adsorb to erythrocytes from other salmonid species such as rainbow trout. No difference in binding is observed between PRV-1 variants of high and low virulence.

The goal of this project is to identify receptors used by PRV-1 to enter erythrocytes. Such knowledge will provide valuable insights for both vaccine development and selective breeding, which may reduce the prevalence of HSMI in Atlantic salmon aquaculture.

Subunit vaccine development for prevention of Viral encephalopathy and retinopathy (VER) in Senegalese sole

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Introduction: Viral encephalopathy and retinopathy (VER) is a viral neurological pathology caused by the Nervous Necrosis Virus (NNV) which provokes high mortalities and important economic losses in Southern Europe. Given that no effective treatments are available for this disease many efforts are being made to develop an effective vaccine for VER prevention. To date, only two NNV inactivated vaccines have been licensed for sea bass protection against RGNNV genotype. The aim of this project was to design and develop a subunit vaccine based on the SJNNV-type coat protein (CP) of NNV and evaluate its efficacy in sole.

Methodology: Two different recombinant proteins were produced: 1) NNV coat protein tagged with histidine (His-NNV), and 2) a recombinant CP loaded into avian reovirus muNS-Nanospheres (NS) by adding an IC-tag to the C-terminal region (NNV-IC). Correct expression and antigenicity was confirmed by SDS-PAGE and ELISA. Immunogenicity of both proteins was assessed in sole by intraperitoneal (IP) injection at three doses (high, medium, and low) and at 30 days post immunization (dpi), a booster injection was performed. In a second experiment postlarvae and juveniles were bath vaccinated. Antibody production against NNV was analyzed by ELISA and expression of immune-related genes was assessed by RT-qPCR.

Results: Encapsulated proteins proved to be safe for fish, as no mortalities or signs of toxicity were observed after administration. Specific anti-NNV IgM production was significantly induced in sole immunized with the highest and medium dose of the NNV-IC, showing values similar to those derived from the immunization with an NNV-inactivated vaccine. Even though the lowest dose of NNV-IC and His-tagged antigen did not cause a significant improvement on the immune response initially, specific IgM levels were markedly enhanced three days after a booster immunization in all immunized groups. Ongoing studies will provide further information about the immune response and its duration.

Conclusion: Encapsulated NNV-IC proteins are an innovative and promising strategy for the development of subunit fish vaccines as the avian nanospheres protect the antigen of interest and act also as adjuvants.

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Exploring Protective Immunity in Tilapia: A Novel Nanovaccine Approach against Tilapia Lake Virus Disease

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Tilapia lake virus (TiLV) causes mass mortality and remarkable economic losses in the global tilapia industry, necessitating the development of an effective vaccine. This study investigates the efficacy of a chitosan nanoparticle TiLV immersion vaccine in laboratory and field trials and explores the adaptive immune response of tilapia following TiLV exposure. Transmission electron microscopy revealed the mucoadhesive properties of the nanovaccine (CN-KV) through fish gills. The CN-KV group demonstrated a higher relative percent survival (RPS) of 68.17% and a stronger TiLV-specific antibody response compared to the inactivated virus vaccine (KV) group and nonvaccinated control group. Additionally, surviving tilapia developed protective immunity and significant antibodies against the protein encoded by the TiLV segment 4. Using an indirect ELISA, high antibody levels were detected in survivors of experimental challenges and following farm outbreaks. The ELISA effectively distinguished TiLV-exposed from unexposed tilapia and monitored anti-TiLV antibody kinetics following infection. Tilapia developed an antibody response as early as 7 days post challenge (dpc), peaked at 15 dpc, and persisted in some fish up until day 110 dpc. Upon re-infection, an increased antibody response occurred within 7-14 days, indicating the development of humoral memory. These findings suggest that the immersion-based nanovaccine offers an easy-to-administer, less labor-intensive solution for mass vaccination against TiLV. The study also provides valuable insights into the immune response of tilapia following TiLV exposure, paving the way for the development of an efficacious vaccine to protect tilapia during the entire grow-out period.

Safety and efficacy of a new vaccine against Moritella viscosa using a laboratory bath challenge model in Atlantic salmon

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Moritella viscosa (M. viscosa) is one of the major etiological agents of winter-ulcers in Atlantic salmon (Salmo salar) in Norway. Outbreaks of ulcerative disease in farmed fish occurs across the North Atlantic region and is an impeding factor for sustainable growth within the industry. Commercially available multivalent core vaccines containing inactivated bacterin of M. viscosa reduce mortality and clinical signs related to winter ulcer disease. Grove et. al (2010) described two major genetic clades within M. viscosa, typical (hereafter referred to as classic) and variant, based on gyrB sequencing. In addition, there are phenotypical traits such as viscosity that may differ between different types of isolates.

Methodology and results: Vaccination-challenge trials using the emerging Norwegian gyrB-variant and classic non-viscous isolates of M. viscosa, demonstrate that the isolates from the classic clade that are included in the multivalent core vaccines, perform on level with PBS control group and thus provide limited cross protection against these emerging strains. Western blot using salmon antibodies showed similarities in binding patterns between Norwegian variant and classic non-viscous isolates, indicating they may be serologically related.

The results of the present study demonstrate serological and clinically relevant differences between Norwegian classic and variant isolates of M. viscosa and also between classic viscous and classic non-viscous types, supporting the need for the new vaccine against winter-ulcer disease. A recently approved vaccine containing inactivated antigen of a variant M .viscosa strain reduces mortality and ulceration caused by infections with variant- as well as classic non-viscous M. viscosa. The new vaccine has also been evaluated for local reactions after co-injection with other vaccines, showing acceptable scores for adhesions and melanisation, demonstrating a good safety profile for the new M. viscosa vaccine. The use of experimental animals in this study was approved by the Norwegian Food and Safety Authority.

The study, and the procedures included in the study, was also approved by the internal Zoetis Animal Ethics Committee.

Vibriosis in the Mediterranean aquaculture caused by Vibrio harveyi and prevention using autogenous vaccines

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During warm months, the Mediterranean aquaculture is at risk of suffering significant losses due to vibriosis, a disease caused by bacteria from the Vibrio genus. Vibrio harveyi is an important pathogen of the genus, affecting the main two main species reared in Greece, the European seabass, and the gilthead seabream with the former being much more susceptible. To better understand how the disease spreads and affects fish stocks, our research aimed to investigate its epidemiology. Additionally, we sought to develop effective tools to prevent vibriosis, which would contribute to safeguarding the sustainability and productivity of the aquaculture industry in Greece. Over the course of seven years, a diverse collection of bacterial strains was assembled, which were associated with disease outbreaks in 27 geographical areas throughout Greece. These strains were collected from various hosts and all rearing stages and underwent molecular identification. Whole genome sequencing was carried out for selected strains to facilitate analysis. During the outbreaks of vibriosis, Vibrio harveyi was the predominant species observed between June and October where the mortalities were the most severe, however other Vibrio species were also implicated complicating further the disease. Through whole genome sequencing of V. harveyi strains from the two aquaculture facilities, we discovered a diverse and genetically blended pool that carries a large array of virulence factors and has a highly homologous antigenic profile. Using this knowledge, we developed two autogenous vaccines for each of the aquaculture facilities. Following the development of adequate challenge models, efficacy of the vaccines was evaluated in European seabass vaccinated using a scheme of initial immersion at 2 g followed by a booster injection at 30 g. Relative percentage survival was above 50% for the first vaccine and 90% for the second while no cross protection was observed when fish were challenged with non-homologous strains. These findings suggest that the autogenous vaccines and the vaccination scheme we developed have the potential to significantly reduce the impact of vibriosis on Greek aquaculture and improve the overall sustainability and productivity of the sector.

The culprits behind bacterial ulcers in sea-farmed Atlantic salmon in Norway

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Ulcer conditions in sea-farmed Atlantic salmon in Norway, particularly when water temperatures are low, represent perhaps the most significant health- and welfare-problem facing Norwegian mariculture today. While undoubtedly often predisposed by external stressors such as mechanical delousing, the presence of Moritella viscosa and/or Tenacibaculum spp. ultimately seems necessary for large ulcer outbreaks to manifest. Complex infections involving multiple bacterial strains/species are nevertheless common, and often complicate diagnostics and epidemiological investigations.

Research projects led by the Norwegian Veterinary Institute (NVI) have in recent years illuminated the genetic population structures of M. viscosa and Tenacibaculum spp. strains isolated in association with Norwegian aquaculture. Considerable overall diversity has been documented within both taxa, but with certain host-specific strains, or clonal complexes, particularly intimately linked to ulcer development in Atlantic salmon sea-farmed in Norway, irrespective of geographic origin on the sub-national level. The underlying basis for these specific dominances remains enigmatic, and one potentially crucial yet essentially unexplored aspect is the demographics of these bacteria outside of commercial fish farming. In other words, how may a fluctuating bacterioplankton composition (and thus infection pressure) influence the risk of ulcer outbreaks? The genetic foundations for host-specific virulence have neither been established. These topics will be addressed in the coming years through a newly started and NVI-led research project financed by the Norwegian Seafood Research Fund (FHF – project no. 901838).

The presentation will provide an update on the current ulcer situation in sea-based salmon farming in Norway, sum up what we know regarding the population structures of bacteria involved in such conditions, and provide an insight into the new project.

Antibiotic resistance and virulence factor profiles of Aeromonas spp. isolated from diseased catfish in Nigeria

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Introduction: Nigeria has a fish supply deficit of over 1 million tonnes despite being the second largest aquaculture producer in Africa. Insufficient capacity for fish health management has led to many fish farmers using antibiotics, sometimes inappropriately, as the primary means of disease prevention and control. Such practice poses a threat to both sustainability and the One Health mantra. This present study aimed at determining virulence potential and antibiotic resistance determinants of bacteria isolated from diseased catfish in two northern states of Nigeria and the capital Abuja.

Methodology: Bacteria were isolated from kidney and skin lesion swab samples of fish collected from 7 farms and 2 fish markets. Isolates were identified using standard phenotypic tests, API 20E, 16S ribosomal RNA gene and whole genome sequencing. Virulence potential was determined using phenotypic assays including haemolysis, protease, DNase, lipase, and gelatinase. Susceptibility to 9 commonly used antibiotics was assessed by CLSI VET03 microbroth dilution method. Presence of resistance determinants was determined by bioinformatics and confirmed by PCR.

Result: Forty-four bacterial isolates were successfully recovered from the 53 fish samples collected. Sixteen isolates, all Aeromonas spp. (including A. hydrophila, A. veronii, A. dhakensis and A. jandaei), displayed enzymatic activities associated with all virulence assays conducted. All these isolates were wild types for gentamicin and erythromycin and non-wild types for oxytetracycline and sulphamethoxazole, while 15/16 were non-wild types for amoxycillin and oxolinic acid. For colistin, enrofloxacin, and florphenicol, 12/16, 11/16, and 2/16 were non-wild types respectively. Antibiotic resistance determinants were located on the chromosome and on plasmids.

Conclusion: Our study emphasises the significant economic and public health ramifications of Aeromonas spp., particularly in the rapidly expanding aquaculture sector of northern Nigeria. The primary bacterial genus identified was Aeromonas, with isolates possessing numerous virulence and resistance genes, some of which were located on mobile elements such as plasmids. The evidence highlights the critical role that aquaculture and the overuse of antibiotics plays in fostering the emergence and dissemination of antibiotic resistance, and the risk of rapid spread.

Columnaris disease is caused by Flavobacterium columnare and three newly described Flavobacterium spp.

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Introduction: Flavobacterium columnare is the causative agent of columnaris disease in freshwater fish. Four discrete genetic groups exist within the species and research has demonstrated associated host and virulence differences. Previous research has suggested that the species designation may require revisions; therefore, the present study determined the taxonomic status of the four genetic groups of F. columnare using polyphasic and phylogenomic approaches.

Methods: A polyphasic approach was taken to confirm previous phylogenetic relationships and to compare phenotypic, biochemical, and chemotaxonomic properties or representative isolates from the four genetic groups. The research followed the proposed minimal standards for describing new taxa of the family Flavobacteriaceae by Bernardet et al.

Results: Phylogenetic analyses of 16S rRNA and gyrB genes using different methodologies demonstrated the four genetic groups formed well-supported and distinct clades within the genus Flavobacterium. The average nucleotide identity (ANI) and digital DNA-DNA hybridization (GGDC) values between F. columnare ATCC 23463(T), genetic group 2 isolate AL-02-36(T), genetic group 3 isolate 90-106(T), and genetic group 4 isolate Costa Rica 04-02-TN(T) were less than 90.84% and 42.7%, respectively. Chemotaxonomic, MALDI-TOF characterization and ANI/GGDC calculations afforded differentiation between the genetic groups, indicating each group is a discrete species. The names F. covae sp. nov., F. davisii sp. nov., and F. oreochromis sp. nov. were proposed to represent genetic groups 2, 3, and 4, respectively, and recently validated.

Conclusion: Since these pathogens (collectively referred to as columnaris causing bacteria, CCB) are globally distributed and have significant impacts on wild and cultured fish species, recognition of the four species will advance and improve research to define host-pathogen-environment relationships, epidemiology, and develop effective control and prevention measures in aquaculture. Such research needs to target the correct bacterial species and research findings can be properly interpreted by correct and consistent taxonomic assignment.

Whole genome sequencing and determination of virulence and antimicrobial resistance genes of bacteria Vibrio harveyi from the Mediterranean Sea

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Introduction: Vibrio harveyi, occurs naturally in marine habitats and has developed into a significant pathogen of wild and cultured marine fish and invertebrates. Although reports on the occurrence of vibriosis caused by V. harveyi go several decades back, outbreaks in the Mediterranean farming of European seabass (Dicentrarchus labrax) and gilthead sea bream (Sparus aurata)start to become a serious economic problem in last fiftheen years.

Methodology: Sixteen V. harveyi strains were isolated from diseased sea bass and sea bream fish farms from several Mediterranean countries: Croatia, France, Italy, Spain, Tunisia, and Turkey using ONT MinION and Illumina HiSeq. The genomes were assembled using bioinformatic tools. For the identification of virulence factors is used VFDB database. The antimicrobial resistance genes and resistance mechanisms were identified by the RGI identifier. The presence of ARGs was compared with results of in vitro susceptibility tests.

Results: Out of 171 determined virulence genes an average of 150 were present in each of the tested strains. The analysis of the virulence genes demonstrated that some strains had genes sitA (4/16), sitB (3/16), sitC (4/16), sitD (3/16), and the ast gene which was not present in other bacteria of the genus Vibrio in the searched database, and they could represent atypical virulence genes. Analysis of the assembled genomes disclosed the presence of five antimicrobial resistance genes: CRP, adeF, E. coli parE, APH(3")Ib, and tet(D). The analysis showed that studied strains were clustered into a closely related separate phylogenetic group.

Conclusion: Presence of virulence and antimicrobial resistance genes are neither conditioned nor related to the geographic origin of the strain within the Mediterranean. It may be explained by the fact that the Mediterranean Sea is a semi-enclosed basin, and there is a frequent movement of different age categories of sea bass and sea bream. The aforementioned facts represent a real danger of the spread of bacteria that are no longer specific to a certain place and do not have typical characteristics related to a certain geographical area but also enable the common regional approach to the prevention and mitigation of the diseases.

Host associated genotypes in Candidatus genus Branchiomonas with proposal of Cand. Branchiomonas mykissi n. sp. from rainbow trout (Oncorhynchus mykiss)

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Candidatus Branchiomonas cysticola has since its first description (Toenshoff et al. 2012) been widely accepted as the primary etiological agent of epitheliocystis in salmon. The obligate, intracellular nature of the bacteria, however, complicates genetic characterization beyond 16S rRNA gene due to the lack of cultivation methods. A recent study (Mjølnerød et al. 2022) has identified several housekeeping (HK) genes through next generation sequencing and fluorescence in situ hybridization (FISH) which has enabled phylogenetic reconstructions of the bacterium at the inter- and intraspecific level.

Multilocus sequence analysis (MLSA) of Cand. B. cysticola and 60 type strains of Betaproteobacteria using newly identified housekeeping genes (dnaK, rpoC, and lepA) and ribosomal subunit sequences (16S and 23S), showed the phylogenetic distinction between Cand. B. cysticola and its closest related type strain to be at the family level. A novel bacterial family named Branchiomonaceae has thus been proposed to include a monophyletic clade of Betaproteobacteria exclusively associated with epitheliocystis in fish. Single locus phylogenies of sequences obtained from samples of salmon from Norway and United Kingdom displayed few genetic differences. The genetic homogeneity observed suggests Cand. B. cysticola from gills of salmon represents a single clonal complex. However, highly divergent genotypes were identified from samples of rainbow trout, suggesting the existence of putative host associated genotypes of Candidatus Branchiomons spp. Sequencing of the rRNA operon of the bacterium from samples of wild marine fish species also culminated in the identification of novel genotypes spesicic to each fish host species.

Elucidation of the bacterium's genetic heterogeneity from spatiotemporal disparate gill samples of different fish host species, may prove valuable in designing new diagnostic tools, identifying strains of varying virulence and/or host adaptations, and aid possible vaccine development in the future.

References:
Mjølnerød EB, Srivastava A, Moore LJ, et al (2022) Identification of housekeeping genes of Candidatus Branchiomonas 28isticola associated with epitheliocystis in Atlantic salmon (Salmo salar L.). Arch Microbiol 204:. https://doi.org/10.1007/S00203-022-02966-Y
Toenshoff ER, Kvellestad A, Mitchell SO, et al (2012) A novel betaproteobacterial agent of gill epitheliocystis in seawater farmed Atlantic salmon (Salmo salar). PloS One 7:e32696. https://doi.org/10.1371/journal.pone.0032696

A new genospecies of bacillus cereus group isolated from Chinese softshell turtles (pelodiscus sinensis), and its pathogenic and genomic features

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Chinese softshell turtle, CST (Pelodiscus sinensis) is a noteworthy freshwater aquaculture species with significant commercial value. CST are widely farmed in Asia, particularly in Taiwan. Nevertheless, diseases caused by the Bacillus cereus group (Bcg) continue to pose a severe problem in modern commercial agricultural systems. Despite the devastation inflicted by this disease in Taiwan, knowledge of its pathogenicity remained restricted. As a result, in this study, we examined the pathogenicity of Bcg strains isolated from clinical CST infected cases. The pathogenicity study indicated that QF 108-045 isolated from CST causing the highest mortality rate and whole-genome sequencing revealed that it was an independent group distinct from other Bcg genospecies. Upon comparing the average nucleotide identity of QF108-045 with other known Bcg genospecies, it was found to be below 95%, indicating that QF108-045 belong to a novel genospecies. Furthermore, several antibiotic resistant genes were detected, including Bacillus cereus beta-lactamase I, class A; B. cereus beta-lactamase II, class B, fosfomycin-resistant enzyme, small multidrug resistance efflux pump, the serine racemases vanW, vanT, and vanY, and ATP-binding cassette, antibiotic efflux pump. The presence of these antibiotic-resistant genes in the Bcg strain isolated from CST underscores the potential risk of antimicrobial resistance in aquaculture and the need for the prudent use of antibiotics in disease management. Further research is needed to investigate the extent of antibiotic resistance in Bcg strains and its impact on the CST bacillosis treatment.

Characterization of a novel toti-like virus in sea bass, dicentrarchus labrax

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Introduction: Sea bass is a major reared species regarding Mediterranean aquaculture. The larval stage, very sensitive to sanitary and environmental conditions, is particularly monitored in hatcheries. An abnormal increase of mortality on very early stages (20 to 35 days post-hatchering - dph) was recently observed in a farm and we investigated a potential infectious etiology.

Methodology: Cell lines (n=11) were inoculated with homogenized affected seabass larvae at 14°C. In case of cytopathic effect (CPE), nucleic acids were extracted from supernatant as well as larvae homogenate and analyzed through Next-Generation Sequencing (NGS). Based on the consensus sequence obtained, a specific RT-qPCR system was developed and used to check various batches of seabass but also seabream larvae. An ELISA assay was complementary developed to determine the serological status of broodstock. Experimental infections were performed (immersion or intraperitoneal injection) on juvenile seabass at different stages of development. Mortality was monitored and fish were sampled for RT-qPCR (organs) and ELISA (blood).

Results: CPE were observed on three cell lines after 3 weeks at 14°C, demonstrating the presence of a viral agent. A 6818 nucleotide-long RNA genome was obtained by high-throughput sequencing. This newly described genome contained six putative ORFs with an organization consistent with the Totiviridae family. It clustered with the newly described Pistolvirus genus, sharing a maximum of identity with other piscine toti-like viruses such as Cyclopterus lumpus toti-like virus (CLuTLV) or piscine myocarditis virus (PMCV). No mortality was recorded on fingerlings experimentally infected. The virus was detected in organs and a specific immune response was observed with detection of antibodies after 7 days of infection. All RTqPCR and ELISA results obtained with the different batches were analyzed in details to find correlations with age, clinical health, and viral amount.

Conclusion: This work enabled the characterization of a new virus named seabass toti-like virus (SBTLV). The monitoring of the hatchery for several years highlighted its presence in numerous batches at the very beginning stage of development, but without evident correlation with the mortality strength and the amount of virus detected in larvae, suggesting a multifactorial and complex etiology.

Evaluation of antiviral activity of coumarin derivative scoparone against VHSV in vitro and in vivo

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Introduction: Viral hemorrhagic septicemia (VHS) severely affects the global aquaculture industry with no adequate measures to control the disease. Coumarin derivatives are currently in interest for evaluating the capacity for rhabdoviral clearance. This study evaluated the antiviral efficacy and mechanisms of 6,7-dimethoxycoumarin, also known as scoparone in fathead minnow (FHM) cells and in olive flounder, Paralichthys olivaceus.

Methodology: Seventeen coumarin compounds were screened to select one potential anti-VHSV compound, scoparone. In vitro antiviral mechanism was studied using a timed plaque reduction assay with 100 µg/mL scoparone in FHM cells. Post infection treatment was studied further by determining absolute copy number of VHSV N gene expression. The in vivo efficacy of orally administered scoparone was tested in olive flounder, 0.1 to 1.0 mg/kg body weight (bw)/day as preventive treatment. Therapeutic effect was tested at 1 to 10 mg/kg bw/day. Ribavirin was tested at 3 mg/kg bw/day as reference control. The in vivo innate immune-related genes were evaluated in olive flounder administered with 1 mg scoparone/kg bw/day for 2 weeks and challenged with VHSV.

Results: Scoparone reduced the cytopathic effect of VHSV-infected FHM cells. Timed plaque reduction assays showed scoparone exerts inhibition of VHSV by: 1. direct virucidal activity, and 2. inhibiting viral replication. The direct virucidal effect increased time dependently up to 32.3 ± 1.3 % by 4 h incubation ($P < 0.001$). Scoparone showed highest plaque reduction, 54.1 ± 0.1 % ($P < 0.001$), when treated to the cells 1 h post-infection (hpi) but was not effective by treating at 5 hpi. VHSV absolute copy number was reduced by post-infection scoparone treatment in FHM cells from 0.5 hpi to 6 hpi, with maximum reduction at 1 hpi treatment, by 90.6% ($P < 0.05$). Scoparone showed 44% relative percent survival, by 1.0 mg preventive and 10 mg therapeutic administration doses, higher than that of ribavirin. Innate immunity related gene expressions in olive flounders suggested promotion of IFN I & II, and ISG15, suppression of TLR, RLR, and caspase 3 in scoparone-mediated prevention of VHSV infection. Overall, scoparone can be considered a potential candidate, with multiple antiviral mechanisms, against VHSV infection.

First detection of Cyprinid herpesvirus 3 and Carp edema virus from Bigmouth Buffalo (Ictiobus cyprinellus)

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Introduction: An acute mortality occurred in mid-May of 2022 in an ongrowing fish farm in Michigan, using mixed cyprinids caught from an inland lake in another Midwestern State. One week after restocking the entire ~36.3 mt stock was lost, including Common Carp (*Cyrpinus carpio*) and Bigmouth Buffalo (*Ictiobus cyprinellus*). After drying the pond for the entire summer, once ready to restock a suspicious mortality rate was reported during the interstate fish transportation. The presence of viral pathogens was investigated as a potential cause for these mortality events.

Methodolog: A large portion of the pond was covered with fish carcasses of varying sizes, including naturally occurring Green Sunfish (*Lepomis cyanellus*), while moribund fish were observed piping and with low escape response. Organs from 6 Bigmouth Buffalo, 5 Common Carp, and 1 Green Sunfish were collected during the first mortality event, whereas 15 Bigmouth Buffalo and 15 Common Carp were sampled from the transportation tank truck right before restocking the pond. The presence of *Cyprinid herpesvirus* 3 (Koi herpesvirus, KHV), Carp edema virus (CEV), and *Sprivivirus cyprinus* (Spring viraemia of carp virus, SVCV) was tested by PCR and followed by nucleotide sequencing for confirmation. Supernatants from pooled homogenized tissues were inoculated into CCB and KF-1 cell lines and incubated at 25°C for 14 days.

Results and Conclusions: The diagnostic investigation indicated that the massive acute mortality event in May was determined by a sudden oxygen depletion, in concomitance with elevated temperatures and lack of oxygenation devices. Although 4 Common Carp tested positive for KHV, and 2 Bigmouth Buffalo were positive for CEV. In September, the pond restoking was preventively avoided. PCR testing detected KHV from 9 fishes (including 8 Common Carp and 1 Bigmouth Buffalo), while CEV was detected from 6 fish (including 15 Common Carp and 6 Bigmouth Buffalo). Sequence analysis showed higher CEV similarity within the genogroup I, whereas KHV had closest similarity to Asian genotypes. Virus culturing and isolation attempts using cell lines were unsuccessful. To our knowledge, this is the first report of CEV in Michigan and the first detection of CEV and KHV in Bigmouth Buffalo. However, PCR detection of viral DNA alone cannot demonstrate an active infection status, and it is important to further characterize the susceptibility of this cyprinid species.

Tilapia Lake Virus: a structured phylogenetic approach

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Introduction: Tilapia Lake Virus (TiLV) is an important emerging pathogen that threatens both wild and farmed tilapia (Oreochromis spp.), the second most important farmed fish species, after carps, for human nutrition. Therefore, the socio-economic implications that the uncontrolled spread of this viral threat could have are easy to predict, particularly in developing countries. To date the scarce availability of TiLV whole-genomes is severely affecting the knowledge on the origin, evolution and epidemiology of this pathogen. Besides, the unknown function of almost all TiLV genes makes it complicated to gain any consensus on how to perform an appropriate phylogenetic analysis to track the viral movements among countries.

Methodology: Tilapia fingerlings were collected in mid-summer 2018 from two Israeli farms during mortality events. Firstly, we investigated the presence of TiLV by rRT-PCR and viral isolation. Two isolates were used to obtain whole-genomes by Sanger sequencing. Furthermore, to determine the phylogenetic relationships between Israeli TiLV strains and those publicly available, multifactorial investigations were carried out with the aim of characterizing each ORF dataset before performing phylogenetic analyses. Therefore, genetic distance and "quartet puzzling" analyses were performed both to determine the phylogenetic signal in each segment and define its evolutionary rate. Finally, we also attempted to investigate the presence of potential reassortment events in all the studied isolates.

Results: Along with the identification, viral isolation and whole-genome sequencing of two Israeli TiLV, we characterized each genetic segment before performing phylogenetic analyses. Indeed, combining results from both likelihood mapping method and genetic variability analysis allowed us to discriminate candidate ORFs (1, 3 and 5) to perform a maximum likelihood phylogenetic analysis. This yielded a fixed tree topology with fully supported nodes and branches. Finally, we also reported a reassortment event detected in segment 3 in one Israeli isolate involved in the present study, and confirmed almost all the other events previously reported.

Conclusions: We presented a multifactorial approach aimed to perform reliable phylogenetic analyses of this new emerging viral species. However, further studies supported by a greater number of TiLV whole-genomes are necessary to better investigate and confirm the obtained results.

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A Nile tilapia strain with resistance to the Tilapia Lake Virus disease - from the immunology to the application considerations

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Introduction: The occurrence of viral diseases that cause very high mortality can disrupt aquaculture production. This recently happened in Nile tilapia aquaculture with the emergence of a disease caused by tilapia lake virus (TiLV), which has dramatically affected tilapia farms around the world. In areas where the virus is endemic, three strategies can be used to limit the losses caused by infection: 1) improved biosecurity, 2) vaccination programmes, 3) selective breeding to increase resistance. To explore the third strategy, we investigated the resistance to TiLV in three genetic strains of tilapia reared in Germany. We used two strains originating from Nilotic regions (Lake Mansala (MAN) and Lake Turkana (ELM)) and one from an unknown region (DRE).

Methods: Nile tilapia juveniles were infected with TiLV by intraperitoneal injection or cohabitation. Immune responses were measured using a Fluidigm array and correlated with viral load and pathological changes.

Results: Infection by injection resulted in 100% fish mortality in all three strains. However, when using cohabitation, we found that the ELM strain did not develop clinical signs of infection and had almost 100% survival. The other two strains showed severe clinical signs and a much lower survival rate of 29.3% for the DRE strain and 6.7% for the MAN strain. Disease resistance in tilapia from the ELM strain correlated with a lower viral load in both mucosal and internal tissues. The lower viral spread was associated with a stronger mx1-based antiviral response in the early phase of infection in the ELM strain. In addition, lower pro-inflammatory responses in the resistant strain may further contribute to its protection against disease-associated pathology.

Conclusion: Obtained results suggest the possibility of using TiLV-resistant strains as a cost-effective ad hoc solution to the TiLV challenge. However, it is important to note that fish of the resistant strain still had a significant viral load in the liver and brain 28 days after infection and could become persistent virus carriers, potentially transmitting the virus to naive populations. Therefore, the resistant strain should be used as part of an integrated approach that includes biosecurity, diagnostic and vaccination measures as appropriate.

Controlled laboratory challenge demonstrates moderate additive genetic variation in resistance to tilapia lake virus in Nile tilapia

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Introduction: Tilapia lake virus (TiLV) is a lethal virus impacting farmed and wild tilapia (Oreochromis spp.). The virus was identified in 2014 and its emergence has resulted in substantial economic losses to the global tilapia industry. Given the lack of treatment options currently available, selective breeding for increased disease resistance may be a viable option for reducing the impact of this pathogen. Therefore, this study was initiated to phenotype Nile tilapia (O. niloticus) families for resistance to TiLV and determine its additive genetic variation and heritability.

Methods: Fish from the eleventh generation of the Spring Genetics Nile tilapia breeding program with nucleus operations in Homestead, Florida, US, were used for this study. A total of 142 full-sib families (mean weight, 142.3 g) were included in the challenge with on average 17 fish per family (range, 4 to 20). All fish were challenged at the USDA-ARS AAHRU with TiLV via intraperitoneal injection with a viral dose corresponding to 2.25×10^4 $TCID_{50}$ $fish^{-1}$ and placed

into a single 5,550 L tank. Mortality data on each individual fish was collected for 21 days post challenge and a univariate animal linear model was used for quantitative genetic analyses.

Results: The accumulated mortality at the end of the experiment was 74.5%. The results revealed high variation in the mean survival of the families challenged with TiLV (range, 0% to 95%). The additive genetic effect for survival to TiLV was significantly different from zero ($P < 0.001$; log-likelihood ratio) and the estimated heritability was $h^2 = 0.29 \pm 0.1$.

Conclusions: The results demonstrated moderate additive genetic variation in resistance to TiLV and suggest promise in genetic improvement of tilapia for resistance to this virus by selective breeding. Genomic analyses are pending to evaluate the potential for genomic or marker assisted selection. In the next generation, families will be produced by assortative mating (high and low estimated breeding values) and challenged to confirm the heritability of resistance to TiLV. The end goal is the production of a high performing strain of tilapia with disease resistance for the global tilapia industry.

Tilapia Lake Virus Infection: Unravelling the complex interplay of mitochondrial dysfunction, hematological imbalances, and cellular signaling

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Tilapia Lake virus (TiLV) is a novel RNA virus that has caused significant economic losses in global tilapia aquaculture. In order to gain a better understanding of TiLV infection, this study focused on examining the clinicopathological alterations, ultrastructural changes, and the role of the MAPK/ERK pathway during infection. Using transmission electron microscopy, the study found that TiLV particles were present in the cytoplasm of fish cells as early as 1 hour after infection, causing progressive swelling of mitochondria and ultrastructural damage to the cells. The study also showed a loss of mitochondrial membrane potential and cell death in infected fish cells, which may be caused by the disruption of mitochondrial structure and function. In addition, the study found that the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway is involved in the early stages of TiLV infection in fish cells. Furthermore, the study investigated the clinicopathological changes during TiLV infection in tilapia. The infected fish showed pale bodies and gills, severe anemia, decreased levels of hemoglobin and hematocrit, and various pathological findings such as pale and friable liver, pale intestine, dark and shrunken spleen, and reduced numbers of red blood cells. Histopathological analysis revealed the presence of multiple necrotic areas in both the spleen and head kidney of infected fish, indicating damage to the hematopoietic organ and likely contributing to the observed anemia. The severity of pathological changes was associated with higher viral loads and the expression pattern of pro-inflammatory cytokines and antiviral genes, including interferon regulatory factor 1 (irf1), interleukin (il-8), radical s-adenosyl methionine domain containing 2 (rsad2), and mx. The study provides new insights into the physiological process and pathogenesis of TiLV in fish cells. The knowledge gained from the study improves the understanding of how TiLV causes pathological and hematological changes in tilapia and provides a basis for developing new strategies to control this virus.

Effects of exposure to sodium fluoride (NaF) on the development, health and virus resistance of Rainbow Trout embryos and larvae

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Introduction: Over the last 20 years, a number of studies have looked at the effects of synthetic chemicals on the human and animal endocrine systems. These endocrine disruptors (Eds), are ubiquitous in terrestrial and aquatic environments and classified as substances of very high concern for health and the environment. While historically the focus has been on Eds that cause effects on sex steroid hormones and affect the reproductive capabilities, there is an increasing interest in the disruption of the thyroid hormone system (THS). In vertebrates, the THS interacts with a wide variety of target physiological systems including the immune system (IS), has a major role in development and can influence microbial diversity in various tissues. The present study is part of SUSPECT project which aims to determine the potential role of sodium fluoride, one of the 16 substances considered as priorities by the French Agency for Food, Environmental and Occupational Health & Safety (Anses) in 2021, on the THS/IS/microbiota triptych of rainbow trout (Oncorhynchus mykiss).

Methodology: In the present study, rainbow trout embryos were exposed for 21 days to 5 different concentrations from 0 to 32.6 mg.L-1 NaF. The animals were monitored daily, and samples and analyses (health status, morphometry, behavior, histology of endocrine/immune organs, RNAseq analysis, …) were taken at different times to evaluate the potential disturbances induced on the thyroid and immune systems. The potential impact of chemical exposure on pathogen resistance in animals was evaluated with an infectious hematopoietic necrosis virus (IHNV) challenge.

Results: First results indicate a decrease of body/head length ratio and a strong impact on behavior at the highest concentration tested. The intermediate concentration tested (10.8 mg.L-1) caused a significant delay in hatching and a significant decrease in virus-related mortality.

Conclusion: NaF appears to have a beneficial impact on resistance to the virus at certain concentrations and negative effects on development and behaviour at higher concentrations. Further analyses are underway to determine the immunotoxicity of this potential ED.

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Pacific white shrimp (Liptopenaeus vannamei) transcriptome analysis after exposure to recombinant Vibrio parahaemolyticus PirA and PirB proteins

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Introduction: Vibrio parahaemolyticus, a Gram-negative bacterium often present in marine and estuarine environments, is endemic among the global shrimp farming industry. V. parahaemolyticus proteins PirA and PirB are known virulence factors that contribute significantly to the development of acute hepatopancreatic necrosis disease. Previous work from our lab has demonstrated the lethality of recombinant PirA and PirB proteins to Pacific white shrimp (Liptopenaeus vannamei).

Methodology: To understand the host response to these proteins, recombinant PirA and PirB proteins were administered using a reverse gavage method and individual shrimp (n=5) were then sampled at 30 minutes, 1-, 2-, 4- and 6-hour post exposure. Shrimp hepatopancreas libraries were generated and RNA sequencing was performed on the control and recombinant PirA/B-treated samples. Differentially expressed genes from each pairwise comparison were subjected to enrichment analyses using both Fisher's Exact Test and Gene Set Enrichment Analyses in OmicsBox.

Results: Following the processing of the raw RNA sequencing data and alignment with the L. vannamei genome, we conducted comparisons between the control and the recombinant PirA/B treatment groups to establish gene expression profiles, which included the sequence libraries from the 30 minutes, 1-, 2-, 4- and 6-hour time points. For each of the different time points, differentially expressed genes were identified among the assayed time points according to the following cutoff criteria (FDR <0.05, fold change >2). Differentially expressed genes that were co-expressed at the later time points (2-, 4- and 6-h) were also identified and gene associations were established to predict functional physiological networks.

Conclusion: Our analysis reveals that the recombinant PirA and PirB proteins have likely initiated an early host response involving several cell survival signaling and innate immune processes that will be discussed. As these Pir A/B protein products harbored in V. parahaemolyticus plasmids have been identified as the causative agent in acute hepatopancreatic necrosis disease, information contained within will provide insight into specific host-toxin interactions after exposure.

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Effect of hydrocortisone implants on development of red mark syndrome in rainbow trout

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Introduction: Red mark syndrome (RMS) is a skin disease affecting rainbow trout, Oncorhynchus mykiss. The disease results in non-ulcerative skin lesions with hyperemia, oedema and often resorption of scales. The disease is not associated with mortality and disease development has characteristics pointing towards host-driven pathology with hypersensitivity-like features.

Formally, the aetiology of the disease is still undetermined but accumulating evidence points to a Midichloria-like bacterium (MLO). The Midichloriaceae is a family within the order Rickettsiales, the members of which are characterized by an obligately intracellular life-style.
Cortisol is a steroid hormone released in response to stress. While the effect of cortisol on the immune system is complex it is generally immunosuppressive.

We hypothesized that cortisol suppresses the response to MLO, thereby leading to less severe RMS pathology. To test our hypothesis, we investigated the effect of slow-release cortisol implants on development of RMS gross pathology, levels of MLO and immune markers.

Methodology: Disease-free rainbow trout were infected with MLO by cohabitation with RMS-affected seeder rainbow trout at 12°C. At this temperature, early signs of RMS are observed at approximately 6 weeks post-exposure, with increasingly more severe lesions developing over the following 4-5 weeks before spontaneously resolving. All fish were individually tagged, allowing treatment groups to be kept in the same tank and thus under the same infection pressure. The experiments consisted of five infected treatment groups: 1) untreated controls; 2) cortisol in coconut oil (vehicle) implanted IP at onset of cohabitation; 3) cortisol in coconut oil implanted IP 5 weeks after start of cohabitation, i.e. just prior to expected onset of clinical RMS. Groups 4) and 5) were similar to groups 2) and 3), except that only the coconut

vehicle was injected with no cortisol. In addition, uninfected control fish were kept in a different tank under otherwise similar conditions.

After 10 weeks the experiment was terminated, RMS gross pathology was scored and samples were taken for histology, gene expression, cortisol quantification and MLO quantification by qPCR.

Initial results appear to support our hypothesis with an ameliorating effect of implants on RMS pathology.

Results will be discussed at the conference.

Nile tilapia (Oreochromis niloticus) resistance to Francisella orientalis is heritable and not genetically correlated to growth performance

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Introduction: Tilapias (Oreochromis sp.) are among the top farmed freshwater fish in the world with an estimated global production value of US $11 billion. Bacterial diseases result in major losses and control relies on health management, vaccination (if available), antibiotics and more recently selective breeding. An understanding of the genetic relationship between traits of economic importance for a widely cultured species such as Nile tilapia (Oreochromis niloticus) is needed, especially the relationship between disease resistance and growth. Francisellosis caused by intracellular Francisella orientalis, results in high economic losses for the tilapia industry not only due to death loss but also due to poor growth and feed conversion in chronically infected fish.

Methodology: Nile tilapia (Spring Tilapia® Strain; 10,167 fish records) were included in the analysis across four generations (G7B1-G10B1) following intramuscular challenge with F. orientalis doses ranging from 9.2 X 10^3 to 2.3 x 10^6 CFU/fish depending on generation. Pit tagged fish challenged for each generation were stocked into a single 5500 L tank and mortality pattern, date of death and time of death recorded. 103,685 harvest weight records (G1B1-G10B1) were used to simultaneously obtain estimates of variance components for estimating genetic correlations between traits.

Results: Analysing four generations, significant additive genetic variation was found for survival to F. orientalis challenge ($P<0.001$; Log-likelihood-ratio test) with an estimated heritability of $h^2 = 0.31 \pm 0.05$. Results of assortative mating groups confirmed that genetic gain could be obtained for survival to F. orientalis challenge since offspring of fish selected with high estimated breeding values (EBV) for survival exhibited higher survival to F. orientalis challenge (e.g., G10 High EBV mean survival = 80 % vs G10 Low EBV mean survival = 14 %). The genetic correlation between harvest weight and survival to F. orientalis challenge was low ($rg= -0.02 \pm 0.13$) and not significantly different from zero.

Conclusion: Survival to F. orientalis challenge was heritable and confirmed via assortative mating. Lack of correlation between F. orientalis resistance and growth suggests multi-trait selection is required to improve both traits.

Double the trouble: Aeromonas salmonicida subsp. salmonicida and Tenacibaculum maritimum co-infection in turbot (Scophthalmus maximus)

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Introduction: Turbot (Scophthalmus maximus) is a flatfish considered one of the most important marine cold-water species in european aquaculture. Aeromonas salmonicida subsp. salmonicida (ASS) and Tenacibaculum maritimum (TM) are two of the main bacterial pathogens in cold-water fish aquaculture, causing significant diseases and economic losses, as well as negatively impacting animal welfare. During a furunculosis outbreak on a commercial fish farm in Galicia, co-infection by ASS and TM was detected. Up to our knowledge, co-infections of fish with ASS and TM have not yet been reported.

Methodology: Fifty turbots displaying skin lesions suggestive of an infection by ASS were selected. Samples from skin and internal organs were collected. Spleen and kidney were tested by qPCR with ASS and TM primers. Routine histological processing was performed on skin lesions and internal organs. Immunohistochemistry procedures were carried out using two specific anti-ASS and anti-TM antibodies on samples chosen based on qPCR and histologic results.

Results: Histological study of the skin lesions showed typical ASS lesions consisted of a granulomatous dermatitis, with the occasional appearance of ASS colonies. In addition, internal organs of one fish showed extended necrosis foci with bacterial colonies. The qPCR studies detected the presence of ASS and TM DNA in the internal organs of the animals. Some fish tested positive for both pathogens in the same organ. The immunohistochemical study of these fish double positive by qPCR revealed that colonies found in the internal organs and skin lesions consisted of a mixed population of bacteria positive for both anti-ASS (in a higher proportion) and anti-TM (in a lower proportion).

Conclusions: Results confirmed a mixed infection by ASS and TM that caused clinical signs and lesions indistinguishable from those caused by ASS alone, and suggest that co-infections could be more common than expected. Research should be conducted to detect more cases of co-infection and deepen into the knowledge on the mechanisms of this interaction.

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Emergence of Streptococcus agalactiae Serotype VII as a Virulent Pathogen in Snakeskin Gourami (Trichogaster pectoralis)

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The expansion of farms and intensive farming practice of Snakeskin gourami (Trichogaster pectoralis) has led to the unexplained mortality and significant economic losses in fish farms in Thailand. In this study, we investigated the unusual mortality of snakeskin gourami at 22 farms in Central Thailand, where the moribund fish exhibited darkened skin, erratic swimming, exophthalmos, and haemorrhaging around the eyeballs, with a cumulative mortality rate of 20-45%. Necropsy findings showed an enlarged liver and anterior kidney, splenomegaly, haemorrhage in most internal organs, pericarditis, and brain congestion. Histopathology revealed haemorrhaging and congestion of blood vessels in the liver and enlarged blood vessels with mononuclear and lymphocyte infiltration in the meninges and cerebral parenchyma. The predominant bacterial isolate from moribund fish was Streptococcus agalactiae, which was further confirmed using mass spectrometry, multiplex polymerase chain reaction assay, pulse-gel electrophoresis, and serotyping. An experimental challenge using three representative isolates of S. agalactiae on snakeskin gourami resulted in clinical signs, gross lesions, and pathological changes, with a high mortality rate exceeding 60%. S. agalactiae was recovered from the spleen, kidneys, and liver of all challenged fish, highlighting its virulence in snakeskin gourami. Our findings provide important information on the potential spread of novel S. agalactiae serotype VII in snakeskin gourami in fish farms, suggesting the need for appropriate preventive measures and control.

Emergence and clonal expansion in Europe of Vibrio aestuarianus lineages pathogenic for oysters

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Crassostrea gigas oysters, with 4.7 million tons harvested per year, represent a considerable food supply worldwide. Since 2001 Vibrio aestuarianus has emerged as a pathogenic bacterium causing adult oyster mortality events in France and Ireland, and its impact on oyster aquaculture has increased in Europe since its reemergence in 2012. To better understand the evolutionary mechanisms leading to the emergence and persistence over time of this pathogen, we conducted a survey with national reference laboratories at the European scale for mollusk diseases. We analyzed genomes of V. aestuarianus isolated from multiple countries, since 2001, with or without bivalve mortalities, and from multiple environmental compartments. Using a combination of comparative genomics and population genetics approaches, we show that two oyster pathogen lineages have almost clonally expanded in Europe, after a probable recent selective bottleneck. Low mutation and recombination rates, as well as purifying selective pressure, have preserved virulent selected genotypes. Furthermore, we identified a specific cus-cop-containing island conferring copper resistance to V. aestuarianus that could have favored the emergence of V. aestuarianus pathogenic lineages adapted to oysters. Clonal expansion of a specialist bacterial pathogen is uncommon in marine environment and aquaculture. These results contribute to unravel its evolutionary story and provide new knowledge essential for better management of diseases affecting aquaculture.

Low salinity triggers a virulence genetic program in the generalist marine pathogen photobacterium damselae subsp. damselae

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Introduction: Facultative marine bacterial pathogens sense environmental signals so that expression of virulence factors is induced upon entry into hosts and downregulated during free-living lifestyle. One of the main environmental signals of this transition is the abrupt drop of NaCl concentration encountered upon entry host. In this study, we used transcriptome sequencing to compare the transcriptional profiles of Photobacterium damselae subsp. damselae (Pdd) a generalist pathogen that causes disease in diverse marine animals and fatal infections in humans, at NaCl concentrations that mimic the free-living lifestyle or host internal milieu, respectively.

Methodology: RNA-sequencing analysis was performed in a highly virulent strain of Pdd grown at 1% NaCl and 3% NaCl. Differentially expressed genes were considered when Fold Change (FC) values were < -1.5 or > 1.5 and a p-value adjusted by FDR≤ 0.05. Functional categories of DEGs were determined using COG and KEGG databases. Additional phenotypic studies, such as carbohydrate utilization and growth with antibiotics under different NaCl, were performed to support the RNA-seq results.

Results: Transcriptome analysis revealed that NaCl concentration shapes Pdd transcriptome and revealed 1808 Differentially Expressed Genes (DEGs) (888 upregulated and 920 downregulated in response to low-salt concentrations). Growth at 3% NaCl, mimicking the free-living lifestyle, upregulated genes involved in energy production, transport of compatible solutes, utilization of trehalose and fructose, and amino acid metabolism with strong upregulation of the arginine deiminase system. In addition, we observed a marked increase in resistance to antibiotics at 3% NaCl. On the contrary, growth at 1% NaCl triggered a virulence gene expression profile that maximized production of the type two secretion system (T2SS)-dependent cytotoxins damselysin, phobalysin P and a putative PirAB-like toxin, observations that were confirmed by analysis of the secretome.

Conclusions: Low salt triggers a virulence genetic program in Pdd by inducing cytotoxin abundance in the secretome fraction as well as other additional virulence-related functions, whilst marine salinity upregulates genes involved in energy production and global metabolism. The results obtained in the present study clearly demonstrates that NaCl constitutes a major regulatory signal in Pdd and expands our knowledge on the salinity-mediated response of this versatile marine pathogen.

Multiple Aeromonas strains associated with Red Skin Disease in Atlantic salmon in Scandinavian rivers

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Introduction: Since 2014, Atlantic salmon (Salmo salar) displaying clinical signs of Red Skin Disease (RSD), including haemorrhagic, ulcerative, and necrotic skin lesions, have been repeatedly observed in Swedish rivers. Although the disease has rapidly spread to new countries, including Norway, Denmark, Ireland, and the UK, no pathogen has been conclusively associated with RSD. This study aimed to investigate the presence of potential pathogens in wild salmon exhibiting RSD symptoms, using bacterial culture and molecular analysis.

Methodology: Nineteen adult Atlantic salmon with RSD symptoms were collected from rivers in Norway and Sweden between 2019 and 2021. The presence of fish pathogens (bacteria, viruses, parasites) was assessed using published and home-designed quantitative PCR assays. The isolation and identification of bacterial pathogens were performed by culturing fresh tissue from ulcers and internal organs, followed by 16S rRNA sequencing. The phylogenetic relationships between the isolated bacteria and previously characterized fish pathogenic species were investigated using Multi-Locus Sequence Analysis (MLSA).

Results: Several pathogens were identified by qPCR analysis. Some were frequently detected, such as bacteria belonging to the genus Aeromonas or Candidatus Branchiomas cisticola, and freshwater parasites, Saprolegnia and Ichthyobodo.

Additionally, cultures from fresh tissues displayed fast-growing bacteria that outcompeted other bacteria. They were identified as Aeromonas species. Phylogenetic analysis demonstrated that all salmon in this study were co-infected with at least two distinct Aeromonas species (A. piscicola and A. sobria).

Conclusion: Although several fish pathogens were repeatedly detected, only Aeromonas species are likely to be associated with the clinical manifestations of RSD. Saprolegnia or Ichthyobodo, also frequently present, are typically considered secondary pathogens, and are therefore unlikely to be the primary cause of RSD.
To fully understand the role of Aeromonas in RSD, additional field samplings and controlled challenge experiments with these bacteria will be required.

Immune response of gilthead seabream infected with wild and mutant nervous necrosis virus reassortants

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Introduction: Viral nervous necrosis (VNN) is a disease that affects farmed fish worldwide. Its etiologic agent is the nervous necrosis virus (NNV) (genus Betanodavirus, family Nodaviridae), currently classified into four species: SJNNV, RGNNV, TPNNV and BFNNV. In Southern Europe, natural reassortants between RGNNV and SJNNV have been isolated from VNN outbreaks affecting Senegalese sole (Solea senegalensis) and gilthead seabream (Sparus aurata). The aim of the present study is to identify differentially expressed genes (DEGs) involved in gilthead seabream response against different NNV reassortants. This research was funded by MCIUI and FEDER under Grant RTI2018-094687-B-C22.

Methodology: Gilthead seabream juveniles (5 g mean weight) were challenged by intramuscular injection using the wild-type (wt) strain Ss160.03, a highly virulent RGNNV/SJNNV reassortant isolated from Senegalese sole, and an attenuated mutant of this strain presenting substitutions in the amino acids 247 and 270 of the capsid protein. Head-kidney and brain samples were collected at 24, 48 and 72 h post-challenge (hpc). Viral multiplication was analysed by qPCR, and the evaluation of the immune response against the infection was carried out using an OpenArray® with 56 gene targets.

Results: At the end of the experiment, viral multiplication in seabream was higher for the wt reassortant ($1.2x10^8$ and $1.2x10^7$ RNA copies/µg RNA for wt and mutant strains, respectively). The number of DEGs detected through the experiment was also higher in the wt-infected animals, being the kinetic of expression different between the organs analysed (early deregulation of genes in head-kidney compared to belated expression in brain). The main differences in gene expression found between fish infected with both reassortants occurred at 72 hpc, being all DEGs up-regulated in wt-infected fish, compared to the downregulation observed within the mutant-infected animals. In addition, the expression of toll-like receptor 9 (tlr9), interleukin 1 beta (il1β), and interferon (ifn) was detected exclusively in brain samples at 72 hpc, and the endothelial leukocyte adhesion molecule (elam) in head-kidney samples at the same time point.

Conclusions: The different profiles of gene regulation detected at 72 hpc in gilthead seabream infected with both viruses could be related to the lower multiplication of the mutant strain.

European sea bass rtp3 genes: genomic characterization and transcription analyses

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Introduction: Fish RTP3, belonging to receptor-transporting protein (RTP) family, has been described as an interferon-α (IFN-α)-responsive gene. However, little information is available about fish rtp3 gene structure and the role of RTP3 proteins during viral infections. NNV (Nodaviridae family, Betanodavirus genus) is the causative agent of the viral nervous necrosis, the main viral disease affecting European sea bass (Dicentrarchus labrax) culture. Betanodaviruses have been classified into four species, although RGNNV is the only one causing high mortalities in sea bass.

Aim: The aim of the study has been to analyse the genomic structure of European sea bass rtp3, and its transcription profile after injection with LPS, poly I:C, or RGNNV infection.

Methodology: A partial sequence of seabass rtp3 gene was used as alignment sequence within European sea bass genome database. The located sequences were used as templates to design primers for full-length rtp3 sequencing. In addition, rtp3 X1 and X2 transcription was analysed in brain and head kidney by relative qPCR.

Results: European sea bass displays two rtp3 genes, X1 and X2, composed of two exons and a single intron (1007-bp and 888-bp long, respectively) within the ORF sequence. The full-length cDNA is 1969 bp for rtp3 X1, and 1491 bp for rtp3 X2. Several ATTTA motifs have been detected in the intron sequence of both genes, whereas rtp3 X1 also showed this motif in both untranslated regions. Regarding transcription analysis, the results revealed a significant level of rtp3 X2 transcription in brain and head kidney after LPS and poly I:C inoculation; however, the induction caused by RGNNV infection is much higher, suggesting an essential role of this protein in controlling NNV infections.

Conclusion: The present study contributes to further characterize the European sea bass response against RGNNV, being the first step in elucidating the role of sea bass rtp3 in the course of infections.

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Carp edema virus infection induces cortisol release and leads to temperature-dependent immunosuppression

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Introduction: Gill diseases have a significant impact on fish health and a very negative impact on aquaculture, mainly due to the multifunctional properties of the gills in fish physiology. Carp edema virus (CEV) is a large DNA poxvirus that primarily infects the gills of common carp (Cyprinus carpio L.), causing a highly contagious and fatal disease known as Koi Sleepy Disease (KSD). Our previous studies have shown that, when experimentally infected with genogroup IIa CEV, different strains of carp exhibit high (Amur wild carp - AS) or low (koi carp) resistance to this virus. The increased susceptibility of koi leads to severe impairment of gill function.

Methods: In the present study, blood parameters, viral load and expression of selected immune-related genes were determined in the gills of both carp strains. The experiments were carried out at two temperatures: 12 and 18 °C. In the case of the koi carp, we also introduced a salt rescue model based on the addition of 0.5% NaCl to the tank water, which prevents mortality of the fish.

Results: Nanoscale qPCR analysis of 40 genes showed that CEV induced a significant antiviral response in the gills of all infected fish groups compared to uninfected controls. We also found that the viral load was higher at 18°C than at 12°C in all fish groups studied, and at both temperatures the highest viral load was present in koi carp compared to the koi salt rescue group and AS carp. Interestingly, CEV-infected koi carp had higher glucose and cortisol levels and lower plasma sodium levels than the koi salt rescue group and AS carp at both temperatures. This clearly indicates that CEV infection in a susceptible strain correlates with high stress parameters that can trigger immunosuppression. Further analisis indicated that indeed the disease severity and higher stress response in koi were associated with immunosuppression, which was reflected in a down-regulation of T-cell responses.

Conclusion: Our data indicate a clear, temperature dependent, relationship between CEV-induced gill disease, stress and immunosuppression in susceptible koi carp.

Role of host immunopathology in the outcome of disease severity during cardiomyopathy syndrome in Atlantic Salmon

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Introduction: Cardiomyopathy syndrome (CMS) caused by piscine myocarditis virus (PMCV) is a severe cardiac disease in Atlantic salmon and one of the leading causes of morbidity and mortality in the Norwegian aquaculture industry. Previous research suggests a variation in individual susceptibility to develop severe disease, however, the link between individual immune response and the outcome of CMS is poorly understood.

Methodology: The study was performed as a large-scale controlled experiment of Atlantic salmon smolts from pre-challenge to 12 weeks post PMCV infection (wpi), during which fish were exposed to intermittent stressors. Heart transcriptome of high responders (HR) with atrium lesion histopathology score ‘4’ and low responders (LR) with score ‘0.5’ were compared by RNA sequencing (RNAseq). In addition, immune gene transcription at 6, 9 and 12 wpi was analyzed by high-throughput real time PCR. Based on RNAseq and PCR results, RNAscope in situ hybridization (ISH) was performed for visualization of IFN-γ- and IFNb-producing cells in affected heart tissue.

Results: A total of 1592 genes showed an increased expression in the heart of HR at 12 wpi. Significant increased immune gene transcription was found in HR at both 9 and 12 wpi, despite a decrease in PMCV transcription between these time points. These included key antiviral response genes and genes associated with a pro-inflammatory immune response including IFN-γ. Based on RNAseq results, an increased expression of chemokines CCL19, CXCL11, CXCL10, CXCL13 and CCL14-like could be responsible for attracting various leukocytes to the hearts of HR. Interestingly, an increased transcription of various pathogen pattern recognition molecules, such as TLR7 and TLR8, and increased type 1 IFN transcription in HR could indicate a presence of plasmacytoid dendritic-like cells, which are high-quantity IFN-secreting cells. Preliminary results from the ISH show the presence of IFN-γ positive cells in heart ventricle of HR.

Conclusions: Following PMCV infection, high responders show sustained host immunopathology and persistent antiviral responses. The immune gene transcription in the heart of HR indicate the presence of high-quantity IFN-secreting cells, which may be responsible for sustaining the antiviral signaling despite reduction in viral counts.

Characterization of carp antiviral immunity to aid development of cell culture infection models with a focus on fastidious carp viruses

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Global aquaculture production is one of the fastest growing food sectors with common carp (Cyprinus carpio) representing 5.2% of aquatic production. Health management is key to sustainable production. However, viral diseases represent a major threat to industry sustainability. Research and diagnosis are hampered by lack of cell lines that efficiently allow replication of several important viral pathogens of carp. The current research presented is to further explore the responses of two different carp cell lines following stimulation with the double stranded viral mimic, poly I:C. The Common Carp Brain (CCB) and Koi Fin (KF-1) cell lines were examined by gene expression (qPCR) analysis based on a panel of genes involved in antiviral responses. Given carp are tetraploid, multiple paralogues of individual antiviral genes were identified from the common carp genome. Analysis revealed significant paralogue specific responses in several key antiviral genes with key differences noted between each carp cell line. Overall, this work will facilitate the targeting of individual antiviral genes for gene editing studies to overcome the current lack of CyHV-3 variant and CEV infectivity and replication in carp cell lines. This work serves to expand the knowledge of differential antiviral immune responses within carp cell lines.

Circulating and neutralizing antibody responses to salmonid alphavirus (SAV) in Atlantic salmon immunized against pancreas disease (PD) using licensed vaccines

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Pancreas disease (PD), caused by salmonid alphavirus (SAV), is an economically important disease affecting seawater reared Atlantic salmon in parts of Norway as well as Scotland and Ireland. Farming of portion sized rainbow trout in freshwater is also impacted by PD in parts of Europe. Seven different subtypes of SAV (SAV1-SAV7), have been described based on the nucleic acid sequences encoding the E2 glycoprotein and the non-structural protein nsP3. In a cross-neutralization study, extensive serological cross-reactivity has been demonstrated between the SAV1 through SAV5 subtypes. For alphavirus in general, neutralizing antibodies and innate immune responses play a pivotal role in protection against disease.

Blood plasma was collected during different laboratory experiments and a field study from fish immunized with different vaccines against PD, including a DNA vaccine and different inactivated whole-virus oil-adjuvanted vaccines. The study designs included either PIT-tag marking of fish (laboratory) or adipose fin-clipping as group marking (field study) with the different treatment groups otherwise cohabitating under identical conditions in the same rearing units. The analysis included both an ELISA, coating the plates with infective SAV subtype 3 (SAV3), and a neutralization antibody end-titer test using CHSE-214 cells and varying SAV subtypes. The antibody response profiles differed significantly between the analytical methods and vaccine technology platforms used. The results will be presented and discussed with reference to protective immunity.

Surveillance of fish pathogens during complex disease outbreak in RAS by high-throughput microfluidic qPCR

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Introduction: Piscine orthoreovirus genotype 3 (PRV-3) was first reported in Denmark in 2017 in association with disease outbreaks with high mortality in rainbow trout (Oncorhynchus mykiss). In 2017-2019, we conducted a surveillance program for PRV-3 in Danish farms, revealing that PRV-3 is widespread. Importantly, disease outbreaks with mortality were only observed in recirculating aquaculture systems, and primarily during the winter. The effect of water temperature has been confirmed experimentally: When comparing PRV-3 infected fish at different temperatures, it was shown that virus levels increased at low temperatures (5°C) compared to higher temperatures (12° and 18°C) as well as a tendency towards worse heart pathology. Field investigations highlight presence of other pathogens during PRV-3 associated disease outbreaks.

In order to deepen the understanding of PRV-3 associated disease, we developed a high-throughput qPCR method (Fluidigm) for simultaneous detection of multiple pathogens. Field samples were collected from a farm with history of PRV-3 associated disease.

Methodology: From March to September 2022, monthly sampling was performed from 10 fish from the same batch. Sample collection consisted of 1) heart, spleen and kidney in RNAlater, 2) gills in RNAlater, 3) blood, 4) kidney swab, 5) heart, spleen and kidney in medium, and 6) water. Swabs and tissue were tested by gold standard methods for virology and bacteriology, and tissue collected in RNAlater along with filters from water were tested by standard qPCR and Fluidigm.

Additionally, production data was recorded during the seven months, including weight, feeding, disease outbreaks, treatments, and water quality parameters.

Results: Bacteriological examination revealed recurrent presence of Yersinia ruckerii and occasionally Flavobacterium psychrophilum. Additionally, PRV-3 was detected by qPCR in connection with significantly increased mortality. The disease outbreak lasted five weeks with an overall mortality yielding more than 2 ton. A comprehensive diagnostic overview combined with the production data will be presented.

Conclusions: PRV-3 associated disease outbreaks in RAS are complex, and sustained by co-infections and possibly stressors induced by farm practices. Interestingly, mortality increased significantly after fish transfer, and was not mitigated by water treatment with salt. Additionally, PRV-3 can be detected by qPCR before disease outbreaks in the prodromal phase.

Development of a high-throughput chip technology to monitor water quality, off-flavour and diseases in recirculated aquaculture systems (RAS)

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Recirculated aquaculture systems (RAS) represent an ambitious approach that can contribute to a more sustainable land-based fish production by optimizing water resources and controlling discharge of nutrients. Despite an increased interest since the 70's, several crucial challenges remain, such as disease outbreaks, maintenance of high water quality, and presence of microbial off-flavour compounds within the facilities. In the Innovative Training Network (ITN) project RASOPTA – Safeguarding future production of fish in aquaculture systems with water recirculation (years 2021-2025), funded by EU Horizon2020 Marie Skłodowska-Curie Actions, these three subjects are scrutinized by 12 PhD students enrolled at universities throughout Europe. The purpose is to optimize conditions in RAS industries. In the project, a chip-based, high-throughput PCR Fluidigm platform will be developed with the aim of rapid mapping of pathogenic microorganisms for early warning of diseases. In addition, microbial water quality indicators will be identified and included in the chip. Finally, to identify emerging off-flavour episodes in the RAS farms, genes encoding the off-taste compounds geosmin and 2-MIB will also be quantified using the chip technology. The chip can hold 48 different assays combined with 48 different water samples generating 2304 data points thus achieving rapid detection of various indicators in a large number of samples. The chip represents a robust and powerful tool capable of monitoring water quality, off-flavour and disease conditions in the RAS facilities allowing improved management before conditions become severe and costly hurdles. Taken together, PhD students, academic institutions, industrial partners and state of the art technologies may bring forward decisive knowledge for the sustainable support of RAS in the future.

Non-invasive monitoring of pathogens in Atlantic salmon (Salmo salar L.) sea cages during mandatory sea lice counting

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Introduction: During monitoring programmes of pathogens by veterinary authorities and the industry in cultured Atlantic salmon (Salmo salar L.), large numbers of fish are sacrificed. This is problematic with regards to both fish welfare and economy and non-invasive screening methods should be explored. In this study, we investigate the potential of replacing fish tissue samples with seawater samples for detection of a selection of salmon pathogens.

Methodology: Faroese regulations require bi-weekly lice counting from 10 representative salmon from every net pen at sea. The fish are taken into a tank with seawater for anaesthetisation and counting of lice. To examine whether seawater from these tanks represents disease status of the fish and can be used for non-invasive disease screening, we tested sites with ongoing disease with cardiomyopathy syndrome (caused by the RNA virus piscine myocarditis virus, PMCV), amoebic gill disease (caused by the protozoan ectoparasite Neoparamoeba perurans), and Ichthyobodosis (caused by parasitic flagellates belonging to the genus Ichthyobodo). Extended sampling was conducted for PMCV, where samples were also taken directly from the net pens. Screenings were accomplished using real-time PCR.

Results: For all pathogens, when samples were taken from water with high biological load, as confirmed by analyses of the endogenous reference gene in Atlantic salmon, elongation factor 1 α, results based on seawater were a good proxy of results based on tissue (or standard gill and sideline swabs). The anaesthetic water from the lice counting tank represents water with high biological load and accordingly, Faroese salmon farming companies have adopted routine screening of these seawater samples for an array of pathogens.

Conclusion: We show for a broad representation of farmed salmon pathogens (RNA virus as well as parasitic amoebae and flagellates) that sampling of fish tissue can be substituted by non-invasive seawater sampling. All major salmon farming countries conduct regular mandatory sea lice counting and we argue that these present excellent opportunities for implementing non-invasive screening procedures for salmon pathogens as taking a water sample at this point would add limited extra work and cause no additional harm to the fish.

Basibranchial structure affecting cardiac morphology in Atlantic salmon (Salmo salar L.)

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Introduction: During the last few years, a cartilaginous blunt spine affecting the ventral part of the pericardium and the ventricle has been observed in routine diagnostic health surveys of Atlantic salmon parr. The prevalence is not known, but it is a common finding in our diagnostic material.

Methodology: Mid-sagittal sections of farmed Atlantic salmon fry revealing the heart, pericardial cavity, and adjacent structures were stained with Hematoxylin and Eosin and evaluated histologically. For reference, ten wild parr of approximately 4,5 cm to 14 cm from two rivers in Norway were histologically examined using the same method as described above.

Results: A characteristic cartilaginous, blunt spine of variable length and thickness has been observed to push dorsally towards the ventral wall of the parietal pericardium, thereby forming a protrusion into the pericardial cavity. Depending on the height of the spine, this will also affect the ventricle forming an indentation into the myocardium. The indentation may be seen along the entire length of the ventral part of the ventricle, but is most frequently seen halfway between the bulbus attachment and the apex. In cases where an indentation is made into the ventricle, a localized inflammatory response may be seen. A similar cartilaginous protuberant structure was observed in all samples from wild fish, but did not appear to affect the heart in these individuals. In production-size farmed Atlantic salmon (1-3 kg), a marked inward thickening in the ventral part of the compact myocardium and chronic epicarditis is often seen histologically, corresponding to the location of the cartilaginous spine. In wild fish, the spine did not appear to affect the heart morphology, a finding that may support the theory of disproportional or too intensive growth in farmed fish.

Conclusion: The spine is most likely a part of the basibranchial bone which is the cranioventral medial connection behind the tongue upon which the gill arches are hinged. The indentation made by the spine on the heart could be the result of disproportional growth of the heart and basibranchial structures. The condition may be assumed to affect the heart function, particularly during stressful and physical challenges.

Longitudinal study of different production regimes revealing risk factor for developing nephrocalcinosis in Atlantic salmon (Salmo salar L.)

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Nephrocalcinosis is a growing concern for fish health and welfare in the salmon industry in Norway. Although several risk factors have been identified previously, different technological innovations in the aquaculture industry have made it timely to revisits predisposing factors.

A longitudinal study from fry to smolts in six different production facilities under normal production conditions was performed, spanning from flow-through to conventional- and zero water exchange recirculating aquaculture systems (RAS). Macroscopic and histological scoring with a semi-quantitative scoring model were used to assess kidney health and disease development, and these findings were compared with available production parameters.

Histology identified nephrocalcinosis at a much earlier stage than macroscopic evaluation, hence proving itself as a useful tool in elucidating time of initiation of disease development. As a general finding, nephrocalcinosis occurred early in the production cycle. Comparing the semiquantitative nephrocalcinosis score with production parameters, high salinity and smoltification strategy emerged as important risk factors. Different morphologies of the precipitation were identified and described.

Farming conditions need to be adapted to the physiology and natural habitats of the species in question. Atlantic salmon parr seems less adapted to brackish water. Although nephrocalcinosis can have many causes, we believe that medium range salinity poses a risk for overload of the nephrons causing dystrophic mineralization of epithelium and nucleation centra for further disease development. Proper and timely smoltification is crucial for proper sea-water adaptation. Histology proves to be a valuable tool to describe early lesions and further disease development; the semi-quantitative scoring scheme makes it easier to see trends, and further characterization of the different precipitations may shed light on composition, cause, and possible prevention of this emerging disease complex.

Ring trial of qPCR assays for Piscirickettsia salmonis in Atlantic salmon in 11 Chilean laboratories identifies test-accuracy deficiencies

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Real-time PCR (qPCR) testing is an essential component of early detection systems for Piscirickettsia salmonis infection in Atlantic salmon farms in Chile. Variation and lack of harmonization of qPCR testing systems (e.g. primers/probe, RNA/DNA as target, and extraction methods) may contribute to interlaboratory variation in qPCR results and an increased frequency of false-negative and false-positive outcomes. The purpose of the interlaboratory ring trial was to compare qPCR results from 11 laboratories routinely testing Atlantic salmon for P. salmonis as part of a national control program in Chile. The standardized panel of 14 samples included duplicates of 3 concentrations of P. salmonis in homogenized head kidney, LF89 and EM90 bacteria and 2 negative controls (a blank and a suspension of Flavobacterium psychrophilum spiked into head kidney). The sample order was randomized across labs, samples were tested blind and analyzed without knowledge of source lab. All participating labs were running Taqman assays with varying protocols and all but one had ISO-17025 accreditation. Four labs used primers and probe reported by Corbeil et al. (2003), 4 used their own design, and 3 used those reported by Karatas et al (2008). Six labs used RNA as template and 5 used DNA as template. Of the 11 laboratories, 8 (72.7%) had at least one incorrect result out of 14 tested samples. Low concentration samples (CT of about 30) were more often incorrectly classified as negative by RT-qPCR (3/6 labs) than by qPCR (0/5). Six (54.5%) labs had at least one false-positive result indicating that cross-contamination was likely during processing. Based on our study's findings, we recommended that (1) harmonizing qPCR protocols would reduce inherent variation associated with use of different assay components, and (2) standard-operating protocols for qPCR testing (including use of appropriate controls) should be reviewed for laboratories with false-positive results to ensure that they meet minimum quality management system standards. Because Renibacterium salmoninarum produces similar gross signs to P. salmonis including white nodular lesions in the kidney we also proposed that a ring trial that considers the accuracy of detection of both agents by molecular methods would be a logical next step.

Using eDNA-based approach to improve knowledge of bivalve parasite life cycles

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Environmental DNA approaches are increasingly used to detect microorganisms in environmental compartments, including water. They show considerable advantages to investigate non-cultivable microorganisms such as the bivalve protozoan parasites Marteilia refringens, Bonamia ostreae and Haplosporidium costale.

Marteilia refringens and Bonamia ostreae were initially detected in flat oysters (Ostrea edulis) in France in 1968 and 1979, respectively. The transmission of B. ostreae beween oysters is direct but this does not exclude the potential existence of reservoirs. In contrast, M. refringens can not be transmitted directly between oysters. The copepod Paracartia grani appears as the best intermediate host candidate although the parasite life cycle still remains unelucidated. Previously considered exotic to Europe, Haplosporidium costale was recently detected in cupped oysters (Magallana gigas) in France. The route of transmission as well as the host range of this parasite are not well defined.

Different studies were carried out using eDNA -based approach to improve knowledge of the environmental distribution of these protozoan parasites. Through integrated field studies, we investigated the seasonal dynamics of these parasites not only in oysters, but also in planktonic and benthic compartments. After optimizing Real Time PCR assays to properly detect parasite DNA in water and sediment, the presence of the parasites was tested in nano, micro and mesoplancton, sediment and associated meïofauna. Results from these studies emphasize the different parasitic strategies used by these protozoan species, M. refringens and H. costale showing a wider environmental distribution than B. ostreae which seems exclusively associated to flat oysters. Our findings highlight the key role of planktonic and benthic compartments in some parasite life-cycles. More generally, we provide here a method that could be useful not only to further investigate non cultivable pathogen life-cycle, but also to support the design of more integrated surveillance programs.

Francisella halioticida's virulence towards the blue mussel Mytilus edulis

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Mortality events of marine mussels (Mytilus spp.) occur in France since 2014. After several rejected hypotheses, a new one arose in 2019 upon the detection of DNA of the bacterium Francisella halioticida in mussels. Detection was achieved in animals from Brittany, Normandy and North-Hauts de France (France) and soon after in England and Netherlands. Already reported in Asia as pathogen of Yesso scallops (Mizuhopecten yessoensis) and giant abalones (Haliotis gigantea), F. halioticida became the main suspect in these mortalities. Several strains of F. halioticida have been isolated in France from moribund mussels and two type strains have been characterized, FR21 and FR22. This study aims to determine the pathogenicity of all isolates towards both juvenile and adult mussels.

The isolated strains were priorly characterized through phenotypic and genomic analyses. Juvenile and adult mussels were injected with each isolate at a high dose (≥106 CFU/mussel). Mortality was monitored twice daily for 11 and 15 days, respectively. Isolates showing the highest virulence were selected for LD50 analysis. Serial dilutions from 103 to 107 CFU/mL were injected in juvenile and adult mussels and monitored for 30 days. Gills from a selection of moribund, surviving and control mussels was plated on specific media and submitted to specific PCR for F. halioticida.

Phenotypic and genomic comparison highlighted differences between the isolates. From the six strains tested, two showed high morbidity with mortality >90% in 7 days in both adults and spats. LD50 for these isolates were at 104 CFU/mussel in 30 days and one isolate showed a LD50 of 103 CFU/mussel in spats. A difference of DNA load was observed between moribund and surviving mussels. Re-isolation was successful for most moribund animals while surviving and control ones did not show growth of F. halioticida.

This study showed that virulence of F. halioticida towards mussels seems to be strain-dependant and that virulent strains show high potential for causing high morbidity in both spat and adult mussels. Virulence factors analysis among high and low virulent isolates is required to start to understand the factors required to predict the high pathogenicity in F. halioticida towards mussels.

Long term study of the impact of the parasite MSX (Haplosporidium nelsoni) on Eastern oyster (Crassostrea virginica) in the Maine

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Introduction: Oyster farming represents a significant industry for the state of Maine with the Damariscotta River Estuary accounting for around 70% of the states oyster production. In 2010 the haplosporidian parasite of Eastern oysters (Crassostrea virginica), MSX (Haplosporidium nelsoni), appeared in the Damariscotta River Estuary, resulting in significant losses to commercial oyster operations. In subsequent years we have followed the presence of the parasite in both farmed and natural bed populations.

Methods: We used published PCR methods to track prevalence in both farmed and natural bed populations. Samples were collected in 2012, 2014, 2016 and 2019. We also surveyed potential intermediate hosts for presence of the parasite and finally we monitored the population genetics of both farmed and natural bed populations, using published microsatellite information, from 2012-2016, to see what impact the parasite may have had.

Results: In 2012, 2014 and 2016 studies on commercial and wild oyster populations indicated a significant prevalence of the parasite despite the introduction of MSX resistant strains. Our most recent survey in 2019 indicated the parasite was almost completely absent, with only 3 positive samples. This was despite testing over 600 oysters that were sampled over an eight-month period, and from several locations. The survey for potential intermediate hosts indicated that tunicates carried the highest prevalence. Our study of the population genetics showed how the populations remained distinct from each other, despite the infectious pressure.

Discussion: The parasite appears to have vacated the estuary. Why that has occurred is unclear. Suggestions of parasite suppression relating to freshwater inputs or temperature impacts, seem in unjustified at this time. We will continue to monitor for the parasite.

Study of susceptibility to perkinsus olseni on three commercial clam species

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Introduction: The protozoan parasite Perkinsus olseni is a global threat to bivalve mollusks, including economically valuable species such as Ruditapes decussatus (RD), Ruditapes philippinarum (RP), and Venerupis corrugata (VC) the most economically valuable species of clams in Europe. The deleterious effect of Perkinsus olseni in growth performance as well as reproduction capacity of the clams was largely documented.
This study aimed to investigate the response of these three clam species to the parasite by exposing their seeds (5-7 mm length) with two different concentrations of parasite to identify the most susceptible species to the disease.

Material and methods: Two different concentrations of parasite´s hypnospores were added to tanks (Low Infection (LI) treatment $5{\cdot}10^5$ cells; High Infection treatment (HI) $5{\cdot}10^6$ cells). After 48 hours, water was renewal and the progression of the infection was followed for one month. Samples were taken over four time periods (48 hours, 7, 14 and 30 days post exposure) to assess short and longer-term responses. Absolute qPCR was used to identify and quantify the presence of the parasite with specific primers.

Results & discussion: The prevalence of infection showed differences among species in the LI treatment. While in HI treatment all clams became infected, in LI treatment the prevalence indicates a higher susceptibility of RD to the disease (98.07% prevalence), followed by RP (74.9% prevalence) and VC (69.64% prevalence). Regarding the parasite load of infected individuals, results showed significant differences in the infection intensity of each clam species to the parasite, with RP showing higher parasite load throughout the experiment. Conversely, VC demonstrated a lower susceptibility to the parasite, with lower parasite load observed at short and long term while RD showed an intermediate response, falling between the other two species but without differences with VC.

In conclusion, these findings highlight the variable response of three clam species to P. olseni, suggesting that VC may be less susceptible to the parasite, while RD may be the most susceptible, but reaching lower parasite load than the infected RP clams. This study provides valuable information for understanding and managing the impact of P. olseni on clam populations.

Antibiotic resistance in the Pacific oyster industry in France: a first picture in a local production area, the Charente Maritime

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Antibiotic resistance is a major concern in human and animal health, with an increasing number of studies looking at this phenomenon at the environmental level. Although antibiotic resistance is monitored through various networks in France, it remains poorly considered in aquaculture, particularly in shellfish farming. Nevertheless, this production is highly exposed to environmental hazards, in particular to anthropogenic pollution including antibiotics through wastewaters. Thus, a study was conducted on Magallana (ex Crassostrea) gigas, in Charente Maritime, the main oyster production area in France, to provide an initial view of the potential existence of antibiotic resistance in wild populations. Site selection was guided by the outputs of a hydrodynamic transport model (MARS3D) to estimate their level of exposure to wastewaters. Six populations, including three more exposed to anthropogenic inputs, were sampled once per season during one year (i) to detect specifically Escherichia coli, resistant to third generation cephalosporins, and (ii) to characterize the antibiotic resistance profile of some Vibrio spp isolated from oysters. Resistance profiles were established using the disc diffusion method for Vibrio or using a specific medium for E. coli. During the survey, no E. coli resistant to third generation cephalosporins were detected in wild populations. Different Vibrio species were isolated and some of them, such as Vibrio alginolyticus, showed resistance to Beta-lactams and quinolones. Resistance profiles of Vibrio were similar among populations whatever their origin more or less exposed to anthropogenic inputs. Further studies will be necessary to obtain an overview of antibiotic resistance in the oyster industry, although ours did not detect E. coli resistant to third generation cephalosporins, as well as did not reveal the emergence of new resistance profiles in Vibrio in comparison with Vibrio strains isolated before 2010.

Full rRNA arrays differentiate between Marteilia refringens and Marteilia pararefringens and identify new/verify current variable regions of interest for diagnostics

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Introduction: The separate species status of the paramyxid parasitic protists Marteilia refringens and Marteilia pararefringens has been a topic of debate for the past two decades. The two parasites were originally classified as distinct species before being 46ynonymized into a single species comprising two 'types': M. refringens M-type and O-type. The two types were redesignated as distinct species (M. refringens and M. pararefringens) in 2018 based on consistent differences in longer regions of ribosomal RNA (rRNA) array sequences, but from only a limited number of samples.

Methodology: Here we generate full rRNA arrays using long-range PCR and next generation sequencing (NGS) from a larger number of M. refringens and M. pararefringens infections spanning the geographical range of their distribution to (1) reinforce evidence that M. refringens and M. pararefringens are genetically distinct species, (2) demonstrate consistent differences within the current diagnostic regions and (3) determine whether newly revealed variable regions outside of the current diagnostic regions could be used as complementary informative marker regions.

Results: Phylogenetic analysis of the transcribed region of rRNA arrays (ETS-18S-ITS1-5.8S-ITS2-28S) provides maximal support for, and separation of M. refringens and M. pararefringens across investigated geographical ranges. Phylogenetic analysis of the most variable regions of the array also supported the two separate Marteilia species.

Conclusions: Robust marker regions that delimit M. refringens and M. pararefringens as two separate species will allow the two parasites to be studied independently from one another to investigate the host preference, pathogenicity, and lifecycle of each parasite.

Single-cell RNA-SEQ of head kidney leukocytes from two rainbow trout isogenic lines with contrasting resistance to viral and bacterial infection

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Infectious diseases represent one of the largest hindrances to the continued growth and welfare standards of the aquaculture industry. We have previously described isogenic rainbow trout lines with contrasted resistance to Viral Hemorrhagic Septicemia Virus (VHSV) and to Flavobacterium psychrophilum (Fp), two key pathogens in European aquaculture. To better understand mechanisms behind genetic resistance, we undertook a global analysis of immune cell subsets within the head kidney of these two rainbow trout lines. Our single-cell RNA-seq data of unstimulated control fish produced 34 clusters that could be separated into 6 major cell lineages: B-cells, T-cells, granulocytes, monocytes, thrombocytes and erythrocytes. All cell lineages from both fish lines displayed closely associated clusters except in granulocytes where a clear separation of the cell clusters from the different isogenic fish lines could be observed. Further investigation revealed clear differential expression of a gene subset between granulocytes of the two isogenic lines, comprising a number of immune genes. Interestingly, several of these differential genes are paralogs with contrasted levels of expression. We then performed a follow up single cell RNA-seq experiment examining the impact of VHSV infection on these defined cell lineages and corresponding gene expression in the same resistant and susceptible rainbow trout families. This study represents an initial step to help further the understanding of how resistance to viral and bacterial pathogens is conferred within rainbow trout immune cells.

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Immune response of DNA vaccinated-gilthead seabream against LCDV-Sa infection: relevance of the inflammatory process

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Introduction: Lymphocystis disease is one of the main viral pathologies affecting cultured gilthead seabream (*Sparus aurata*) in the Mediterranean area. Recently, we have developed a DNA-vaccine based on the major capsid protein (MCP) of the *Lymphocystis disease virus 3* (LCDV-Sa). The immune response triggered by LCDV-Sa or the vaccine in gilthead seabream has been previously study. In infected fish, the response is characterized by a slightly and transitory activation of type I IFN system and a lack of systemic inflammatory response, while a systemic inflammatory process and a humoral adaptive immune response have been observed for vaccinated fish. In the present study, a comprehensive evaluation of immune-related gene expression in vaccinated fish after viral infection has been carried out to identify immune genes involved in the vaccine-induced protection. This work was funded by Junta de Andalucia and FEDER (Grants P12-RNM-2261 and UMA20-FEDERJA-076).

Methodology: Gilthead seabream specimens (5 g mean weight) were distributed into 3 experimental groups; two of them were inoculated with the vaccine and the empty plasmid at 0.1 µg/g fish dose, respectively, whereas fish in the control group were inoculated with PBS. Thirty days post-vaccination, fish were intramuscularly injected with the virus at 10^6 $TCID_{50}$/fish. Samples of head-kidney, spleen, intestine and caudal fin from 6 fish per group were individually collected at 24, 48 and 72 h post-challenge. The expression and quantification of viral DNA in fins of fish challenged with LCDV-Sa were carried out by a qPCR assay targeting a viral structural gene (putative myristoylated membrane protein, MMP). Immune response was studied by an OpenArray® platform of 56 gene targets.

Results: The global effect of vaccination was a significant decrease of viral replication in vaccinated fish compared to fish in the control group, and the differential expression of immune genes related to viral recognition (*tlr9*), humoral and cellular response (*rag1* and *cd48*), inflammation (*csf1r*, *lam*, *il1β*, and *il6*), antiviral response (*isg15*, *mx1*, *mx2*, and *mx3*), cell-mediated cytotoxicity (*nccrp1*), and apoptosis (*prf1*).

Conclusions: The exclusive modulation of the immune response provoked by the vaccination seems to control the progression of the infection in the LCDV-Sa challenged gilthead seabream.

Development of a vaccine against CMS

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Piscine Myocarditis Virus (PMCV) is the causative agent for Cardiomyopathy syndrome (CMS) in Atlantic salmon. CMS is a serious cardiac disease affecting Atlantic salmon during sea water stages. There is currently no available vaccine against the disease in the market. PHARMAQ will present an update from the work with developing a DNA vaccine against CMS. The presentation will include description of the laboratory challenge model and efficacy data of three vaccine candidates challenged up to 21 weeks post immunization, both supported by PCR and histopathology data. We will also present results from a biodistribution study of vaccine candidates where the plasmid is monitored in several internal organs for 12 weeks post vaccination.

Initial work for the development of an mRNA vaccine against infectious salmon anemia

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Introduction: The success of mRNA vaccines against Covid-19 has generated considerable interest in utilizing this technology for other viral diseases. Most mRNA vaccine research has been conducted in mammals; but it could potentially be implemented in aquaculture to combat viral diseases such as infectious salmon anemia (ISA). The objective of this study is to design and test an mRNA vaccine against ISA including assessing its effectiveness in preventing disease. The study started recently and are in the initial phases focusing on method optimization and testing proof of principle.

Methodology and results: To test the principle, i.e., to ensure that in vitro transcribed mRNA can be transfected and express proteins in salmon, we started out with the generation of nucleoside-modified mRNA encoding a reporter protein (EGFP). The mRNA sequences have a 120 nucleotide Poly-A tail, Cap1 modification and incorporation of N1-methylpseudouridine.In addition, the ORF sequences are framed in by either naturally occurring untranslated regions (UTRs) of ISAV or by UTRs from a putative salmon β-globin sequence, to determine which of these UTR that yields the most efficient protein expression. Transfection of mRNA induced protein expression in salmonid cell cultures, and the transfection efficiency was better for mRNA than for corresponding plasmid DNA.

The mRNA expressing EGFP was encapsulated in lipid nanoparticles (similar lipid composition as Moderna's COVID-19 mRNA vaccine) and intramuscularly injected in Atlantic salmon to assess the efficacy of mRNA delivery in vivo.

Detection of protein expression in salmon would demonstrate the ability of the lipid nanoparticles to efficiently deliver mRNA.

Further, mRNAs encoding the ISAV hemagglutinin-esterase and the fusion glycoprotein will be used in challenge experiments against ISA. The aim is to obtain protection against disease, and possibly against infection.

Conclusion: The study is ongoing, and the results will be presented at the meeting.

Keywords: Atlantic salmon, mRNA vaccines, infectious salmon anemia

Funding: FHF – Norwegian Seafood Research Fund, # 901746. SINTEF funded LNP formulation and encapsulation of mRNA.

Moritella viscosa in Canada: isolate characterization and challenge studies

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Winter ulcer disease caused by Moritella viscosa is a major problem for the Canadian salmon farming industry as it causes animal welfare challenges and significant economic losses due to mortality and downgrades at slaughter. All commercial vaccines currently available in Canada with a winter ulcer disease component contain a classic/typical strain of this pathogen. Field use of commercial vaccines in Canada has indicated moderate to good protection against the variant strains identified.

In this study we have characterized several Canadian field isolates from multiple regions in both Western and Eastern Canada using genetic sequencing as well as antibody-based methods. Furthermore, we have assessed the virulence

of some of these Canadian isolates and assessed the efficacy of commercial multivalent vaccines against Canadian Moritella viscosa field isolates in laboratory challenges.

Our results show that Canadian Moritella viscosa is serologically similar to the strain used in the multivalent core vaccines despite there being genetic differences. Furthermore, Canadian Moritella viscosa was shown to be virulent, but displayed some regional variability. Lastly, we found that commercial multivalent vaccines do elicit protection in challenges with Canadian variant Moritella viscosa.

The use of experimental animals in this study was approved by the Norwegian Food Safety Authority. The study was also approved by the internal Zoetis Animal Ethics Committee

Immune memory development, AID expression, and affinity maturation revealed in oil adjuvant vaccinated giant groupers (Epinephelus lanceolatus)

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Teleostei is the most primitive infraclass of vertebrates with a functional adaptive immune system similar to mammals, based on major histocompatibility complexes, T cell and B cell receptors. Vaccination exploiting this trait has been effective in preventing diseases in aquaculture. In addition to the specific antigen, effective vaccines comprise an adjuvant to improve and prolong protective immunity, but the mechanisms of adjuvant effects are not well understood. Here we compare the effects of two water-in-oil adjuvants (Montanide ISA 763 A VG and ISA 660 VG) on adaptive humoral immunity in a warm water perciform fish, giant grouper (Epinephelus lanceolatus) using a hapten-carrier model deliver by intraperitoneal injection. During a 142-day prime-challenge experiment, fish were injected with DNP-KLH as aqueous or water-in-oil emulsions and were periodically challenged with DNP-KLH antigen to stimulate secondary response. Fish serum antibody analysis showed that adjuvanted vaccines were able to stimulate an elevated and prolonged primary antibody production post vaccination. Only fish injected with adjuvanted antigen developed immune memory to DNP, evident by the magnified and rapid secondary antibody responses. Further, groupers injected with antigen in adjuvant emulsions produced antibodies significantly higher avidity than those injected with aqueous DNP-KLH. There was also a strong positive correlation between serum antibody avidity and the expression of activation-induced cytidine deaminase (AID) expression in grouper head kidney. This is first evidence connecting the binding performance of IgM with AID expression. As Teleostei do not have a lymphatic system based on lymph nodes and germinal centres, we examined the oil-adjuvant-induced granulomas in the peritoneum. Microscopy with immunohistochemistry revealed progressive degradation of the emulsion and the presence of IgM/B cell receptor together with melanomacrophage pigments within the granulomas. AID and DC expressions were also detected by qPCR. It is possible that germinal centre-like activity is enabled ad hoc at the site of injection during prolonged antigen release within the emulsion-dependent granulomas.

Conserved Pro-inflammatory transcriptomic response in salmonids similarities and differences between closely related species

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Infectious diseases represent one of the most pressing threats to modern aquaculture on a global scale. Control of disease outbreaks supports maintaining fish health and welfare, essential for a sustainable and profitable farmed fish production. The early innate immune response conditions the outcome of infection. Here we advance previous work to profile responses both in vivo and in vitro within head kidney leukocytes to an immunological challenge (heat-killed Vibrio anguillarum) that drives a pro-inflammatory antibacterial response in Atlantic salmon and rainbow trout. This whole transcriptome analysis by RNA-seq revealed a core set of cytokines involved in the innate immune response against bacterial stimulation. We analysed all paralogs of up and down regulated cytokines belonging to IL1, TNF, and type I/II subgroups. We found key differences in the expression of orthologous genes/gene sets between salmon and trout. Data from this investigation will increase our understanding of the evolution of the innate immune response in salmonid species in relation to their lineage-specific gene retention and expression following the most recent salmonid whole genome duplication.

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Characterization of immunoglobulin T in Asian Seabass (Lates calcarifer) and mucosal immune response to viral infection

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Introduction: Mucosal-associated immunoglobulin T (IgT) plays a critical role in the protection of teleost fish against infection, but mucosal IgT of economically important aquaculture species unique to Southeast Asia remained largely unknown. This study describes the identification of IgT from Asian Seabass (ASB) and its role in the mucosal immune response to nervous necrosis virus (NNV) infection.

Methodology: The secretory IgT (sIgT) gene of ASB was identified and expression profile was analyzed in a series of mucosal and non-mucosal tissues by real-time quantitative PCR for healthy and NNV-infected ASB. Further, anti-CH2-CH4 IgT antibodies were generated to validate the presence of IgT-positive cells in the ASB gill and intestine. Also, localized secretion of IgT was measured in the intestines and gills in response to a viral infection.

Results: IgT of ASB possesses the characteristic structure of immunoglobulin with a variable heavy chain, four CH domains (CH1-CH4), a secretory tail and conserved cysteine and tryptophan residues necessary for the formation of intra/interchain disulfide bonds and proper immunoglobulin architecture. The constitutive expression of ASB IgT was characterized in different tissues and in response to nervous necrosis virus NNV infection. The highest basal expression of secretory IgT (sIgT) was observed in the mucosal and lymphoid tissues such as the gills, intestine and head kidney. Following NNV infection, IgT expression was upregulated in the head kidney and mucosal tissues. In addition, immunohistochemical analysis revealed that mouse anti-CH2-CH4 IgT antibody recognizes IgT-positive cells in the ASB gill and intestinal tissues. Also, a significant increase in localized IgT was found in gills and intestines of infected fish on day 14 post-infection. Interestingly, a significant increase in NNV-specific IgT secretion was only observed in the gills of the infected group.

Conclusions: Our results suggest that ASB IgT may play an important role in the adaptive mucosal immune responses against viral infection and could potentially be adapted as a tool for the evaluation of prospective mucosal vaccines and adjuvants for the species.

Evaluating operational welfare indicators as an objective assessment of the welfare of salmonids in ras

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Introduction: During the last couple of decades, there has been a shift in the land-based production of finfish from traditional flowthrough water systems to recirculated aquaculture systems (RAS). However, there is some uncertainty as to how this production type affects the health and welfare of the farmed fish. In the IntelliRAS-project1, our aim is to integrate data on production and environment with observations of health and welfare in order to move from experience-based to knowledge-based decision making.

As a part of this, veterinarians have investigated how to use operational welfare indicators (OWI) for objective health and welfare evaluation across RAS farms.

Methodology: Four land-based RAS study farms were included: 2 with Atlantic Salmon and 2 with Rainbow Trout. The farms had different degrees of recirculation, and produced fish for different sizes. In each farm, 3-4 batches of fish were followed. Health and welfare evaluations of 100 fish of each batch were performed at minimum 2 timepoints.
Morphological evaluations were performed on sedated fish, using the scoring system of the FishWell handbook2.

Results: The variation in welfare parameters were both batch-specific and farm-specific. In addition, it was possible to observe a development over time in some batches on some of the health parameters.
In one farm, 97-100% of the fish in each batch had fin lesions, and this did not change during the production. However, the severity of fin lesions did change: at first scoring most lesions were active, whereas in the end most lesions were healed. In the same batches, the amount of fish with scale loss increased from <10% to around 50% over time. Other injuries and abnormalities were either absent (snout ulcers), detected only a few times (jaw or vertebrate deformities, eye injuries, skin lesions) or only in a few percent of the fish (operculum deformities).
In another farm, operculum deformities were observed in more than 80% of the examined fish.

Conclusions: Fish health managers can use OWI as an objective tool to assess welfare in RAS. However, each farm must over develop their own benchmarking procedure, using OWIs relevant for them.

1: https://www.vetinst.no/en/research-and-innovation/ongoing-research-projects/intelliras
2: http://www.nofima.no/fishwell/english

Comparison of seaway distance and particle tracking as measures of hydroconnectivity for modelling waterborne spread of viruses among salmon farms

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We developed a hybrid model (HydroEpix) which simulates within- and between-farm waterborne spread of viruses. In prior studies, we evaluated alternate surveillance, detection, vaccination, and culling strategies at the pen and farm level for IHNV and infectious salmon anaemia virus (ISAV) in farmed Atlantic salmon populations in Canada. HydroEpix uses a decay function to allow a lower probability of between-farm transmission of ISAV as distance increases. This study reports on application of the model to marine spatial planning and risk ranking of farms for enhanced surveillance and testing for ISAV in Liverpool Bay, Nova Scotia (NS). A single company submitted a licensing application to the NS Department of Fisheries and Aquaculture (NSDFA) for 3 sites in the bay each with 20 net-pens of 33,000 salmon. Seaway distance (SD) between the 3 lease locations are 1.3, 1.7, and 2.7 km. SD is a useful measure of hydrodynamic connectivity but it considers equal transmission risk in both directions and it doesn't account for ocean currents which are influenced by winds, tides, and freshwater flow. For comparison with SD simulations, particle tracks (PT) for the same farms were extracted from the FVCOM coastal circulation model. Tracks were obtained for 2 days (maximum survival of ISAV) based on 432 releases of 500 particles every hour for 18 days, Data were expressed as particle contact hours at the recipient farm site after release from each of the 3 source sites. There were 6 combinations of between-site transmission based on hydroconnectivity matrices and 3 runs from HydroEpix for comparison. PT data were superior to seaway distance and in the case of one farm pair, PT data showed that unidirectional transmission from the same source to a recipient farm that was 2.7 km distant was more frequent (75.9% vs. 65.9%) than to a closer farm (1.3 km distant) because of the direction of currents at the mouth of the bay. Results of the seaway distance and particle tracking model outputs have been shared with NSDFA personnel to support decision-making for siting of Liverpool Bay farms and with veterinarians for preparedness planning for ISAV outbreak mitigation.

The history and relevance of the Scottish Production Surveys to animal welfare and sustainable aquaculture development in Scotland

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Introduction: Quality data on aquaculture production is essential for management and policies to ensure sustainable aquaculture. The Scottish fish farm annual production survey reports production as Official Statistics and has been carried out by Marine Scotland (MS) since 1979. The survey analysis and is based on returns obtained from all authorised Scottish aquaculture businesses. The survey include statistics on production of salmon, trout and other fish species and incudes data on employment and fish escapes. A second survey provides a complementary data set for production of shellfish species.

Methodology: In December a questionnaire is sent by MS staff to all the registered fish and shellfish producers. This questionnaire requests details of production and employment activities on all the sites. The questionnaire is followed up by email and phone reminders as required. Returns are quality checked, with follow up for clarification from the aquaculture businesses where required. The survey are then analysed and published under the standards of official statistics with data calculated at national and regional levels.

Results: The data in the surveys are published in annual reports which are available on the Scottish Governments website (www.gov.scot) where the historical reports can also be found. The data tables are also published as open data on the MS data pages (https://data.marine.gov.scot/).
Key production and employment data can now be visualised through a shiny app https://scotland.shinyapps.io/sg-aquaculture-production-surveys/. Visualisation allows exploration of trends to help in setting policy in a changing system and assessment of achievements against objectives.

Conclusions: These Official Statistics are used in support the SG's Marine Atlas and National Marine Plan and are key to a Blue Economy being developed by the SG. Data are used by the UK government, for example in food security assessment, and are provided to international organisations such as the Organisation for Economic Co-operation and Development (OECD) and the Food and Agriculture Organisation of the United Nations (FAO).

Changes in epidemiology of blood fluke infections in ranched southern bluefin tuna in the last twenty years

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Introduction: Southern Bluefin Tuna (SBT) is a commercially important species in Australia. Wild SBT 4-5 years old are caught in Great Australian Bight and towed to ranching area where they are held in ranching pontoons for about 6 months to improve their condition and market price. Few health problems have been reported during ranching, one of the most common is blood fluke infection. Cardicola forsteri was first discovered in 1997, whereas Cardicola orientalis was first reported from SBT in 2012. In this presentation we report similarities and differences in epidemiology of the infections based on our published and unpublished data.

Methodology: In the last 20 years blood fluke infections have been monitored in ranched SBT on regular basis including at harvest during most of ranching seasons. Prevalence and intensity of infection has been monitored by counting adult flukes in SBT heart. More recently, qPCR for both species of flukes have been developed and applied to heart and gill samples. Additionally, eggs have been counted in the gills to add another measure of infection severity. Fish weight and length have been measured to determine condition factor.

Results: Infection prevalence and intensity in wild SBT continues to be very low. Blood fluke infection occurs in the first 4-6 weeks post transfer. In the past, two peaks of infection were observed before harvest, however now the second peak is usually absent unless the SBT are held for extended period of time. Cardicola orientalis was dominant until 2012, but since then Cardicola forsteri has become much more common.

Conclusions: While some of the differences in prevalence and intensity patterns may be due to temporal variation, others are caused by introduction of praziquantel as a treatment against blood flukes in 2012. Introduction of molecular methods to detect the blood flukes and quantification of blood fluke eggs in the gills has increased our understanding of the infection.

Estimating transmissibility of White Spot Syndrome virus in Marsupenaeus japonicus in the setting of aquaculture pond

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White spot syndrome virus (WSSV) triggers white spot disease which causes quite high mortality in farmed shrimp. To control WSSV, understanding WSSV epidemic character is required. To this end, the epidemiological data of WSSV in the setting of aquaculture is required especially for measuring transmissibility including basic reproduction number (R0). However, the detailed epidemiological data is difficult to obtain due to the rapid and high mortality. In this study we proposed a framework for estimation of transmissibility of WSSV from the combination of i) the epidemiological data in the early phase of outbreak, ii) the infection experiment of WSSV and iii) the feeding experiment of dead shrimp eaten by healthy shrimp using a mathematical model describing WSSV transmission by cannibalism of dead and infected shrimp. We measured the transmissibility of WSSV by R0 using the epidemiological data of WSSV outbreak in aquaculture ponds observed in the island in Japan. Our estimate of R0 suggests the transmissibility of WSSV in the setting of aquaculture is quite high and the urgent intervention is required when a WSSV infection is confirmed in an aquaculture pond.

Rapid risk assessments of aquatic pathogens are key in anticipating sudden epidemiological changes

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The evolution of existing pathogens or the emergence of new diseases are a major threat to fish populations. Specifically, anthropogenic activities and environmental factors can rapidly change the epidemiological situation of aquatic environments and exacerbate detrimental disease impacts on aquaculture production. Disease horizon scanning has been developed to identify such imminent aquatic animal health threats and to feed into rapid risk assessments (RRAs), designed to quickly estimate the risk that an identified pathogen of interest could cause to aquatic populations.

This work presents a RRA tool developed specifically for aquatic pathogens and explores its practical application to risk communication. The main objective of the RRA is to combine likelihoods of the introduction, establishment and potential consequences of a pathogen in order to 1) assess its pathogenicity, 2) anticipate its ecological, economic and social impacts, 3) advise on disease control management, and 4) communicate risks. The RRA is based on the best scientific evidence available, supported by the literature and expert opinion. The assessment gathers essential knowledge about pathogen characteristics, host specificities, feasible routes of introduction, conditions for establishment, potential consequences, and mitigation measures. Additional information on diagnostic methods is also provided when available. Often performed with limited information and subject to high uncertainty, the RRA provides valuable insights to direct the collection of additional data, further pathology research, in-depth risk assessments and policy decision.

The recent application of the RRA to salmonid diseases, specifically pilchard orthomyxovirus, the causative agent of salmon orthomyxoviral necrosis in farmed Atlantic salmon Salmo salar, illustrates how the tool provides key information for import risk assessments in a comprehensive, structured and reproducible summary. By combining qualitative and quantitative data, it provides a robust framework for the assessment of risks from aquatic emerging diseases and associated uncertainty in a short-term period, allowing for a rapid and effective response from decision-makers to imminent disease risks. Finally, the RRA tool is flexible and easily adaptable to support efficient and timely decisions and communicate risks in different contexts, encompassing commodity trade, farming expansion and diversification, and emergency preparedness under changing environmental conditions such as climate change or non-native species introduction.

Sampling bias in fish health monitoring and research

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Introduction: Successful disease control and mitigation in farmed animals require representative sampling for health surveillance. This is particularly challenging for fish grown in commercial sea pens. In research, the use of statistical methods requires random sampling where all fish in the pen have an equal probability of being sampled. This can only be achieved by removing all the fish from the pen or tank and making a random selection. For non-invasive assessment (e.g., weight or skin health) the same can be achieved by capturing, anesthetizing and assessing the whole pen content and then taking a sample from the randomized data set. These options are usually not practical in commercial aquaculture. Fish are often sampled for health surveillance by hook and line or dip netting.

Methodology: We evaluated sampling bias for methods used for weight checks and skin lesions assessment in farmed chinook salmon, including hook and line sampling of 4 fish and manual assessment of the first 60 fish from the whole

pen. We compared those with whole pen data (for each fish) and data for 4 or 60 randomly selected fish from the whole pen data.

Results: Both hook and line and manual assessment methods showed sampling bias. The manual assessment method produced a significant weight bias with a clear tendency towards oversampling of larger fish, while hook and line sampled fish had lower weight than the whole pen. Neither hook and line or manual assessment provided a representative sample of the skin health of the pen population. Random selection of 60 fish was representative of the pen population with regard to skin lesions.

Conclusions: Understanding the sampling bias of these methods is necessary to improve experimental design for studies based on commercial farms and to inform investigators of the potential validity of commercial data for epidemiological studies. Furthermore, the results will help the industry in interpretation their health monitoring results.

Epidemiology and ecology of crayfish plague in Switzerland

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Aphanomyces astaci is the causative agent of crayfish plague in native European crayfish, leading to high mortality. Invasive North American crayfish serve as carriers of the agent. Although disease severity depends on genotypes (A-E) involved, detection and genotyping methods are complex and so far unsatisfying and disease dynamic in rivers is largely unknown.

Our aims were to (1) optimise detection and genotyping methods, (2) monitor A. astaci genotypes in Swiss crayfish populations, native and invasive, and (3) characterise disease dynamics in natural waters following crayfish plague outbreaks.

DNA was extracted from the exoskeleton of 30 crayfish plague affected native crayfish, using various commercial kits specific for animals (Ak), plants (Pk) and insects (Ik). DNA concentration, fragment length and quality, as well as A. astaci DNA detection probability by qPCR and PCR were compared.

DNA originating from pure cultures of A. astaci genotypes A-E were sequenced using Pac-Bio HiFi-Long-Read-Sequencing. Whole genome comparative analysis was performed to identify sequences suitable for A. astaci detection and/or genotyping.

A retrospective study using archive material from 1960 till 2020 aimed to investigate A. astaci occurrence and genotypes by qPCR, PCR and Sanger sequencing on formalin-fixed paraffin-embedded (FFPE) material.
A Prospective study was performed between 2020 and 2023. 15-20 individuals per population were sampled, A. astaci occurrence and genotypes were investigated as described above.

After a crayfish plague outbreak, eDNA was investigated over a period of 12 months by filtering water (1800-2400ml) followed by qPCR.

Total DNA concentration was higher in samples extracted with Ak or Pk. Fragment length was highest using Ik. While qPCR detected A. astaci in all samples, PCR showed higher detection probability in samples extracted by Ik or Pk.

In the retrospective study, crayfish plague was diagnosed in 40/212 populations, with a first case from 1980. Three different genotypes were identified (B, D, E), as early as 1994.

In the prospective study, 1020 crayfish were sampled. So far, crayfish plague was confirmed in 4 native crayfish populations.

Preliminary eDNA results indicate strong influence of season and river characteristics on A. astaci eDNA.

Virome diversity in native and invasive crayfish populations in Switzerland

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Introduction: Native crayfish populations in Switzerland are, as in most parts of Europe, declining. Besides degradation and loss of habitat, or competition with invasive crayfish, native species are faced with the introduction of exotic pathogens, such as Aphanomyces astaci, the causative agent of crayfish plague. However, little is known about other pathogens and their impact on wild crayfish. Among these, viruses can act as potent pathogens and some, such as the White Spot Syndrome Virus, are known to have devastating impacts on crustaceans. Viruses can be primarily pathogenic or have a debilitating effect on their host, permitting secondary infections and increasing likelihood of disease. In recent years, metagenomic studies focusing on invertebrates have revealed a high diversity of viruses, but their importance and host-pathogen interactions still need to be elucidated.

The aim of this study is to (1) compare viral populations of two species of crayfish present in Switzerland, the noble crayfish (Astacus astacus), a native species and the invasive signal crayfish (Pacifastacus leniusculus), (2) assess the geographical distribution of viruses of interest and (3) investigate differences between crayfish plague infected and non-infected individuals, revealing possible interactions with A. astaci.

Methodology: As part of a nation-wide project on crayfish plague in Switzerland, 6 populations of native noble crayfish and 29 populations of invasive signal crayfish were sampled between June 2021 and June 2022. From each individual (n=540), a pool of organs containing hepatopancreas, heart, gills and green gland was conserved in RNAlater and frozen at -80°C until subsequent RNA extraction and next-generation sequencing (NGS). NGS reads were then analysed by an in-house virus discovery bioinformatics pipeline.

Results and conclusion: Preliminary results indicate the presence of large numbers of different virus species in noble crayfish populations. Interestingly, Bunya-like Brown Spot Virus (BBSV) RNA was detected in diseased noble crayfish. BBSV is a negative-strand RNA virus described from a mass mortality event in the native white-clawed crayfish (Austropotamobius pallipes) in the Eastern part of France (Grandjean et al., 2019).

Our results could indicate a possible underestimation of the importance of viral infections as the cause of clinical disease and mortality in crayfish.

Multiple cutaneous soft tissue sarcomas in a goldfish population: pathological features and virological investigations

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Introduction: Dermal Soft Tissue Sarcomas (STS) are common cutaneous lesions, detected in ornamental and reared fish. In goldfish (Carassius auratus), they may appear as a single cutaneous mass, even though more frequently, multiple presentations have been reported. So far, few described cases of fish STS had been studied through cytology, a rapid and low-cost method that in the diagnostic process should be paralleled by histology, which provides the details of neoplastic tissue architecture. Fish neoplasia may be the result of a genetic mutation, after exposure to environmental agents or oncogenic viruses. Concerning dermal STS in goldfish, a hypothetic viral-induced condition has been previously suspected, but did not find confirmation. The aims of the present study were characterize multiple cutaneous masses affecting a pond-living goldfish population by means of pathological and virological investigations.

Methodology: Multiple cutaneous masses affecting goldfish population were analyzed by means of cytology, histology and Transmission Electron Microscopy. Regarding virological investigation, the pathological cutaneous tissue and spleen, kidney and liver samples were inoculated onto Koi-Fin1 cell culture, to evaluate the possible development of cytopathic effect (CPE). Furthermore, molecular investigations were conducted, using PCRs, in order to detect the presence of viruses usually associated with the development of proliferative cutaneous lesions such as Lymphocystis Disease Virus (LCDV) and Cyprinid Herpesvirus-1 (CyHV-1).

Results: the cytological diagnosis of the cutaneous masses was spindle cell-tumors, which were histologically classified as dermal STS. LCDV and CyHV-1 were not detected by PCRs. Viral isolation reported no CPE. Viral particles were not detected at ultrastructure.

Conclusions: Although cytology prompted diagnosis of the tumors, their multiple distribution did not allow opting for any therapeutic option. In fish, the association between tumorigenesis and infectious agents (i.e. herpesvirus and retrovirus) ranges from weak (i.e the mere detection of reverse transcriptase activity or ultrastructurally-evident viral particles in tumor samples), to reasonably strong (i.e. virus isolation and sequencing, and successful generation of the neoplasia by exposure of fish to cell-free filtrates derived from homogenized tumor tissue). In this study, the cutaneous masses affecting goldfish population were diagnosed as multiple, dermal STS, without any current association with viral presence.

Characterisation of a new genotype of Marteilia cocosarum linked to high mortalities of cockles (Cerastoderma edule) in the Wash Estuary

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Background: Marteilia parasites have been associated with recurrent and significant infection outbreaks of commercially important bivalve molluscs. Prior to 2022, the only known Marteilia parasite infecting cockles (Cerastoderma edule) was Marteilia cochillia – the causative agent of mass mortalities and cockle fishery collapse in Spain. However, marteiliosis in British cockles was reported by Skujina et al. (2022), caused by a novel Marteilia species, M. cocosarum. The Wash Estuary, located in Norfolk, UK, is a socio-economically and ecologically important estuary embayment, supporting several major molluscan fisheries. Since 2008, C. edule stocks in the Wash have suffered

unusually high mortalities, particularly affecting larger, market size cockles, leaving beds dominated by smaller juvenile stocks.

Methods: Samples of healthy (buried in the sand) and moribund (weak, unable to bury) cockles obtained from the Wash Estuary in 2021/2022 underwent parasite PCR screens. High prevalence of a Marteilia parasite was detected across all sites, with up to 95% prevelance in moribund cockles. Lower prevalence of Marteilia was detected by PCR in buried cockles. We characterise this Marteilia species by molecular and histopathology methods, and link its prevalence to present and historic mortalities in the Wash.

Results: Long-range PCR, sequencing and phylogenetic analysis provided evidence to suggest a novel genotype of M. cocosarum was infecting cockles (C. edule) in the Wash estuary, named here M. cocosarum WE1 (Wash Estuary 1). Both M. cocosarum strains demonstrated an identical pathology and mode of infection upon their host, with histopathological analysis and in situ hybridisation identifying a high prevalence of Marteilia cells within the gill and mantle tissues. Further analysis of cockle samples taken from a 2009 mortality event indicated that Marteilia parasites were also present at this time and may have been implicated in long-term stock declines.

Conclusions: The presence of a novel Marteilia species is associated with mortalities of cockles in the Wash Estuary. Future work is focusing on further analysis to assess the association between cockle mortalities and the emergence of the novel Marteilia in the Wash, as well as the influence of potential confounding environmental variables associated with the mortalities.

Haplosporidian sequence diversity associated with European bivalves, including the description of Minchinia cerastodermae from the common cockle Cerastoderma edule

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Introduction: Haplosporidians (Ascetosporea, Haplosporida) are major protozoan parasites of aquatic invertebrates, including commercially exploited bivalve molluscs and crustaceans. Several species are well known for their association with oyster mortalities, and in recent years Haplosporidium pinnae has been associated with catastrophic mortalities of the now critically endangered fan mussel Pinna nobilis, but little is known about the diversity and geographic distribution of haplosporidians in bivalves.

Methodology: We use a molecular-led approach, with supporting histopathology, to explore the sequence diversity of haplosporidians associated with bivalve tissues collected in the United Kingdom, Ireland, Norway and Spain. A targeted, haplosporidian-specific nested primer set – previously used to great effect to explore haplosporidian diversity associated with different environmental compartments – was used to screen DNA extracts from bivalve tissues, and the sequences generated were placed in a phylogenetic framework including all described, sequenced haplosporidians, uncharacterised haplosporidian sequences and environment-only lineages.

Results and Conclusions: We detected sequence types related or corresponding to high impact oyster parasites Haplosporidium nelsoni and H. costale, and novel lineages identified in previous haplosporidian-targeted environmental DNA surveys. Based on sequence and morphological data, we describe Minchinia cerastodermae, detected in cockles collected in the UK and Ireland, and previously reported in Spain. We also report for the first time infection of the parasitic copepod Mytilicola intestinalis in host Mytilus edulis with crustacean parasite H. orchestiae, originally described from Orchestia spp. amphipods. Additionally, we identify 17 novel lineages across the order Haplosporida, including lineages showing strong associations with bivalves Cerastoderma edule and Crassostrea gigas. Phylogenetic analysis demonstrates the wide diversity of these novel, bivalve-associated haplosporidian lineages. We discuss the potential of haplosporidians as causative agents of emerging diseases, as threats to both aquaculture production and conservation. We also highlight the implications of increased awareness of parasite molecular diversity for diagnostic assays and standards.

Perkinsus olseni requires glycine betaine from the host Manila clam Ruditapes philippinarum for proliferation

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Introduction: Perkinsus olseni is a protozoan endoparasite known as an industrially significant pathogen of Manila clams Ruditapes philippinarum. So far, in vitro propagation methods have been established for this species, and our recent study showed that the proliferation of P. olseni was enhanced by supplementation of the tissue extract of host clams, suggesting the presence of host factor(s) required for its proliferation. Thus, this study first aimed to isolate and

identify such factor(s) in Manila clams. Additionally, we investigated the effects of the identified factors on the proliferation of other congeneric Perkinsus species and then examined their biosynthetic pathway of the identified proliferation factor.

Methods: Using a fetal bovine serum (FBS)-deficient medium PBMΔF in which P. olseni did not proliferate well, the effect of Manila clam tissue extract on P. olseni proliferation was examined. Then, Manila clam tissue extract was fractioned through high-performance liquid chromatography (HPLC), and the effective compound was identified by high-resolution electrospray ionization mass spectrometry (HRESIMS) and nuclear magnetic resonance (NMR). Subsequently, the proliferation of six Perkinsus species was examined under the presence of the identified compound as well as its biosynthetic precursors.

Results: Active proliferation of P. olseni was confirmed in PBMΔF supplemented with Manila clam tissue extract. A single fraction showing a high effect on P. olseni proliferation was recovered by HPLC, and the effective compound was identified as glycine betaine (=trimethyl glycine). Of six examined Perkinsus species, P. marinus, P. chesapeaki, P. mediterraneus, and P. honshuensis showed rapid growth under the presence of glycine betaine and its precursors, choline and betaine aldehyde, while P. olseni and P. beihaiensis proliferated actively only under the presence of glycine betaine.

Conclusion: This study revealed that P. olseni required glycine betaine for proliferation, and this species utilized this compound of its host Manila clams. It was also revealed that P. olseni and P. beihaiensis have little activity to biosynthesize glycine betaine de novo, whereas the other four congeneric species retain this biosynthetic pathway.

Identification of the gametogenesis of razor clams (E. siliqua) in Scotland

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Introduction: Razor clams (Ensis sp.) are marine bivalve molluscs commonly found around the coast of Europe. Razor clams are harvested by fishers in Scotland using electrofishing, and mainly exported to supply markets abroad, including Europe and the Far East.

Since 2018, Marine Scotland Science have participated in a scientific trial to gather local level information on razor clam populations and stocks and to ensure the fishery is sustainable. As part of the scientific trial, this project aimed to investigate the gametogenic development of the razor clam and their spawning season using histological examination.

Material & Methods: Between 2019 and 2022, twenty razor clams were collected every two/four weeks from different areas by divers. A section of the foot muscle which included the gonadal and visceral mass was dissected from an area close to the anterior abductor muscle and immediately fixed in Davidson's seawater for 24/48h. The samples were processed by standard histological techniques.

Results: A total of 1036 razor clams were analysed by histological examination; 458 identified as male, 515 as female, and 3 as hermaphrodite. It was not possible to identify the sex of 60 of the razor clams because the individuals were in stage '0'. The examination of histological sections of gonad identified six different stages of gonad development.

In the first quarter of the year (January to March) the majority of razors were identified as stage II with increased follicles. In April and May razor clams were observed to be stage IIIA and IIIB; this is when individuals are ripe and spawning. For the summer months (June, July and August) the razor clams were identified as stage 0 with the gonads at rest. Razor clams were also observed at stage IV (exhaustion) between April and September. From October to December the razor clams were classified at stage I.

Conclusion: The knowledge of the razor gametogenesis of the razor clam (E. siliqua) is important as identifying key stages of spawning can allow fishery managers to consider options for the fishery (including closed seasons). This is vital to ensure that any potential future fishery is harvested sustainably.

Effect of a loss of mda5/ifih1 gene on the antiviral resistance in a Chinook salmon Oncorhynchus tshawytscha cell line

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Cells are equipped with intracellular RIG-like Receptors (RLRs) detecting double stranded (ds)RNA, a molecule with Pathogen-Associated Molecular Pattern (PAMPs) present in the cell during the life cycle of many viruses. Melanoma Differentiation-Associated protein 5 (MDA5), a helicase enzyme member of the RLRs encoded by the ifih1 gene, binds to long dsRNA molecules during a viral infection and initiates the subsequent production of type I interferon (IFN1) that orchestrates the antiviral response. In order to understand the contribution of MDA5 to viral resistance in fish cells, we have engineered a clonal cell line invalidated for the ifih1 gene by CRISPR/Cas9 genome editing in CHSE-EC, a cell line derived from chinook salmon Oncorhynchus tshawytscha . We demonstrated that IFN1 induction is impaired in this cell line after infection with the Snakehead rhabdovirus (SHRV) or the Salmon Alphavirus (SAV). The cell line, however, does not show any increase in cytopathic effect when infected with Infectious Pancreatic Necrosis Virus (IPNV), Gold Shinner Virus (GSV), Epizootic Haemorrhagic Necrosis Virus (EHNV), Infectious Haemorrhagic Necrotic Virus (IHNV), Infectious Salmon Anaemia Virus (ISAV), Viral Haemorrhagic Septicaemia Virus (VHSV), Channel Catfish Virus (CCV), Blotched Snakehead Virus (BSNV), SAV or SHRV. These results illustrate the redundancy of the IFN1 system that has evolved to circumvent viral evasion strategies.

Yersinia ruckeri infection activates local skin and gill B cell responses in rainbow trout

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Introduction: Teleost fish lack organized structures in mucosal tissues such as those of mammals, but have been shown to contain dispersed B and T cells with the capacity to respond to external stimuli. Nonetheless, there is still a great lack of knowledge regarding the nature and functionality of mucosal B cells. To gain insight on this issue, in the current study, we have characterized the IgM/D-expressing subsets in the skin and gills of rainbow trout (Oncorhynchus mykiss), to then determine how they responded to an experimental infection with Yersinia ruckeri.

Methodology: We studied the presence of different IgM/D-expressing B cell subsets in the skin and gills of rainbow trout by flow cytometry and confocal microscopy, and performed a transcriptomic analysis of each subset in comparison to naïve blood B cells. The levels of expression of MHC II and the antigen-processing abilities were also compared through flow cytometry. We then conducted a bath challenge with a strain of Y. ruckeri serotype I and analyzed the transcription of genes related to B cell function in both mucosal surfaces. Additionally, we evaluated how the infection affected the presence and size of B cells in both skin and gills, as well as the number of plasmablasts secreting total or specific IgMs though ELISpot.

Results: Although most B cells in teleost systemic compartments co-express IgM and IgD on the surface, cells exclusively expressing either of the two Igs were the main IgM/D-expressing subsets in skin and gills. The transcriptional profile of IgM+IgD- and IgD+IgM- B cells corresponded to that of cells that have started a differentiation program towards plasmablasts. Yet, IgM+IgD- B cells retained high levels of surface MHC II and antigen-processing abilities, while these were much lower in IgD+IgM- cells. In response to Y. ruckeri, the transcriptional profile obtained points to the local differentiation of B cells to plasmablasts / plasma cells. Interestingly, these plasmablasts / plasma cells were shown to secrete specific IgMs as soon as 5 days after the exposure.

Conclusions: These findings contribute to a further understanding of how B cells in the periphery respond to bacterial pathogens in teleost fish.

Assessing Immunological response of Ballan wrasse (Labrus bergylta) to Differential Aeromonas salmonicida Vaccination Regimes

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Introduction: Ballan wrasse (Labrus bergylta) are cleaner fish that are deployed as a highly efficacious management strategy for sea lice (Lepeophtheirus salmonis) infections of Atlantic salmon. Atypical Aeromonas salmonicida has severely hampered production, particularly in fry, and autogenous vaccines are currently being applied through different vaccination regimes at commercial sites in Scotland. Typical commercial practice involves immersion vaccination of juvenile fish <15 g, prior to injection (IP) boosting. However, the efficacy of immersion vaccination is unknown as previous studies have shown immunocompetence biomarkers being expressed from 35 days post-hatch (dph) although immersion vaccination did not protect fish of up to 170dph (ca 1.5g) against experimental challenge with atypical A. salmonicida. The objective of the current study was to establish the immunological response of vaccinated ballan wrasse over the course of 2 commercial vaccination regimes including with/without immersion priming and whether multivalency impacts antibody response (i.e. antigenic competition).

Methodology: Fish from two commercial hatcheries were followed through vaccination regimes. Prime immersion vaccination was conducted at 3-5g with a boost immersion at 6-8g and finally IP at >15g. Samples of mucus, sera and tissue were collected at multiple time-points throughout the regime. Mucus was 60erotransf via protein concentration for alkaline phosphatase, lysozyme and peroxidase assays. Sera was serially diluted and investigated for its reactivity to target strains of A.salmonicida using ELISA. Gene expression was analysed in spleen, gut and liver by qPCR targeting IgM, MHCII, RAG2 and 60erotransferrin

Results: Two novel vaccine formulations were compared: A multispecies vaccine and a monospecies vaccine targeting atypical A.salmonicida. Assays and biomarkers were developed to investigate the innate and adaptive immune responses of L.bergylta. The innate assays were affected by fish development instead of vaccination. ELISA showed strong reactivity post IP and an increasing response from 30dd (degree days) post IP to 600dd. Preliminary analysis suggests enhanced antibody responses in fish previously immersion vaccinated.

Conclusion: Sera response to IP vaccination improves from 30dd-600dd post IP and may be enhanced by immersion vaccination. Mucosal innate responses were unaffected by vaccination status. Initial qPCR analysis indicates that 60erotransferrin and IgM may be affected by immersion vaccination.

The Two-Fold Mechanism of a health-promoting additive to support prevention strategies against Ectoparasitic Infestations in Fish

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Controlling ectoparasitic infestations is a significant challenge in fish farming, and health-promoting additives have been shown to enhance mucus defense and reduce the incidence of ectoparasites. This study aimed to better understand the skin immunity regulatory mechanisms of the phytobiotic-based additive APEX® BRANCHIA (Adisseo) to protect fish from ectoparasite infection.

The mode of action of the additive in fish skin was evaluated using the Guppy-Gyrodactylus infection model, followed by a shotgun proteomics approach. Guppies (Poecilia reticulata) were maintained in individual containers (n= 40) and fed with feed supplemented with the additive during 14 days. Each fish was infected with two Gyrodactylus turnbulli and continuously fed with additive-supplemented feed for 17 days. The parasite number of each fish was recorded every 48 hours for 17 days. Fish were categorized into responsive, resistant, and susceptible based on their susceptibility to the infection. Fish skin was sampled on day 13 and day 17, representing the period when the immune responses are most reactive and the end of the infection, respectively. Protein identification and quantitation were performed using nano LC-MS/MS. The expression of proteins differentially regulated by the additive supplementation was examined in responsive and resistant fish to gain insight into the mode of action of the additive based on fish response to infection.

Two key mechanisms to combat parasite infection were identified in this study. In responsive fish, where the parasite number peaked at the middle of infection (day 13) but drastically dropped until the end of infection, APEX® BRANCHIA induced skin cornification. Skin cornification is a process of programmed cell death resulting in the formation of a tight barrier of dead cells that effectively blocks parasite penetration. In resistant fish, which consistently showed a low parasite load from day 0 to day 17, the additive activated the complement system. This is a proteolytic cascade reaction that supports the elimination of invading microorganisms.

In conclusion, APEX® BRANCHIA supplementation effectively strengthens the defense mechanisms against ectoparasite infection in fish by providing physical barrier protection and enhancing immunocompetence. As demonstrated in field trials, these mechanisms are key to supporting prevention strategies against ectoparasitic infestations.

Dietary intervention to improve wound healing in heat stressed Atlantic salmon

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During summer, farmed Tasmanian Atlantic salmon are often exposed to sea water temperature that is close to their upper thermal limit which can disrupt the nitric oxide wound healing pathway. Compromised skin integrity of sea cage farmed fish is unavoidable due to parasite and cnidarian damage, handling for gill disease treatments and general physical contact with infrastructure and cohabitants. Even minor lesions can lead to secondary infection resulting in mortality. We investigate whether dietary supplementation of amino acids which underpin the nitric oxide wound healing pathway and can become limited during heat stress will improve the inflammatory response and rate of tissue repair in heat stressed Atlantic salmon. Atlantic salmon post-smolt (200 g) were distributed across 16 x 350 L tanks (17 fish/tank) comprising four treatments with four tank replicates. Treatments include diets supplemented with an additional 1.5% L-arginine (ARG), 1.5% L-citrulline (CIT), 1.5% L-ornithine (ORN) and a control diet (Ctl). All fish were tagged and measured for length and weight at the beginning of the trial. After acclimation at 15^{o}C, fish were fed their experimental diets and the water temperature was increased 1^{o}C per week until a maximum of 20^{o}C was reached and held for a further 4 weeks. By week 9, all fish were weighed and measured again, and half the fish had their gills abraded. At 24 h post-wounding, 8 fish per tank (4 wounded and 4 control) were sampled to assess the baseline level of injury and inflammatory response. The remaining fish were sampled 8 days post-wound to assess the rate of healing and immune response between diet treatments through histological and molecular methods. ARG, CIT and Ctl fish performed the same in terms of weight gain, FCR, specific feed rate and condition index, while ORN fish significantly underperformed. Fish fed additional citrulline had the highest survival in heat stressed conditions pre- and post-wounding. Plasma osmolality data indicate dietary differences in gill function. Results to date indicate that nutritional support may be a viable preventative solution to gill injury and disease rather than reactive treatments such as antibiotics.

Inclusion of methanotroph bacteria in Nile tilapia diets improves growth, health outcomes, and disease resistance

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It is widely recognised that there is a need to find new and novel sources of protein to fill the protein gap in food production. These sources need to be sustainable, climate friendly, and, in an ideal world, provide additional benefits including improved growth and health for the animals. One approach currently under investigation at an academic and commercial level is the use of single cell proteins (SCPs). These so-called alternative proteins include bacteria, algae and fungi. Whilst there has been a huge investment in developing these SCPs, few have been successfully commercialised. One protein source, developed by Calysta, is FeedKind(R), a dried bacterial biomass derived from Methylococcus capsulatus, a bacteria that uses methane as its primary feedstock. Under laboratory conditions, five groups (each with five replicates) of Nile tilapia were fed diets containing increasing levels of the bacterial biomass, ultimately replacing 100% of the protein in the diet. Fish were fed for six weeks and subsamples taken for histology, microbiome, biochemistry and molecular studies, alongside growth measures, at the start and end of the feed trial. A subset of these animals were subsequently exposed to Streptococcus agalactiae Ib and observed for up to 3 weeks post-challenge. Samples as previous were collected. No differences in growth parameters or in feed conversion ratios were noted across all treatment groups confirming that protein could be replaced with FeedKind without impacting growth outcomes. Likewise, growth was not impacted in the challenge groups suggesting that fish were able to maintain growth despite the infection pressure. Although there was no statistically significant difference in survival following infection, there was a numerically lower survival in the two groups with high FeedKind inclusion comapred with the control and low inclusion groups (60% vs 65%). Outcomes are discussed in relation to pathologies seen, blood biochemistry and gut microbiome and contextualised in the future requirements for protein in aquaculture.

Counteractive effects of tryptophan dietary supplementation on neuroendocrine-immune communication pathways

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Introduction: Amino acids, such as tryptophan, play several functions on key metabolic pathways important to immune and neuroendocrine responses. This study aimed to assess the links between tryptophan nutrition and the network that regulates the bi-directional pathways between neuroendocrine and immune systems in European seabass.

Methodology: European seabass juveniles (12.02±2.77g) were distributed in two independent recirculating seawater systems with a density of 5kg/m3 (control) or a stressful density (10kg/m3). Fish were fed a control diet (CTRL) and a CTRL-based diet supplemented with tryptophan (0.3% DM basis; TRP) for 15 days. Fish were sampled at the end of the feeding period and at 4, 24 and 72 hours post intraperitoneal injection with Photobacterium damselae piscicida. The hypothalamus, pituitary gland and head-kidney were sampled for gene expression analysis.

Results: Hypothalamic gr1 expression was significantly induced by injection in stressed fish fed CTRL, but no such response was observed in stressed fish fed TRP, which expression levels at 4 hours post-injection were lower than in CTRL counterparts. Moreover, as opposed to CTRL, TRP-fed groups did now show an increase in hypothalamic il6 expression levels after injection. In the pituitary, the expression of htr2a was higher in stressed fish fed CTRL compared to those fed TRP. Moreover, tph1α was not induced by injection in stressed, TRP-fed fish, and was lower in these fish at 24 hours post-injection than in their CTRL counterparts.

Conclusions: Taking into account genes that are related with neuroendocrine and serotonergic pathways, in non-stressful conditions, TRP-fed fish displayed a molecular profile more similar to that of CTRL-fed, stressed fish (neuroendocrine activation and immunosuppression); in contrast, TRP in stressed fish promoted a scenario resembling that of non-stressed fish (lower cortisol and serotonin reactivity). Results unveil modulatory effects of tryptophan dietary intervention in molecular patterns that might have sustained changes in cortisol levels (parallel approach), pointing out a serotonergic activity (changes) as a key regulatory mechanism.

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Using egg microinjections for experimental induction of IPN infection in brook trout and rainbow brook trout interspecific hybrid

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Introduction: Microinjection is a micromanipulation technique that has been used in various fields and a variety of species, including teleost fish, for over a hundred years. The paper describes an experimental study of a microinjection technique for the administration of Infectious Pancreatic Necrosis Virus (IPNV) in fertilized eggs of brook trout, Salvelinus fontinalis (Mitchill), and a rainbow trout Oncorhynchus mykiss (Walbaum) hybrid, Salvelinus fontinalis ♂ × Oncorhynchus mykiss ♀. The oocytes and embryos of these salmonids have complex structures that require modifying the method commonly used in zebrafish, Danio rerio (Hamilton), research.

Methodology: Two sets of microinjection apparatus were tested. The first one, used in the pilot study, was the InjectMan NI2 system with FemtoJet Express (Eppendorf) with single-channel borosilicate glass capillaries with reinforcement filament (outer diameter 1.00 mm, inner diameter 0.78 mm, length 10 cm). The second system, used in the main study, contained a 10 µl single-channel borosilicate glass syringe (outer diameter 6.60 mm, inner diameter 0.46 mm) mounted with a repeating semi-automatic dispenser for syringes (Hamilton) with stainless steel needles with an SN-RN connection, a size of 26sG (outer diameter 0.47 mm, inner diameter 0.13 mm, length 19.0 mm), angle of 30°, and point style 4 (Hamilton).

All-female triploid fertilised eggs (pilot study n=1728, main study n=3456 oocytes) of brook trout and rainbow brook trout interspecific hybrid were divided into three groups: a control group (untreated eggs), a placebo (virus-free vehicle) injected group, and an IPNV-injected (The Spjarup reference strain from the National Veterinary Research Institute in Poland; GenBank accession number: AM889221) group.

Results: In the pilot study 4 hours after microinjections, 100% mortality was observed in the glass needle procedure, compared with no mortality in all groups of the stainless-steel needle procedure. In the main study 14 days after fertilization, there were no distinct differences in the influence on viability and mortality in groups without microinjections compared to ones with placebo and IPNV that could have been caused by microinjection.

Conclusions: The modified microinjection method can be used successfully to administer to salmonid eggs infectious substances that simulate the vertical transmission of pathogens.

Paradigm shift in the temperature range of Infectious Hematopoietic Necrosis

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Introduction: In recent years, Infectious Hematopoietic Necrosis has spread in Europe, with outbreaks occurring in previously disease-free countries such as Denmark and Finland. The strain of IHNv responsible for outbreaks in Denmark has been isolated from infected fish in follow-up sampling at water temperatures close to 20°C. The IHNv genotypes U and M can replicate in cell culture and induce disease in rainbow trout, Oncorhynchus mykiss, and sockeye salmon, O. nerka, at temperature higher than 14°C. This feature has never been demonstrated for European IHNv strains, which belong to genotype E.

Methodology: A selection of IHNv strains, including the Danish and some Italian isolates, were analysed for their replication fitness at different temperatures of incubation. The panel of isolates was subjected to titration by end-point dilutions assays on EPC cell monolayers at 15°C, 20°C, 22°C and 25°C. Cytopathic effects were monitored daily and titers were calculated according to the Spearman-Karber formula daily up to seven days post inoculation. Based on the results, a subset of IHNv strains was selected to be tested through an in vivo challenge in order to confirm the influence of water temperature on their pathogenicity in rainbow trout. Finally whole genome sequencing was performed to look for genetic markers of temperature adaptations.

Results: The titers yielded in the in vitro testing showed a high degree of variability of IHNv strains in relation to their sensitivity to temperature. Danish isolate and some Italian strains replicated without significant reduction when incubated at 22°C and could replicate even at 25°C that was considered a restrictive condition for this virus. Notably, the temperature tolerance appeared linked to the environment of origin rather than to the year of isolation. The in vivo experiment is on going.

Conclusions: A wider temperature range for IHNv strains belonging to genotype E has been demonstrated in vitro and will be soon confirmed in vivo. Based on results obtained a change of paradigm in the surveillance of IHN should be considered also at the regulatory level.

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Salmonid alphavirus accumulates deletion variants localized to specific regions of the viral genome during serial passages in vitro

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Introduction: Salmonid Alphavirus (SAV) is the causative agent of pancreas disease (PD), one of the most serious diseases in farmed Atlantic salmon and rainbow trout in Norway. There are currently two subtypes of the virus circulating in Norway, SAV2 and SAV3. We have shown in previous studies that the SAV3 genome can recombine during infection and generate so-called defective viral genome variants (DVGs). These DVGs are mainly characterized by deletions of varying in size, occurring in different parts of the genome. Still, some deletions have been found to occur at certain positions with higher frequency than in others. It has been shown for other RNA viruses that certain DVG types can be packaged, exit the cell, and reinfect new cells. Recent studies on mammalian viruses such as Chikungunya and Zika virus have shown that both the composition and number of DVGs produced during infection can influence the host immune response and disease severity.

Methodology: We present results from SAV3 genome sequence analyses following transfection of a plasmid-based infectious clone after serial passaging in Chinook salmon heart (CHH-1) cells. The sequence analyses were performed combining next generation sequencing and a computational approach previously used to detect DVGs in Chikungunya and Zika virus infections.

Results and conclusions: The sequencing data revealed a large number of different deletion types with sizes ranging from a few nucleotides to deletions covering most of the viral genome. Both the type and frequency of deletions generally increased with increased passaging with some deletion types accumulating to high frequencies. Some of the larger deletions, in particular those encompassing parts of non-structural nsp3/4 coding regions and/or all portions of the structural genes, reached high levels during later passages compared to the other deletion types. The putative mechanisms involved in generating these deletions and the potential future significance and applicability of DVGs in controlling PD, will be discussed.

Metagenomics: a new era of diagnostics for aquatic veterinary medicine

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Aquatic veterinary medicine services an expanding aquaculture industry. As aquaculture grows, so too does the battle against infectious aquatic diseases. PCR and histopathology excel at pathogen recognition but are reactionary processes that offer little insight into causation. A new era of diagnostics is emerging whereby total microbiomes are resolved via metagenomics, providing complete resolution of the microbial landscape, before, during and after health challenges. As metagenomics becomes affordable it has the power to provide veterinarians and health managers novel diagnostic insights and if implemented correctly, the power to foresee future health challenges. Here I will discuss how metagenomics yields insights PCR and histopathology cannot and explore the preventative capabilities of surveillance metagenomic programmes.

FORTIOR Genetics, a collaborative and innovative platform dedicated to improving farmed fish genetic resistance to diseases: overview and prospects

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Introduction: Genetic selection for disease resistance is an effective strategy to reduce the use of antibiotics and improve the health status of farmed fish but it implies to kill candidates or sibs in authorized ethical and welfare conditions. After R&D simulations and validation in mixt families breeding designs, the ANSES national reference laboratory for regulated fish diseases and SYSAAF created the FORTIOR Genetics platform in 2017 with the aim to improve genetic resistance and robustness towards the consequences of climate changes of the major species of farmed fish produced in Europe. ANSES facilities, hosting the platform, can supply tanks of different sizes with filtered, disinfected and thermoregulated fresh- or sea-water. They were recently up-graded through public funding.

Methodology: 1200 to 2000 sibs of commercially selected lines from 4 species (sea bass, sea bream, rainbow trout, turbot) can be challenged to 7 pathogens (Nervous necrosis Virus (NNV), Vibrio harveyi or anguillarum, Photobacterium damselae subsp.piscicida, Viral hemorrhagic septicemia virus, Infectious pancreatic necrosis virus, and Edwardsiella tarda) or 2 environmental factors (acute hypoxia or hyperthermia). Each challenge provide individual performances on “day of mortality” (disease challenge) or “time of loss of equilibrium” (hypoxia or hyperthermia challenges). Fin samples are collected for genotyping using 1k to 665k SNP arrays to establish their pedigree.

Results: Since 2017, 36 challenges have been conducted to estimate genetic parameters and breeding values (EBV or GEBV) for breeding companies or in national or EU R&D projects. For example, GWAS ran on data from sea bass/NNV challenges identified a major QTL close to IFI6 interferon candidate gene. Heritabilities of resistance to acute hypoxia or hyperthermia were computed (0.37 ± 0.04 and 0.32 ± 0.04, respectively) and GWAS identified several QTL.

Conclusion: To keep on the relevancy of the phenotyped traits, challenges are designed in a model of continuous improvement and protocol standardization. The platform is one of the first European platform supporting aquaculture breeding companies for disease resistance or climate change’s robustness phenotyping. Its operating model, involving public and private partners, is particularly relevant for generating synergy and developing innovative approaches.

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Seasonal and salmon farm production-specific variation in pathogen profiles in western Norwegian Fjords using environmental DNA surveillance

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Introduction: Infectious disease transmission is among the most pressing threats to sustainable aquaculture in Norway and many other aquaculture producing regions. There is a great deficit of knowledge regarding the spread of infectious agents in seawater between domestic and wild populations and among aquaculture sites or how infection risk varies throughout aquaculture production cycles. In an ongoing collaboration between researchers and industry partners (fish farm companies), the PATHDNA project consortium is adapting environmental DNA methods for viruses, bacteria, and

parasites to assess when, where, and how disease agents are spread to and from net pen salmon farms in western Norway.

Methodology: We are conducting a large-scale quantitative molecular investigation of 41 important pathogens across 20 production sites using Edna methods. In addition, assays targeting marine fish and salmonids are included to provide information on potential for pathogen exchange between wild and farmed fish. The surveys are replicated seasonally throughout the production cycle and fallowing periods, and combined with meta-data from the farms, used to determine how environmental pathogen profiles vary with seasonal and production-specific factors.

Results: Twenty-nine distinct pathogens have been detected in water samples collected from farm sites. Preliminary results have revealed spatial and temporal variation in the diversity and abundance of pathogen communities. We are exploring the relative influence of natural seasonal variation (e.g. temperature, salinity, turbidity) and production-specific variation on the dynamics of this diverse group of pathogen species.

Conclusion: This study provides a new approach for effective monitoring of infectious disease agents in aquaculture. Overall, this project is working to develop a holistic understanding of pathogen dynamics in Norwegian aquaculture that can be translated to improved disease management in this and other salmon aquaculture-producing regions world-wide.

Keyword: Edna, salmonid aquaculture, fish pathogens

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Evaluation of water environmental DNA and RNA for the prediction of enteric redmouth disease (ERM) in rainbow trout (Oncorhynchus mykiss)

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Introduction: Over the past recent years, there has been a growing interest in applying environmental DNA (eDNA) in various research fields like pollution response, air quality monitoring and invasive species detection. Aquaculture and in particular recirculated aquaculture system (RAS) is a promising food sector relying mostly on monospecific production. Consequently, outbreaks caused by various pathogens (e.g. bacteria, parasite, fungi, virus) represent a serious challenge to animal welfare and economic losses. The present study is part of the EU project RASOPTA which aims to increase knowledge regarding water quality, off-flavor, pathogen detection in RAS and develop a biomonitoring tool based on water sampling.

Methodology: In the present experiment, we investigate the sensitivity of water eDNA and eRNA to predict the onset of enteric redmouth disease (ERM) caused by Yersinia ruckeri, in rainbow trout. One week prior to infection, half of fish were subjected to unpredictable chronic stress to evaluate the effect of stress on fish immune response and mortality rates in relation with eDNA and eRNA levels of the bacteria. The fish were either mock-infected or expose to low (1E5 CFU/mL) or high (1E7 CFU/mL) dose of bacteria by bath infection. Fish spleens were sampled to evaluate immune response and bacterial load. Clinical signs were equally recorded to monitor disease occurrence. Water and fish were sampled regularly before the start of mortality and after the mortality had ceased.

Results and Conclusions: Results from the experiment will be compared and discussed to bring forward the optimal methods between eDNA and eRNA to evaluate occurrence of enteric redmouth disease in rainbow trout. Outcome from this study will contribute to extend knowledge which will provide a basis to develop technologies to predict and prevent outbreaks in RAS.

Antimicrobial susceptibility of aeromonas salmonicida subsp salmonicida isolated from rainbow trout (onchorhynchus mykiss) in france toward major aquaculture antibiotics

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Introduction: Furunculosis is a disease caused by a Gram-negative bacterium Aeromonas salmonicida subsp salmonicida (ASS), which can affect rainbow trout production at all stages of farming and requires sometimes the use of antibiotics. Unfortunately, limited data are available on the sensitivity of ASS to antibiotics.

Material and Methods: 372 bacterial strains of ASS isolated exclusively from diseased farm fishes were collected over a period of 10 years (2012-2021) in France. The identification at level specie of each isolate was carried out by combination of three techniques: specific characteristics of the bacterial culture (GSP coloration and TSA pigmentation), PCR and Maldi-Tof confirmation. All MICs data were obtained by the microbroth-dilution method in accordance with CLSI recommendations. The eight most commonly used antibiotics in fish farming were tested: florfenicol, oxolinic acid,

flumequine, enrofloxacin, oxytetracycline, doxycycline, sulfadiazine alone or in combination with trimethoprim. Provisional epidemiological cut-off (COwtpro) were calculated through the NRI method.

Results: For sulfadiazine alone, all strains studied were resistant, with very high MICs, while the association with trimethoprim revealed three distinct subpopulations, with a COwtpro of 4.8/0.25 mg/L. For quinolones, 2 subpopulations were observed, of which the most susceptible strains accounted for the majority of the strains (between 78 and 86%). COwtpro were 4 mg/L, 4 mg/L and 0.5 mg/L for oxolinic acid, flumequine and enroflocacin respectively. For florfenicol, 78% of the strains have a MIC below the COwtpro of 4 mg/L. In contrast, for tetracyclines, strains with MICs below the COwtpro (1 mg/L) represent only 43% of the strains. Over the period studied, the proportion of sensitivity of bacteria to the different molecules remains stable.

Conclusions: The complete absence of sensitivity to sulfadiazine raises questions about the relevance of its use for this indication, including in combination with trimethoprim, as in vivo synergy has never been demonstrated in trout. The high prevalence of ASS with high MICs for tetracyclines confirms the need to carry out antibiograms before using these drugs. Finally, the determination of provisional COwt can help to re-evaluate dosing regimens or to refine the clinical breakpoints for antibiograms and help veterinarians in choice of treatment/posology.

Surveillance of Gyrodactylus salaris with a non-lethal method in England and Wales

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Introduction: Gyrodactylus salaris (GS) is a monogenean parasite of Atlantic Salmon (Salmo salar) that can cause high levels of infection and mortality and is arguably the most important disease threat to salmon populations in the United Kingdom, which has recognised national Gs-free status. The Cefas Fish Health Inspectorate (FHI) conduct a surveillance programme under the Aquatic Animal Health Regulations (England and Wales) 2009 to maintain this status. Since 2018 a novel non-lethal sampling method developed by Cefas has been applied (Thrush et al., 2019) which replaces the previously destructive collection of fish for whole-body examination which impacted on already fragile wild salmon populations.

Method: Groups of 30 Atlantic Salmon parr per are sampled annually from 10-12 river catchments (in England and Wales) in collaboration with the Environmental Agency (EA). These are exposed to a hydrogen peroxide bath treatment for 3 minutes causing parasites to detach from their hosts which are collected by filtering the treatment water. Filter papers are stored in ethanol for molecular diagnostic analysis.

Result: This work has provided an important step forward in surveillance for this parasite, removing the need for destructive testing of juvenile salmon in threatened populations, reducing the resources required to implement testing and improving the sensitivity and confidence of surveillance designed to demonstrate freedom from disease. The method has been introduced into the national routine surveillance programme by the FHI and included in the World Organisation for Animal Health manual for diagnostic tests for aquatic animals.

Conclusion: The new methodology underpins a robust and defensible surveillance strategy for Gs which may be applied in other territories.

Reference: Thrush M.A., Taylor N.G.H, Hill T. (2019). Development of a non-lethal hydrogen peroxide bath treatment for surveillance of Gyrodactylus salaris on trout farms and its application to testing wild salmonid populations. Transboundary and Emerging Diseases. 66(5) 2107-2119.

Tumour investigation with various diagnostic imaging techniques in rainbow trout (Oncorhynchus mykiss, Walbaum 1792)

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The broodstock is of great value in the aquaculture. As it has been involved in production for a longer period of time, a number of diseases that may not affect the younger specimens can occur. In their case, cancers caused by the environment (water quality, feed) and/or pathogens also appear. Various diagnostic techniques are used in veterinary medicine to keep the population healthy and prevent from the diseases. Diagnostic imaging techniques could provide a new aspect of the ante mortem and post mortem diagnostic on the fish farms. Ultrasonography, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) could be used to examine internal organs and malformations. These methods can provide more information about the soft and hard tissues. The authors used diagnostic imaging techniques to evaluate and describe the tumours in rainbow trout broodstock. The fish were examined with portable Mindray M9Vet

and C5-1s convex ultrasound transducer, Siemens Somatom Definition AS+ CT scanner and Siemens Biograph Mmr scanner. The first animal was lethargic and showed muscular dystrophy and cachectic signs. The ultrasound investigations were performed under anesthesia. CT and MRI scans and tumour identification were performed during the post mortem investigation. Histopathology and immunohistochemistry were also implemented. The results showed soft tissue masses in the gastrointestinal system, which has proven to be adenocarcinoma. The malformations subsequently led to digestion and absorption disorders. Neoplastic cells of carcinoma revealed E-cadherin and pancytokeratin positivity by immunohistochemical staining. The authors started to use the ultrasonography routinely on the field as a diagnostic method to eliminate the affected animals from the broodstock.

First report of megalocytivirus in ornamental fish in Italy

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Introduction: The trade in marine ornamental fish is a multi-billion-dollar industry involving over 50 exporting and importing countries. Europe is one of the three major importers of marine ornamental fish, along with the United States and Japan. Megalocytiviruses are associated with severe mortality in ornamental fish aquaculture, including both freshwater and marine species. The international trade in live ornamental fish has aided the spread of these viruses. So far, megalocytiviruses have been detected in few European countries (Belgium and Germany), but, to the best of our knowledge, they have never been detected in Italy.

Methodology: In the context of a health surveillance programme on imported ornamental fish, histological lesions consistent with megalocytivirus infection were pointed out in two fish dead during the quarantine period. Case 1# referred to one neon damselfish (Pomacentrus coelestis) out of 10 subjects involved in a 100% mortality event in June 2018, whereas Case 2# was a prickly leatherjacket (Chaetodermis penicilligerus) dead after showing lethargic behaviour in June 2021. To investigate the presence of the megalocytivirus genome, FFPE tissue samples, including the areas with enlarged cells, were subjected to DNA extraction and PCR/real-time PCR analysis using two protocols previously described. PCR products were sequenced and subjected to phylogenetic analysis.

Results: The histological investigation showed in both species the presence in the kidney, spleen, liver, heart, gills, intestine of multiple hypertrophic cells containing granular to smudgy basophilic intracytoplasmic inclusions. Often, these cells were clearly endothelial cells. The presence of megalocytivirus DNA was pointed out in the two fish samples resulting positive to the real-time PCR and being identified as megalocytiviruses. Phylogenetic analysis of a fragment of the MCP gene from Case 2# showed its clustering within the RSIV-like group.

Conclusions: The presence of megalocytivirus was pointed out in imported marine ornamental fish for the first time in Italy. The viral DNA was detected in Pomacentrus coelestis and Chaetodermis penicilligerus, two marine ornamental species that, so far, have never been associated with megalocytivirus infection. Due to the high risk of importing megalocytiviruses through the ornamental fish trade, a strengthening of the surveillance programmes and quarantine measures is recommended.

Detection and characterization of Heterophyidae infections in the endangered Spanish toothcarp

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Introduction: Spanish tooth carp Apricaphanius iberus is an endemic and endangered fish species distributed in coastal lagoons along the Mediterranean coast of the Iberian Peninsula, suffering a decreasing trend in their populations.

In frame of the project Aphanius, unexpected mortalities were detected in wild specimens. An extremely large number of metacercaria were observed in the gills suggesting a relevant impairment of respiratory and osmoreguratory functions and allostatic capacity in these fish. Their structure and morphology highly suggested an Heterophyidae family of digenean trematodes. This study is focused on the characterization of these parasite populations at three nearby coastal lagoons with different environmental conditions.

Methodology: Spanish toothcarp specimens were sampled from three coastal lagoons at La Pletera salt marshes (L'Estartit, Costa Brava) during 2022 and 2023. Additionally, specimens of cohabitating mosquitofish and snails (Hydrobia sp.) were also sampled. The left gills of the fish were examined for the presence of parasite and the rest of the specimen and snails were fixed in 10% buffer formalin for histological studies. Some metacercariae were preserved in 100% ethanol for molecular studies. Some snails were maintained alive during a week to test emergence of cercariae.

Results: Metacercariae were found in the gills of all the analyzed Spanish toothcarps, located in both filaments and gill arches. High mean abundances were detected in all lagoons, but with great disparity among them, ranging between 66 to more than 500. Cohabitating mosquitofish species did not present any metacercaria, suggesting a high host-specificity of the parasite.

Cercariae emerging from the snails were also identified, thus strongly suggesting that they act as first intermediate host of the parasite cycle.

By histological studies, metacercariae were also detected in different internal organs of fish and sporocysts could be also identified within snails.

Conclusions: The present study indicates that heavy infections by Heterophyidae digenean metacercaria in Spanish toothcarp should be considered as an added risk for the survival of wild populations of this endangered species. The influence of environmental conditions and ecological traits of the different lagoons on the parasitic infection will also be discussed.

Impact of Eye Flukes on Freshwater Fish: Field and Experimental Investigations

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Introduction: Eye fluke infections in freshwater fish are common, resulting from the penetration of cercariae released from freshwater snails. High infection pressures may be associated with mortalities. Our field investigations in two Danish lakes clearly indicated the pathogenicity of these parasites. We further performed a laboratory study, by infecting zebrafish with eyeflukes and subsequently followed the reactions. The results supported this notion.

Methodology: To investigate the prevalence and impact of eye fluke infections, we examined 77 freshwater fish from two lakes. The infection level suggested a high cercarial infection pressure in the Danish lakes. To further elucidate short-term effects on the fish host, we infected zebrafish Danio rerio with cercariae of the prevalent species Diplostomum pseudospathaceum.

Results and Conclusions: The dominant genera identified in the fish samples from the Danish lakes were Tylodelphys and Diplostomum, covering a range of species identified by PCR and sequencing of the 18S (partial)-ITS1-5.8S-ITS2-28S (partial) of the rDNA. Exposure of zebrafish to 200-400 cercariae did not result in abnormal behavior, but dosages of 600 and 1,000 cercariae/fish proved lethal. When exposed to sublethal dosages, 19 out of 27 immune genes were significantly regulated. At 3 hours post-infection (hpi), three genes encoding cytokines (IL 4/13B, IL-6, and IL-8) were upregulated, whereas others were downregulated, particularly at a later time point. We suggest that direct massive cercarial penetration of fish surfaces may be detrimental and may represent a threat to fish populations.

Spinal damage in European eels - Incidence and possible causes

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Introduction: Eels are catadromous migratory fish that migrate downstream to reach their spawning grounds in the sea. On their way, they are exposed to numerous risks, such as passages through hydropower plants. Due to their body shape and length, as well as their swimming behaviour and preference to swim with the main current, eels are more susceptible to injury from a turbine passage than other fish species.

Methodology: Four groups of eels were studied. The first group was caught just downstream of a hydroelectric power plant and showed no external abnormalities. The second group of eels, which also showed no abnormalities, was caught not in the vicinity of a hydroelectric power plant. The third group were animals from group 2, which were released back to the river and recaptured using standard fishing techniques to evaluate the effects of fishing on spinal damage. The fourth group of eels was caught in the area downstream of a hydropower plant, with most of the captured animals showing clear impairments in their swimming behaviour. All eels were examined for external damage and anaesthetised, X-rayed and examined for spinal damage.

Results: In 47 % of the eels from group 1 spinal damages were detected radiographically. Animals of group 2 showed spinal column damage in 19 %. After recapture, only one of the eels showed an additional minor compression of two vertebral bodies. Eels of group 4 had 88 % spinal column damage. Not all animals with spinal damage also had external injuries.

Conclusion: Fishing techniques do not seem to be responsible for spine damage in eels. Rather, a correlation between passage through a hydropower installation and the occurrence of spinal damage can be observed. However, an external examination of eels does not allow any conclusion on the presence of spinal damage. Damage to the spinal column could lead to impaired swimming behaviour and increased losses during further migration to spawning grounds in the sea.

Interest of non-lethal sampling methods in detection and genotyping of Carp Edema Virus in Koi trade

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Introduction: Carp Edema Virus (CEV, Poxviridae family), is an emerging pathogen associated to a disease causing high mortalities in common and koi carps (Cyprinus carpio). CEV has been detected in many countries, sometimes associated with trading context. For koi, CEV surveillance, which requires lethal sampling methods, is usually not performed in non-symptomatic batches. This study aimed to develop new non-lethal sampling methods for CEV early detection.

Methodology: All samples were collected in imported koi batches from Japan to a French wholesaler facility (2019 - 2022). Various shipping environmental samples (water and bag swabs) were collected. In 2022, gill swabbing was performed shortly after arrival and gills of naturally dead fish were analyzed too. After DNA extraction, CEV detection and quantification were performed by qPCR. Positive samples were genotyped (partial P4a gene).

Results: CEV DNA was detectable in most (45 – 100%) of shipping water and/or shipping bag swabs of batches coming from different Japanese breeders. Unexpectedly, most of dead fish gills and gill swabs from positive shipping water batches were CEV-negative, suggesting that monitoring water was more sensitive than analyzing individuals.

As expected, all the analyzed samples clustered in genogroup II which is usually associated with koi. Despite all batches has originated from Japan, sequences were very similar to strains reported in various countries.

Conclusions: Shipping water is an easy-to-collect and effective sample for early detection and genotyping of CEV in imported batches of koi. We will keep monitoring CEV strains imported into the area, looking for partial P4a sequence and other genes variation over time.

Diving into fish pathology: the case of merluccius merluccius from the Catalan coast (NW Mediterranean sea)

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Introduction: Merluccius merluccius is one of the most important target species of commercial fisheries in the NW Mediterranean. Assessing the potential diseases that fish stocks may contract is fundamental for establishing baselines for evaluating future changes. Pathology and histopathology are effective diagnostic techniques to detect diseases, parasites and alterations that can occur in these populations. Hence, the aim of this study is to describe the gross pathology and histopathological alterations identified in juvenile specimens of M. merluccius from the Catalan coast (NW Mediterranean).

Methodology: A total of 108 hakes were collected on the continental shelf of the Catalan coast (NW Mediterranean) in 2007 (n=46) and 2019 (n=62) and were immediately fixed in toto in 10% buffered formalin. Fish external surfaces and internal organs were inspected macroscopically for alterations and parasites under a stereomicroscope. Then, samples of liver, spleen, and gills were processed by routine paraffin histology. Moreover, for specimens collected in 2019, a portion of gonads, kidney, stomach and intestine were also processed. Each sample was screened and examined under a microscope for histopathological assessment.

Results: No macroscopic alterations were found in any organ but several parasites (i.e. copepods, monogeneans, nematodes) were detected. Microscopically, in 2019 specimens, alterations identified in gills included foci of inflammation and hyperplasia (67.7%), extensive hyperplasia and diffuse inflammation (21.0%) that were potentially related to gill parasites, cysts of unknown aetiology (46.7%), and lamellar inflammation associated to Aporocotyle spinosicanalis eggs (11.3%). Granulomas, pigmented macrophages and inflammatory focus were slightly detected in the liver, spleen, kidney and stomach apparently associated with the presence of nematodes. Coelozoic myxosporean parasites were detected within the renal tubules (63%). A temporal comparison of the prevalence of alterations with respect to specimens from 2007 is provided.

Conclusions: Most of the alterations and pathologies detected on M. merluccius are similar to those described in other gadoid species (i.e. Gadus morhua, Micromesistius poutassou). Alterations found in gills and internal organs were usually related to the presence of ecto and endoparasites. Although the prevalence was high, the intensity and extension of the lesions found were limited. No alterations related to other potential causes were detected.

Black spot syndrome in wild rock cook (Centolabrus exolatus; Labridae)

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Introduction: Wild caught wrasse are used as cleaner fish in seawater farming salmonids, removing sea lice (Caligidae). These fish may be transported and released into net pens in geographical areas distant from their origin. There is always a risk of transmitting disease when translocating live fish. Over the last years there has been an increase in the observations of black spots on wild rock cook (Centolabrus exolatus) in the Austevoll area in Norway. We refer to this condition as “black spot syndrome” since a causative agent has not been identified. The individuals develop various types of black spots (hyperpigmentation).

Methodology: The syndrome has been routinely monitored in a scientific survey from 2017 until present. In addition, samples of affected tissue (black spots) were fixed in both Karnovsky fixative, and 10% neutral phosphate buffered formalin for histological examination.

Results and conclusions: The black spots are variable in size and in some cases, most of the body may be covered. There is a positive correlation between fish size and the black spots prevalence. In addition, females seem to be more affected than males. The prevalence increased considerably in 2018, followed by a marked reduction in the abundance of rock cook in 2019, with no signs of recovery since. In the same period and area, there have been sporadic observations of black spots on other wrasse species, the corkwing wrasse (Symphodus melops), the goldsinny wrasse (Ctenolabrus rupestris) and Cuckoo wrasse (Labrus mixtus).
Histopathological examinations of the hyperpigmented areas and affected tissue show changes in both epidermis and dermis with infiltration of inflammatory cells and melanomacrophages, oedema, connective tissue and development of granulomas.

We will present our updated prevalence data and our histopathological findings.

First identification of mycobacteriosis in Atlantic mackerel (Scomber scombrus)

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Introduction: Mycobacterium infections in fish is a well-known disease problem globally, mainly in farming of ornamental fish or fish for food. Less is known about prevalence, distribution, and the effect such infections have on wild fish species. Presumptive mycobacteriosis has previously been observed in Atlantic mackerel (Scomber scombrus). From 2018 there has been an increase in reports of a granulomatous kidney disease in Atlantic mackerel with the suspicion of this being mycobacteriosis.

Methodology: Institute of Marine Research received six individuals for further examination. These were caught in the North Sea by either commercial fishing vessels or during the International Ecosystem Summer Survey in the Nordic Seas (IESSNS research cruise) 2018-2020. Samples for both histological and molecular analysis were collected from affected inner organs. Histological sections were stained with Ziehl-Neelsen AFB-colour staining kit (cold staining). DNA was extracted from tissue samples and, 16S rDNA-ITS and selected protein genes were amplified and sequenced.

Results and conclusions: On the basis of rDNA and protein gene sequences, we detect a likely novel Mycobacterium species in tissue samples from Atlantic mackerel with this condition. The same unnamed bacterium seems to have been found in some Pacific marine fishes. Histological examination of affected tissue samples confirmed presence of granulomas with acid-fast bacteria. The macroscopic and histological manifestation of the disease will be described. Over the past years there has been an increase in reports of mycobacteriosis worldwide and climate change has been suggested as one of the driving forces as these bacteria prefer warm water.

Deciphering Sparicotyle chrysophrii extracellular vesicles: an inside-out journey within a monogenean parasite

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Extracellular vesicles (EVs) are membrane-bound structures classified as exosomes or microvesicles according to their size and origin. EVs of parasitic helminths participate in communication with its conspecifics, the host and the surrounding microbiota. There is a knowledge gap on how the monogenean Sparicotyle chrysophrii (Microcotylidae) interacts with its fish host. Thus, this study focuses on an in silico screening of S. chrysophrii EV's biogenesis machinery, EVs identification and location within the parasite, and the analysis of their protein cargo, with emphasis on therapeutic target candidates.

Adult S. chrysophrii specimens (N= 200), obtained from experimentally infected gilthead seabream, were incubated in vitro in sterile filtered marine water for up to 3 days. Parasite EVs were isolated by ultracentrifugation and size-exclusion chromatography (UC-SEC) and characterised via nanoparticle tracking analysis (NanoSight NS300). Purified EVs underwent liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis coupled to a TimsTOF pro mass spectrometer to identify their protein cargo. Furthermore, sequences of all known proteins involved in human EVs biogenesis were obtained from the UniProt database and S. chrysophrii draft genome was mined for homologs using Basic Local Alignment Search Tool (tblastn). In addition, three adult specimens were high-pressure frozen (HPF/FS) and observed under the transmission electron microscope (TEM).

The highest concentration and purest EVs sample was obtained on the second day of the parasite's in vitro maintenance (2.33e+08±1.12e+07 particles/ml and 6.89e+08 particles/µg of protein, respectively), with a higher prevalence of ≈200 nm size nanoparticles population. From the 77 proteins involved in human EVs biogenesis, 57 orthologs were found in S. chrysophrii draft genome. Finally, the protein cargo analysis identified 17 structural proteins related to EVs biogenesis and 24 potential therapeutic target candidates; proteases, hydrolases, iron transporters, enzymes involved in haem detoxification, and some unknown proteins exclusive to S. chrysophrii. TEM showed that EVs were located in cephalic channels adjacent to the oral suckers.

In this study, we infer the presence of EVs in S. chrysophrii, their biogenesis machinery and location in the parasites, and identify several candidate therapeutic targets among EV's protein cargo, offering opportunities for target-based drug discovery approaches.

Host-parasite interactions: Innate immune responses of zebrafish (Danio rerio) and immune evasive behavior of a fish parasite

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Ichthyophthirius multifiliis is a protozoan fish parasite causing white spot disease in a wide range of freshwater fish species worldwide. It infects epidermal surfaces such as fins, gills and skin and causes high morbidity and mortality. I. multifiliis resides just below the outermost epidermal layer of the fish, which gives us a unique opportunity to visualize host-parasite interactions. The zebrafish has a level of natural resistance towards the parasite, which allows investigations on protective mechanisms in this unique model organism. Therefore, zebrafish were utilized to elucidate acute immunological reactions towards I. multifiliis. Larvae from a double transgenic reporter line with green-fluorescent neutrophils and red-fluorescent macrophages were infected with the parasite and the host-parasite interactions were studied using in vivo real-time imaging. In addition, immune-relevant gene expression of the host was investigated using real-time qPCR. Different host-parasite interactions were observed and are discussed. It was documented how neutrophils and macrophages, in some instances, were able to kill the parasite and it was evident that neutrophils utilized neutrophil extracellular traps (NETs) in the process. The parasites created an interstitial space where they rotated vigorously, and we propose that this behaviour is an immune evasion strategy to avoid direct contact with host innate immune cells. Whole body gene expression revealed that a mild acute immune response was induced in the larvae, indicating that strong responses were focused locally around the parasites. In this study new knowledge on host-parasite interactions was obtained and once again the zebrafish proved to be an excellent model organism to investigate in vivo reactions.

Unraveling molecular interactions in a host-parasite model: Integration of different omic approaches in gilthead seabream infected with Sparicotyle chrysophrii

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Sparicotyle chrysophrii is a monogenean parasite that infects the gills of gilthead seabream (Sparus aurata) causing hypoxia, anemia and emaciation. It is an important economic problem in Mediterranean aquaculture with limited management, mitigation and treatment strategies. A deeper knowledge of the host response during S. chrysophrii infections is crucial to develop preventive or therapeutic strategies. Here, we study the molecular pathways occurring at local and systemic levels upon severe sparicotylosis and integrate results from different omic approaches to improve our understanding of the mechanisms behind the disease.

Illumina RNAseq was performed on gills, spleen and liver samples of gilthead seabream with severe sparicotylosis (>75 parasites/fish, mean hemoglobin 2g/dL). Healthy individuals kept under the same conditions were used as control. Differentially expressed transcripts (DET) were identified and pathway analysis was conducted. Results were integrated with gill microbiota, histopathology and plasma proteomics information available for the same animals in order to unravel the most relevant pathways and holobiont interactions in this host-parasite model.

A total of 759, 337, and 603 DET were found in gills, spleen and liver, respectively, with 16 commonly regulated transcripts, including important genes related to the pathogenesis of the parasite, such as hemoglobin subunits or hypoxia-inducible factors. The most relevant regulated pathways in infected fish were immune system; response to stress, starvation and hypoxia; apoptosis; and hemostasis. Twenty-nine DET were shared with a previous plasma proteomics study, highlighting hemoglobin subunits, immunoglobulins, complement proteins and apolipoproteins. In liver, 54% of the regulated pathways were shared with the proteomics study. Gill microbiota and histopathology observations showed that, upon parasite infection, there is secondary infection by a bacteria genus causing epitheliocystis. Integrating all the results, we separated genes and pathways specifically affected by the primary (monogenean: e.g. Hemostasis, Complement activation) and secondary (bacterium: e.g. Extracellular matrix organization) infections.

These results evidence a series of host molecular pathways involved in the pathogenesis of sparicotylosis, such as hemostasis, response to hypoxia and lipid metabolism. Key genes and interactions identified using this integrative approach contribute to the understanding of the disease and can constitute targets for further research pointing to solutions for this infection.

Immune modulation – a comparison between the generalist Caligus elongatus and the specialist Lepeophtheirus salmonis

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Both salmon louse (Lepeophtheirus salmonis) and Caligus elongatus have glands with secretory ducts ending at the host-parasite interface assumed to produces immunomodulatory and anti-coagulant substances. As knowledge of these substances can result in new and better counter measures against lice, we have conducted morphological and functional studies on these glands in both salmon louse and C. elongatus. Both lice species have the same gland types, where the salivary gland is of special interest. The salivary glands are developed at the copepodid stage when both louse types are ready to infest a host and secrete their products distally in the mouth tube near the mandible teeth. The saliva is thus expected to be deposited onto the feeding site of both louse types. We have confirmed the expression of several genes in the salmon lice's salivary glands, and many of these are also identified in the salivary glands of C. elongatus. Functional studies in both species have been performed to elucidate the function of these salivary gland proteins, and particularly one protein seems to be very important to down modulate the immune response by inducing apoptosis in salmonid leucocytes. The salmon's immune response when co-infested with salmon lice and C. elongatus at different stages were also conducted, and correlates to the function and expression level of the salivary gland genes.

Ubiquitination in response to Infectious salmon anaemia and Infectious pancreatic necrosis virus in Atlantic salmon (Salmo salar)

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Atlantic salmon (Salmo salar) is one of the most globally economically important marine aquaculture species1. Improving our understanding of the teleost immune response to pathogens could help combat the high disease burden in the aquaculture sector. Ubiquitination is an essential post-translational modification and is known to play critical roles in the innate immune system in mammalian species2. High levels of conservation of the amino acid sequence of ubiquitin, spanning from yeast and to humans imply that its immune role of ubiquitin in humans may be conserved in other vertebrates3. Despite evidence of many virus-inducible ubiquitination genes in fish4 and the observed upregulation of the ubiquitination pathways in infected fish5, the role of ubiquitination in infection is still poorly understood.

Method: By combining RNA sequencing with mass spectroscopy proteomics, we demonstrate here dramatic changes in the ubiquitination state of host proteins upon different infections.

Results and Conclusions: Upon ISAV infection we see moderate transcriptomic response (157 differentially expressed genes at 24hr), yet a large increase in ubiquitinated host cell proteins, including known immune effector proteins such as TRIM256. In contrast to this, upon IPNV infection we see massive upregulation of gene expression (1033 differentially expressed genes at 24hr), yet a large downregulation of ubiquitination response, relative to uninfected cells. These results also highlight a direct interaction between the host ubiquitination machinery and viral proteins. By combining transcriptomic and proteomic techniques, we hope to start to understand ubiquitination's role in orchestrating the immune response in Atlantic salmon. This understanding may lead to the development of selective breeding candidates, gene editing targets, and improvement in vaccination.

Acute inflammatory response to Koi herpesvirus is characterized by pro-inflammatory M1 type macrophages

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Introduction: Koi herpesvirus (KHV, CyHV-3) establishes a long-life infection through latency in Koi and common carp (Cyprinus carpio L.). Although white blood cells (WBC), particularly IgM-positive B lymphocytes, can support KHV latency, the molecular mechanisms of KHV infection in WBC remain largely unknown. This study aimed to identify key molecular processes occurring during acute KHV infection in WBC before a humoral response and to identify leukocyte subset differentiation in response to the viral infection.

Methodology: Common carp were bath-exposed to KHV at 17°C. Blood samples were collected at 10 days post-challenge and WBCs isolated using a percoll gradient. Illumina-indexed sequencing libraries of WBCs were prepared with ribo-zero rRNA depletion and sequenced on an Illumina NovaSeq platform.

Results: Transcriptome analysis identified host and viral transcripts that were differentially expressed between the cells of infected and control fish. The most abundant viral transcripts were the ORF68 a myosin-like protein, the ORF35 an unknown viral protein, the ORF92 a major capsid protein, and the ORF62 a putative polypeptide substrate binding site. Hierarchical clustering of host differentially expressed transcripts identified clear different patterns between naïve and infected WBC. Gene Ontology annotations and KEGG pathways enrichment showed that biological processes related to macrophage chemotaxis, cytokine-mediated cytokine pathways, and ligand binding were strongly activated, whereas oxygen transport, glutathione metabolic processes, lysine degradation, iron transport, and basal transcription factors were strongly suppressed on infected leukocytes. We also report strong activation of CD86+ macrophages and CD59 regulatory complement, and down-regulation of CD8+ T cells, while the surface receptor urokinase plasminogen activator and the inhibitory receptor programmed cell death protein 1-like were strongly up-regulated during KHV infection.

Conclusions: Our data showed that acute KHV infection in susceptible carp is characterised mainly by activation of macrophages and that iron deprivation might play a role towards an M2-like anti-inflammatory phenotype of activated macrophage polarization. These data could be utilised to identify targets for the development of therapeutics for this aquatic disease.

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Microsporidium Hepatospora eriocheir - an emerging pathogen of aquatic invader Chinese mitten crab from European lagoons and estuarine areas.

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Introduction: Emerging diseases can be defined as infectious diseases that recently expanded their geographic or host range, or prevalence. The role of biological invasions in the emergence of diseases is still under debate. Many invasive species lose their pathogens during the invasive process, other taxa introduced novel parasites into colonised areas. The microsporidia are a diverse parasite phylum infecting hosts from all major taxa in all environments, ranging from the beneficial insects and aquatic animals, to important parasites of humans. Hepatospora eriocheir was first identified in 2007 in the cultured E. sinensis from China, as a pathogen suspected of causing hepatopancreatic necrosis disease with a high mortality rate. Here, we describe infection of a microsporidian parasite Hepatospora eriocheir in invasive Chinese mitten crabs (Eriocheir sinensis).

Methodology: In years 2020-2022 internal organ samples (hepatopancreas) were collected from crabs caught from the Vistula Lagoon (Baltic Sea) and estuarine areas of rivers Schelde (Belgium) and Elbe (Germany) in North Sea. Pieces of the tissues preserved in ethanol were lysed and DNA was isolated. Molecular detection of the partial sequence small subunit ribosomal RNA (SSU rRNA) revealed the presence of the parasite at all three sampling sites.

Results and Conclusions: Our study is a first report on the presence of microsporidian parasite Hepatospora eriocheir infecting non-native Chinese mitten crabs from the Baltic Sea and estuarine areas of the North Sea. Phylogenetic analysis based on DNA coding conserved regions of microsporidian small subunit ribosomal revealed that the parasites had almost 100% sequence identity to that of H. eriocheir from the United Kingdom and indirectly from China. This supports the theory that they were introduced with the invader crab during its first invasions to European waters and Baltic Sea in the early 1900s.

Marker assisted selective breeding as a tool to increase health of aquacultured fish

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Introduction: The susceptibility/resistance of fish to a series of infectious diseases is genetically determined. Genetic tools such as the 57 K microarray for detection of single nucleotide polymorphisms in the rainbow trout genome have facilitated marker assisted selective breeding of fish with elevated resistance to a number of diseases. We here show examples for bacterial and parasitic diseases.

Methodology: The basis for the process is experimental infection of rainbow trout (preferably of 1000 fish) with a specific pathogens. We have conducted the experimental infection for 1) Yersinia ruckeri, 2) Aeromonas salmonicida, 3) Vibrio anguillarum, 4) Flavobacterium psychrophilum, and Ichthyophthirius multifiliis. During each of these infection experiments we surveyed the fish around the clock and sampled moribund fish for DNA-typing using the 57 K microarray (Axiom®Trout). Subsequent analyses pin-pointed chromosomes associated with some level of resistance to the specific disease. Samples were also taken for gene expression analyses in order to describe immune pathways activated in fish with clinical signs, fish with no clinical signs and surviving fish.

Results: The resistance of rainbow trout against Vibrio anguillarum was associated with genes on Omy21, resistance against Flavobacterium was associated with genes on Omy25 and resistance against Ichthyophthirius was associated with genes on Omy 16 and 17. The resistance against Aeromonas and Yersinia was polygenic. We subsequently validated the finding by producing new generations based on male and female parents carrying the SNPs associated with resistance. The gene expression analyses gave indications of how various immune organs and different immune pathways were involved against the different pathogens examined.

Conclusions: The use of marker assisted selective breeding has a potential to elevate the health of fish in aquaculture. The knowledge on the precise location of genes associated with disease resistance will make it possible to establish fish lines with elevated resistance to several diseases.

Transcriptomic Analysis on the Rock Bream Oplegnathus Fasciatus Spleen under Red Sea Bream Iridovirus Infection

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Red sea bream iridovirus (RSIV) which is listed by the World Organization for Animal Health (OIE) as having an infectious impact on Japanese aquaculture and causing high economic losses. The aim of this study is to investigate the kinetics of RSIV and the host gene expression during infection in rock bream Oplegnathus Fasciatus, a species highly sensitive to the virus. After intraperitoneal injection of the virus, viral genome copy numbers in the spleen were evaluated by qPCR on 3 and 5 days post-viral injection (dpi). The tissues were applied to total RNA extraction and transcriptome analyses were performed using the next-generation sequencer MiSeq. The reads were mapped to the viral genes to evaluate the virus gene expression profiles, while for the host gene expression profiling, the reads were assembled using the Trinity program, and differential gene expression analyses were performed. The virus genome copy number in the spleen were 4.7 ±0.2 and 5.9 ± 0.4 copies/μg DNA on 3 and 5 dpi, respectively. The viral gene transcripts were detected on both 3 and 5 dpi, and 6 genes including RING-finger domain-containing protein and laminin-type epidermal growth factor-like domain genes were significantly expressed on 5 dpi. Among the 6 genes, ORF539R is a novel and highly expressed gene. On the other hand, 334 of the host genes showed differentially expressed compared to those before infection. The genes were clustered into four groups based on their expression profiles: the first group showed up-regulation at 5 dpi, the second showed up-regulation at 3 and 5 dpi, the third showed down-regulation at 5 dpi, and the last showed down-regulation at 3 dpi. By focusing on the differentially expressed genes involved in immunity, interferon-stimulated genes were more prevalent in the first and second groups. In contrast, the fourth group included granzyme and eosinophil peroxidase genes. This study not only identified a new candidate antigen gene but also provides new insights into the mechanism of RSIV infection.

Novel cell culture and single-nuclei RNA-sequencing methodologies for the study of WSSV response in Pacific whiteleg shrimp

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Introduction: Shrimp are one of the most important groups of animals in global aquaculture. The Pacific whiteleg shrimp (Litopenaeus vannamei) is the most cultured species, accounting for over 50% of the shrimp worldwide production. Unfortunately, the industry's efficiency and sustainability are threatened by significant annual losses due to infectious diseases, 60% of which are caused by viral pathogens such as white spot syndrome virus (WSSV). Efforts to limit the impact of disease caused by this pathogen are impeded by a lack of effective treatments available.

Methodology: In order to establish whiteleg shrimp primary cell cultures, we extracted haemolymph, lymphoid organ and hepatopancreas samples from sterile shrimp and cultured them as dissociated cells or explants in a range of cell media mixes while monitoring the growth and survivability of cells. For RNA sequencing, we isolated nuclei from snap-frozen hepatopancreas tissue and processed it using 10x Chromium Next GEM Single Cell Dual Index kits. The sequenced samples have been analysed and the cell atlas was built using cell cluster marker genes.

Results: Our team has established an in vivo infection model in juvenile and adult stage shrimp and developed primary cell culture systems for the study of host response to WSSV using haemocytes, hepatopancreas and lymphoid organ tissues from adult L. vannamei. Additionally, we have established a novel protocol for shrimp nuclei isolation and single nuclei RNA-sequencing, and generated a hepatopancreas cell atlas for the species.

Conclusion: The novel protocols will help us use high throughput transcriptomic analysis to identify priority candidate genes for WSSV resistance via single-nuclei RNA sequencing of WSSV-infected and non-infected shrimp. The results will serve as a foundation for future studies using in vitro models and single-nuclei sequencing in whiteleg shrimp and set a new base for developing therapeutic strategies to combat WSSV in shrimp aquaculture.

Genotypic Characterization of Infectious Spleen and Kidney Necrosis Virus (ISKNV) in Southeast Asian Aquaculture

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Infectious spleen and kidney necrosis virus (ISKNV) is a species within the genus Megalocytivirus (family Iridoviridae), which causes high mortality disease in many freshwater and marine fish species. ISKNV was first reported in Asia and is an emerging threat to aquaculture with increasing global distribution, in part due to its presence in ornamental fish with clinical and subclinical infections. The species ISKNV includes three genotypes: red seabream iridovirus (RSIV), turbot reddish body iridovirus (TRBIV), and ISKNV. There is an increasing overlap in the recognized range of susceptible fish hosts and the geographic distribution of these distinct genotypes. To better understand the disease caused by ISKNV, a nucleic acid hybridization capture enrichment was used prior to sequencing to characterize whole genomes from archived clinical specimens of aquaculture and ornamental fish from Southeast Asia (n = 16). The method was suitable for tissue samples containing 2.50 × 104–4.58 × 109 ISKNV genome copies mg−1. Genome sequences determined using the hybridization capture method were identical to those obtained directly from tissues when there was sufficient viral DNA to sequence without enrichment (n = 2). ISKNV genomes from diverse locations, environments, and hosts had very high similarity and matched established genotype classifications (14 ISKNV genotype Clade 1 genomes with >98.81% nucleotide similarity). Conversely, two different genotypes were obtained at the same time and location (RSIV and ISKNV from grouper, Indonesia with 92.44% nucleotide similarity). Gene-by-gene analysis with representative ISKNV genomes identified 59 core genes within the species (>95% amino acid identity). The 14 Clade 1 ISKNV genomes in this study had 100% aa identity for 92–105 of 122 predicted genes. Despite high overall sequence similarity, phylogenetic analyses using single nucleotide polymorphisms differentiated isolates from different host species, country of origin, and time of collection. Whole genome studies of ISKNV and other megalocytiviruses enable genomic epidemiology and will provide information to enhance disease control in aquaculture.

Atlantic salmon resistance to Piscirickettsiosis: Comparison of GWAS between vaccinated and non-vaccinated fish under laboratory and field conditions

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Introduction: The use of vaccination and selective breeding of unvaccinated fish is widely recognized as an effective approach for disease prevention and control in global aquaculture. However, there is limited research demonstrating the complementary nature of these methods. Piscirickettsia salmonis, an intracellular bacterium, has been particularly challenging to control through vaccination in Chile, leading to the implementation of selective breeding of vaccinated and unvaccinated fish. This work aims to assess the effectiveness of these methods in preventing P. salmonis infection and to identify potential challenges.

Methodology: We report and compare two genomic association studies (GWAS) of vaccinated and non-vaccinated smolt fish challenged against Piscirickettsia salmonis under laboratory conditions with GWAS performed on vaccinated adult naturally challenged against Piscirickettsia salmonis in sea farming conditions. Samples come from the salmon breeding program of Salmones Camanchaca and search for SNPs associated with resistance against Piscirickettsiosis in the Lochy strain.

Results: Heritability of resistance against P. salmonis ranged from 0.29 to 0.58 (0.29 in unvaccinated smolt; 0.39 in vaccinated smolt; and 0.58 in vaccinated adult). Fish that survived the natural outbreak of the disease showed lower frequency of external lesions compared to fish that died. GWAS performed under laboratory conditions (smolt) revealed significant QTLs for P. salmonis resistance on chromosomes 11, 12 and 26. QTLs exclusively associated to P. salmonis resistance in vaccinated smolt and adult were found on chromosome 12 and 2 respectively. Candidate genes were associated with receptors in immune response pathways, with immune system homeostasis, with regulation of B cell function, apoptosis and Proinflammatory cytokines.

Conclusions: This research supports the hypothesis that resistance to Piscirickettsiosis is a polygenic trait, and confirms that vaccination, the farming environment, and perhaps the age of the fish can modify the genetic architecture of this trait. Thus, identifying an adequate selection strategy against this pathogen remains a complex and challenging issue.

Rapid, Cost-Effective SNP Genotyping Using Standard BioTools SNPtype Assays and X9 Real-Time PCR System

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Introduction: The salmon research community has been specifically hampered by the cost barriers, and would benefit from the technology for conservation and management purposes. Using chum salmon (Oncorhynchus keta) as an example, we describe a simple workflow using Standard BioTools SNPtypeAssays, the X9 System, and 96.96 Dynamic Array™Integrated Fluidic Circuits (IFCs) for Genotyping to achieve cost-effective and rapid development of a SNP genotyping panel.

Method: For the chum salmon panel development, a candidate list of 143 SNPs was selected based on their ability to provide population structure and harvest composition information within the chum species of salmon. The sequences around these SNPs were submitted to the Standard BioTools Assay Design Group for design and manufacture of allele-specific PCR primers. TaqMan® assays were also ordered for the same SNPs for comparison. Assays were run with 95 samples from three different locations in Washington State (HammaHammaRiver –24, Kalama River –24, Skookum River –23), and one from British Columbia (SquakumCreek –24).

Results/Conclusions: All 143 SNPtype Assays were tested with the 95 samples mentioned in the Introduction, and 107 high-quality assays were selected, which was more than the final panel size of96. Data obtained showed >90% correlation between Taqman and SNTtype Assays and demonstrates how integrated fluidics circuits can be used as a cost-effective and rapid system for genotyping salmon species.

Immune priming in the Pacific oyster as an approach to improve resistance against the wide-spread pathogen Vibrio aestuarianus

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Introduction: Bivalve molluscs represent an aquaculture sector of high socio-economic value, yet effective management of disease outbreaks remains challenging. For Pacific oyster (Crassostrea gigas) production, the most notable pathogens of concern include ostreid herpesvirus 1 and the bacterium Vibrio aestuarianus, which have been associated with mass-mortality events in recent years. Immune priming, whereby the invertebrate immune system is trained to respond to infection through previous pathogen exposure, could offer an opportunity to improve disease resistance in bivalve aquaculture. This project aims to investigate whether exposure to inactivated V. aestuarianus during early life stages of C. gigas primes an immune response to provide resistance upon re-exposure later in life, and aims to investigate the molecular mechanisms underpinning this process.

Methodology: We primed Pacific oyster veliger larvae and young spat with heat-inactivated V. aestuarianus, via bath exposure. Spat were challenged at 6-months-old via a bath exposure of a fully virulent V. aestuarianus, for 40 hours. Survival was monitored for 2 weeks and samples were taken for molecular analysis prior to the challenge, and at 16 hours, 72 hours, and 2 weeks post-challenge. We conducted transcriptomic analysis using RNA-Seq on the Illumina NovaSeq platform using whole spat samples collected 72 hours after the initiation of the challenge to identify any alterations in gene expression between the primed and non-primed treatments.

Results: Survival to 2 weeks following challenge initiation did not differ significantly between the primed and non-primed treatments. Analysis of RNA-Seq data is now being conducted to characterise the responses to V. aestuarianus challenge and whether this differed between naïve and primed oysters at either veliger larvae or spat stages.

Conclusions: If we successfully identify alterations in the transcriptional response to infection associated with immune priming, we will conduct further work to investigate the hypothesis that they arise via epigenetic mechanisms. We expect the results to contribute to our understanding of the mechanisms via which priming of invertebrate immunity occur and how this can be utilised for improving disease resistance in oyster aquaculture.
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Comparing single cell/nucleus genomics to study Atlantic salmon head kidney cellular heterogeneity

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Introduction: Single cell RNA sequencing is rapidly becoming more accessible for the study of non-model organisms like Atlantic salmon. The technology allow us to study cellular changes and responses in diseases. We can generate detailed atlas of the cell composition of an organ, enabling us to describe the transcriptional profile of each cell type suggestive of its function and network activity. Single-cell transcriptomics is achieved by analyzing individual cells, using either the whole cell (scRNA-seq) or only the nucleus of a cell (snRNA-seq).

Methods: We have investigated the cellular composition of the healthy Atlantic salmon head kidney, comparing single-cell and single-nucleus transcriptome data to highlight the differences and similarities between the two techniques.

Results: SnRNA-seq captured a higher diversity of cell types than scRNA-seq. By scRNA-seq, we identified eight cell populations: granulocytes, hematopoietic stem cells, erythrocytes, mononuclear phagocytes, thrombocytes, B cells, NK cells and T cells. Along with all cell clusters identified by scRNA-seq, four additional clusters were identified by snRNA-seq: endothelial, stromal, mesenchymal and interrenal-like cells. SnRNA-seq also resulted in a greater diversity of genes, probably because a higher proportion of genes from scRNA-seq were ribosomal/mitochondrial genes. The stress of whole cell dissociation may influence the expression of genes, while nuclei are more resistant to mechanical dissociation without simulated transcriptional stress responses.

Multiple B and T cell subsets were identified in both datasets. Salmon head kidney displays a great variety of B cells, ranging from early development B cells to plasma cells. Head kidney T cells were identified as cd4+, cd8+ and different stages of γδ T cells.

Conclusions: We show that both snRNA-seq and scRNA-seq are useful to analyze different cell types in HK of Atlantic salmon. ScRNA-seq shows some disadvantages when compared to snRNA-seq. Some cells are more vulnerable to tissue dissociation or are harder to isolate, resulting in their underrepresentation or absence in the final data of our scRNA-seq. The technique of choice will depend on the biological question, cell types of interest as well as sample availability and logistics (frozen x fresh).

As water temperatures rise, is there a possibility of a climate-stress-induced host jump of tilapia lake virus to salmonids?

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Introduction: Many salmonids are thermally sensitive and are therefore particularly vulnerable to thermal stress caused by rising temperatures during summer heat waves. We hypothesised that the combination of thermal stress and the presence of a new viral pathogen could lead to a potential host jump of the pathogen if the fish are susceptible. Tilapia Lake Virus (TiLV) can be considered as one of the most dangerous emerging viruses affecting warm water aquaculture. This pathogen has a global distribution and a host range that remains to be defined, but preliminary in vitro data indicate that the virus replicates in a variety of cell lines, including those derived from salmonids. Therefore, the aim of this study was to evaluate the potential of TiLV to infect salmonids in a range of water temperatures that can be reached during summer heatwaves in continental Europe.

Methods: The susceptibility of several cell lines was assessed in different temperatures. The susceptibility of juvenile rainbow trout and brown trout to infection with TiLV was investigated in infection experiments based on cohabitation of both species with infected fish or intraperitoneal (i.p.) injection of the virus at elevated water temperatures of 20°C and 25°C. The behaviour, pathology, virus load and antiviral responses were measured.

Results: TiLV can replicate in vitro in salmonid cells over a wide range of temperatures from 15°C to 25°C. The infection experiments showed that the susceptibility of rainbow and brown trout to the virus was low, considering the ability of the virus to enter the organism. Exposure of these fish to the virus by cohabitation did not result in high levels of virus in the liver and brain. However, the permissiveness, i.e. the ability of the virus to replicate in the body of the fish, is high because i.p. injection of TiLV resulted in high levels of virus replication in the internal organs.

Concussion: TiLV has some pathogenic potential in salmonids, which may be enhanced by climate change and anthropogenic activities. Further studies should determine whether factors affecting the mucosal barrier allow the virus to spread to the already permissive, thermally sensitive salmonid species.

Climate change, dams and disease: the effect of temperature-dependent myxozoan parasite T. bryosalmonae on Swedish brown trout

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Introduction: Infectious agents play a major role in ecosystem functioning but knowledge of their interactive effects with climate change and habitat fragmentation is limited. Proliferative kidney disease (PKD), is caused by a temperature-dependent infectious agent, the myxozoan parasite Tetracapsuloides bryosalmonae (T.b), and is associated with the decline of brown trout in European rivers.

Due to climate change, the spatial extent of rivers reaching critical water temperatures for severe PKD cases is increasing. Whilst PKD distribution is relatively well-known in central Europe, the extent of PKD in Northern Europe is less well documented and, in Sweden, is currently unknown.

We aim to understand the bryozoan host and parasite distribution and assess the impact of PKD on Swedish brown trout recruitment using long-term national fish monitoring and temperature datasets. Later work will explore the implications of dams to the spread and severity of PKD.

Methodology: We sampled 128 locations with 1-20 individuals per site, totalling nearly 1000 individuals that span Sweden. Renal hyperplasia was measured from ethanol/isopropanol stored samples before quantification of parasite presence and load using multiplex qPCR.

Environmental attributes exploring the contributions of morphology, land use, and river fragmentation are extracted for the sampling sites and their catchments are extracted using a GIS. Furthermore, the use of spatial statistics tests the presence and significance of spatial patterns and clustering while spatial modelling methods assess environmental and anthropogenic contributions to infection severity.

Results: Results detail that T.b infections are present from the southernmost sites to the arctic circle, spanning coastal, agricultural and mountainous habitats yet severe cases occur more frequently in southern Sweden.

Infection rates differ at adjacent sites and absences of T.b infection exist within typically severely infected regions suggesting that T.b. infection, whilst influenced by large-scale variation of temperature regime and geography, can also vary substantially at a reach scale.

Severe cases were associated with the presence of dams, smaller catchments, agricultural land use and proximity to the coastline.

Conclusions: T.b. presence and PKD symptoms are extensive throughout Sweden with severe cases common throughout populated coastal regions suggesting high susceptibility to climate change and anthropogenic impacts.

Is PKD linked to cold water preference in Salmo trutta?

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This study aimed to test whether fingerlings of Salmo trutta are able to actively seek out cooler water areas and whether this “cold-water preference” is attenuated by a temperature-dependent salmonid disease, PKD. We hypothesised that fish with PKD would actively search for colder water as this would increase their chances to survive the infection. To test this, fish were kept in two runways with different water temperatures (range: 13-16°C). The fish could move free between areas and actively choose where to stay, as at the end of the runway the fish could switch between lanes. The position of the fish was recorded by camera. Brown trout (S. trutta f. fario) showed a stronger site preference in the lanes and showed a stronger preference for the cold water source than the closely related lake trout (S. trutta f. lacustris). In support of the hypothesis, the tendency to seek cold water was found to be stronger in brown trout experimentally treated with Tetracapsuloides bryosalmonae, the PKD-causing parasite, than in brown trout that were not treated with the parasite. The experiment underlines the importance of protecting cold water refuges as a management strategy for the protection of trout populations in a warming climate.

A histomorphometric assessment of the impacts of thermal stress on the mucosal epithelia of seawater-stage Atlantic salmon (Salmo salar)

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Mucosal epithelia are essential in protecting against foreign substances and pathogens. In ectothermic animals, the immune function and physiology of these barriers are temperature dependent. Understanding the performance of these epithelia cells at the upper limit of their thermal range has importance in economically significant species, such as Atlantic salmon. This issue remains of particular concern to Atlantic salmon aquaculture in Tasmania, Australia, where the water temperature can approach and even exceed the species' upper thermal limit during summer. A problem that is increasing as ocean warming is driven by changing climate.

Here we exposed Atlantic salmon post-smolts to a temperature ramp from 15 to 20°C for six weeks and then held them at 20°C, while a control group was kept at 15°C. After four weeks of exposure to 20°C, samples from skin, gill, mid- and distal intestine tissues were collected and analyzed histologically. Staining with Alcian Blue and Periodic Acid Schiff’s was used to visualize mucins, and the mucin-producing cells of different types were quantified in these epithelia semi-automatically with ImageJ.

There was a significant enlargement in the epidermal thickness of the skin, along with atrophy in the surface area of simple folds in the distal intestine and a tendency to reduction in folds in the mid-intestine and gill lamellae length. These reductions were consistent with the decrease in mucous cells within these epithelia. Additionally, the abundance of both acidic and non-acidic mucous cells decreased across all mucosal tissues, although the ratio of acidic to non-acidic mucous cells did not significantly change.

Exposure to thermal stress at a temperature of 20°C affects mucosal epithelia in Atlantic salmon. The most prominent histopathological change was in the size of mucous cells, which tended to experience hypoplasia and hypertrophy. Given the critical nature of these barriers in defense against diseases, such changes are likely to impact Atlantic salmon’s physiological function and health, evidenced by increased skin, gill, and gut diseases at elevated sea

temperatures. Further research is needed to understand the mechanisms underlying these effects to develop strategies for mitigating the negative impacts of thermal stress on Atlantic salmon mucosal epithelia.

Microbiome dynamics during antibiotic treatment of Bacterial Gill Disease in farmed Rainbow trout (Oncorhynchus mykiss)

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Introduction: Bacterial Gill Disease (BGD) severely impacts salmonid aquaculture systems worldwide, including freshwater. Characteristic is the presence of filamentous bacteria consistent with Flavobacterium species on the gill surface, associated with a complex biofilm. Despite the considerable amount of research produced, the precise predisposing conditions are still undefined, and information on the dynamics of the microbiome during BGD outbreaks and treatment is lacking.

Methodology: A cohort of rainbow trout (Oncorhynchus mykiss) affected by BGD was monitored during an oxytetracycline treatment using canonical and cutting-edge laboratory techniques. Before and after treatment, healthy and moribund fish underwent necropsy, histology, bacteriology, antimicrobial sensitivity testing, and virology, and their gill microbiome was analyzed using the 16S Next Generation Sequencing (NGS) technique.

Results: In both pre- and post-treatment groups, histology revealed severe gill hyperplasia and spongiosis, with clusters of filamentous bacteria and sporadic amoeba in the pre-treatment, while bacteriology detected Yersinia ruckeri in the internal organs and Flavobacterium spp., Pseudomonas spp., Aeromonas sobria, and Y. ruckeri in the gills.

NGS clearly clustered the samples into pre- and post-treatment groups, with distinct pathogenic and environmental bacteria populations. Interestingly, statistical analyses revealed that the numerous bacterial populations discriminating between pre- and post-treatment belonged predominantly to environmental bacteria species. Instead, bacteria known to be associated with disease (i.e., Y. ruckeri, F. psychrophilum, F. branchiophilum, and A. sobria), showed variation between healthy and moribund fish but were not main discriminators between the pre-treatment and post-treatment. Other exams were unremarkable.

Conclusions: This study is the first to investigate the gill microbiome of rainbow trout and show changes in bacteria populations during the treatment of a BGD outbreak. These results shed new light on the approach to gill diseases and highlight some limits of histology and bacteriology in explaining the underlying complex reality. Surprisingly, there was little contribution of bacteria commonly associated with fish disease in discriminating the groups before and after treatment, while the difference in several environmental bacteria populations was of greater statistical significance. These preliminary findings reiterate the complexity of the causes underlying BGD and pave the way for new approaches to its treatment and management.

Effects of management measures on the microbiome in recirculation aquaculture systems for Litopenaeus vannamei

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Introduction: Tropical king prawns are kept more and more frequently in closed recirculation aquaculture systems in continental Europe. Some of these systems are using the Biofloc technology. As there is still little experience with these systems there is a particular need for research in relation to the development of the microbial community and its impact on the animals.

Methodology: In a biofloc facility for Litopenaeus vannamei, samples of the shrimps, the biofilm of the tank surfaces and the water were taken during different operating conditions and analysed for the composition of the microbiome using Next Generation Sequencing. The effects of stocking postlarvae of different origins on the microbiome in the facility were investigated. During operation, the effects of different aeration techniques on the microbiome were examined in a further approach. In smaller experimental tanks, it was investigated whether the use of habitats, which serve as a place of retreat for the shrimps and should thus lead to a reduction in stress, has an influence on the microbiome in the tank.

Results: A comparison of the microbiome of postlarvae from different sources showed significant differences. During the growth phase, the microbial community on the gills of the shrimps changed significantly. The proportion of Protebacteria decreased, while the proportion of Bacteroidea and Actinobacteriota increased. The diversity according to the Shannon index increased slightly at first and decreased again in the further course. The microbiome also differed

significantly from each other during the growth phase in all samples. An analysis of the beta diversity of the microbiome on the gills of shrimp from tanks with different aeration showed a statistically significantly different species composition. The diversity of the community as well as the composition of the microbiome did not differ between shrimp from tanks with or without habitats.

Conclusions: Shrimp of different origins introduce significantly different microbiomes into a facility. The bacterial composition in the facility changes after stocking and the bacteria that were originally present in the facility become part of the microbiome of the animals. Different aerations can lead to changes in the microbiome.

Optimisation experiments in Pacific oyster (Crassostrea gigas) larvae

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Pacific oysters are one of the world's most heavily cultivated species with potential to provide a sustainable global protein source. However, oyster cultivation is heavily reliant upon consistent production of seedstock, a process frequently hindered by large scale larval mortality events. The microbiome is an important yet understudied aspect of larval health which could help better understand and limit these events which are so detrimental to the industry. Here we aimed to (1) investigate the effects of temperature stress on larval heath and microbiome, (2) preform a wide scale screening of bacteria to assess their potential pathogenic role to oyster larvae.

Experimental work was carried out at the Cefas laboratories in Weymouth UK, where strip spawning's were preformed in order to raise oyster larvae. (1): D stage larvae were raised under temperature stress by alternating their water temperature between 20 and 27ºC at daily intervals. Samples were then taken daily over the next 7 days in order to record mortality rates, growth rates, CFU counts and to allow for microbiome analysis via short read 16S sequencing. (2): using the current bibliography, possible larval-pathogenic bacterial species were identified – many of which were belonging to the Vibrio genra, such as V. splendidus, V. aestuarianus and V. coralliilyticus. These bacteria were then used in bath challenges with oyster larvae, which were observed for mortality rates 48 hours post incubation.

(1): results will compare growth rates, mortality rates, CFU counts and microbial make-ups of this temperature stress over time. Data will be presented in parallel to negative control larvae which were reared in the same experimental set up and held consistently at 22ºC. (2): mortality rate data of oyster larvae incubated with different candidate bacteria and at varying concentrations will be presented.

This data will allow for discussion of the link between larval health and microbiome in the context of climate change and hatchery practices. Aim 2 will also provide essential information for hatcheries in the possible detection of pathogens and recommendation of microbial control measures.

Shewanella putrefaciens Pdp11 extracts protect against betanodavirus infection

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Introduction: Viral encephalopathy and retinopathy is caused by the nervous necrosis virus (NNV, Betanodavirus genus), a naked virus composed of two single-stranded, positive-sense RNA segments. Betanodaviruses are classified into four species, being RGNNV highly predominant in the Mediterranean area. RGNNV causes high mortality in several fish species, including European seabass. In fact, there are two commercialized vaccines designed to protect seabass against RGNNV infection. In this regard, the development of strategies able to protect different fish species against different viruses, such as the use of probiotics, is a key issue for the aquaculture industry. Shewanella putrefaciens Pdp11, SpPdp11, is a fish probiotic with proven positive effects on gilthead seabream and Senegalese sole, protecting those species against bacterial pathogens; however, its antiviral activity is unknown. This study is a step forward in the use of probiotics against viral infections, evaluating the anti-RGNNV activity of sonicated-SpPdp11 extracts in vitro and in vivo.

Material and Methods: The in vitro evaluation was performed on E-11 cells following three assays: (i) neutralization, (ii) 6-h pre-adsorption, and (iii) post-adsorption, determining the inhibition percentage of RGNNV-induced CPEs and quantifying viral replication. The immunostimulatory activity of SpPdp11 extracts was also examined, analysing the transcription of mx, hsp70, tnfα, e3 and tlr3 in E-11 cells.

For the in vivo evaluation, two European seabass groups were established: (i) control group, receiving commercial feed, (ii) experimental group, fed with commercial pellet supplemented with SpPdp11 extracts. Animals were fed for 30 days and subsequently challenged by intramuscular injection. Results were expressed as cumulative survival.

Results: SpPdp11 extracts compromised RGNNV replication in E-11 cells (67.3% and 55% CPE inhibition in 6-h pre-adsorption and post-adsorption assays, respectively), and modulated the transcription of all the E-11 immune-related genes examined. The highest induction was obtained for mx gene.

Regarding the in vivo results, 82% of fish fed with the SpPdp11-supplemented diet survived to RGNNV infection, whereas the survival rate of fish fed with the control diet was 64%. These results suggest that SpPdp11-supplemented feed can be a promising prophylactic tool against RGNNV infection.

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The use of probiotics to mitigate Atlantic sea scallop (Placopecten magellanicus) mortality following challenge with pathogenic Vibrio species

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Introduction: The Atlantic sea scallop supports one of the most economically important fisheries in the state of Maine. As demand for sea scallop meat increases, wild harvest efforts must be supplemented with farming. Scallop farmers rely on spat acquisition to continue production, yet wild spat collection is inconsistent. Hatcheries could produce spat year round but struggle with mortality events, presumably from pathogenic Vibrio species. Probiotics have decreased mortality amongst infected bivalve larvae in other fisheries, and could be implemented to decrease larval sea scallop mortality.

Methodology: Identification of probiotic candidates and potential sea scallop pathogens is a crucial first step. Probiotic bacterium effectiveness will be studied in vitro before in vivo experimentation. Bacterial competition assays were conducted to examine inhibition of selected pathogens by probiotic candidates. The impacts of probiotic treatment on the growth and development of larval sea scallops will be monitored. Promising candidates will be used to treat challenged sea scallop larvae in small scale trials.

Results: Eight probiotics and three pathogenic bacteria have been identified and cultured. One probiotic candidate has shown success inhibiting pathogens in vitro. Probiotic physiology and methods of inhibition are being identified. The effects of probiotic treatment on sea scallop larvae is underway.

Conclusion: Initial experimentation on probiotic candidates has been informative. Inhibition of pathogenic bacteria by probiotic candidates has been displayed in vitro. The effects of successful probiotics on larvae growth and in larval challenge experiments are currently being assessed. Probiotics displaying inhibition of pathogenic bacteria, no inhibition of sea scallop larvae, and success at decreasing larvae mortality will be included in hatchery scale experiments on scallop larvae survival.

Potential of marine strains of pseudoalteromonas to limit biofilm formation and improve resistance to vibrio harveyi in European sea bass

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Introduction: The European Sea bass Dicentrarchus labrax represents one of the most produced marine fish species in Europe. Its early stages of life are relatively susceptible to pathogens, with episodes of mortality increased on farms due to high densities. As part of the search for alternatives to antibiotic treatments, the PaqMan project (EMFF PFEA470019FA1000012) explored the protective potential of marine bacterial strains belonging to the genus Pseudoalteromonas, already known to produce antimicrobial peptides, in European Sea bass.

Methodology: Juvenile Sea bass were impregnated fortnightly during 2 months for 4 hours in static hyperoxygenated seawater containing different concentrations of probiotics candidates. Four experimental conditions were included: a mixture of two strains (hCg42-hOe125) described for their capacities to produce Alterin; one strain with antibiofilm properties (3J6); a strain of the same genus but with no identified probiotic effect (RA15); and without bacterial strain (Control). At the end of the impregnation phase, the animals were intraperitoneally injected with a dose of 4.6 x10E8 PFU of V. harveyi and mortality was monitored for 15 days. Biofilm formation was analyzed during the probiotic exposition phase using glass slides implanted in a floating device and immunological analyzes were carried out during the infectious challenge.

Results: The probiotics strains were detected at different times at the end of the 4 hours period of impregnation in gills and mucus of the fish. Biofilm maximum thick was significantly lower for all probiotic conditions as compared to the Control condition after 2 months of soaking. No statistical difference of mortality was observed between the control and 3J6 conditions after infection with V. harveyi. The conditions RA15 and hCg42-hOe125 showed an improved survival of 10 and 25 %, respectively, compared to the Control group. No significant difference in blood formulations, leukocyte mortality or phagocytose activity was observed between the different conditions.

Conclusion: This work highlights the particularly interesting probiotic potential of marine bacteria naturally present in aquatic organisms. Additional analyzes have been carried out to characterize the microbiome of impregnated animals to better understand the effects and mechanisms of action of these marine strains.

Intestinal Bacillus velezensis successfully simulates European seabass peripheral blood leukocytes

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Introduction: In an era of rising antimicrobial resistance and a limited number of curative measures for disease outbreaks, aquaculture struggles to find environmentally friendly and cost-effective alternatives to various chemotherapeutics. One such alternative is the use of health-promoting agents, such as probiotics. They are known to have a beneficial effect on fish health at local and systemic levels, by improving health status, disease resistance and host and environmental balance.

Methodology: We isolated a Bacillus spp. strain from Adriatic aquaculture fish and characterized it by whole genome sequencing (WGS), combining Illumina and Nanopore sequencing data and assessing its biosynthetic potential using the antiSMASH web interface. The same isolate was tested for its immunostimulatory efficiency on European seabass peripheral blood leukocytes (PBLs). The PBLs were isolated by hypotonic lysis and stimulated with live bacteria for 3h, 6h and 18h. In addition, a batch of PBLs was treated with lipoteichoic acid (LTA) from B. subtilis as a positive control. Finally, expression of selected immune-related genes (Il1-β, Il6, Il10, Tnf-α and Tlr2) in stimulated PBLs was quantified by real-time PCR.

Results: Based on Genome Taxonomy Database, the isolated strain was identified as Bacillus velezensis. antiSMASH analysis identified biosynthetic gene clusters (BCGs) for most of the usual B. velezensis specific secondary metabolites, including surfactin, bacillaene, macrolactin, fengycin, difficilin, bacillibactin and bacilysin. However, antiSMASH could not separate between two possible BCGs in “region 8” due to overlapping genes, thus possibly suggesting that biosynthetic potential for iturin is also present. Gene expression analysis showed that the most perturbed genes were those coding for canonical pro-inflammatory leukocytes, indicating a positive effect on innate immunity.

Conclusion: In conclusion, B. velezensis and/or its identified secondary metabolites positively stimulate European seabass peripheral blood leukocytes indicating its potential use as a probiotic in seabass aquaculture.

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Marine bacteria as relevant probiotics for fish farming: genomic characterization, antimicrobial screening and in vivo assessment on European sea bass

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Introduction: Intensive aquaculture and globalization of trade contribute to the emergence and spread of infections in fish farms. In a context of restriction of antibiotics use, probiotics could represent an interesting bioalternative to control infections and improve the zootechnical performances while minimizing the environmental impact. We investigated the probiotic potential of sporulated bacteria extracted from the marine environment on sea bass, Dicentrarchus labrax, one of the major marine fish species produced in Mediterranean.

Methodology: Bacillus strains were screened in vitro and fully sequenced to identify their potential probiotic properties and assess their safety. The efficacy of probiotics was evaluated by dietary supplementation of juveniles sea bass (average initial weight : 5g) with the candidates of interest for several months at 21°C. During the supplementation, their ability to persist in the intestine, their effects on growth (length and size) and immune system (blood formulation, phagocytosis activity) were evaluated. After two to five months of supplementation, some fish were experimentally infected with Vibrio harveyi, Vibrio anguillarum or nervous necrosis virus (NNV) to evaluate their disease resistance.

Results: Genome analyses confirmed the absence of antibiotic resistance and pathogenicity genes and revealed interesting capacities of production of amino acids, vitamins and digestive enzymes. Strains selected showed antibacterial properties in vitro against various genus of pathogenic bacteria isolated from farms, associated with the identification of gene clusters encoding secondary metabolites with potential antimicrobial activities.

The survival of probiotics in the intestinal tract of supplemented fish was confirmed with the detection of concentrations up to 1.10^5 CFU per intestine at different time. Probiotic supplementation increased the survival rates after infection with Vibrio harveyi and Vibrio anguillarum. However, survival rates were not impacted for NNV infection. Furthermore, fish supplemented with probiotics showed significantly higher leukocytes counts, with an increased proportion of phagocyte (expression of immunity genes ongoing).

Conclusion: The in vitro and bioinformatics analyses performed confirm the probiotic potential and the safety of the marine strains of Bacillus selected. These probiotics have beneficial effects on components of the immune system and improve resistance to bacterial pathogens of juveniles sea bass.

Mimicking nature for sustainable aquaculture- Fulvic acid strengthens the mucosal immunity of fish

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Introduction: Aquaculture already covers over 50 % of the human demands for dietary fish. Simultaneously, diseases are an eminent problem in intensive aquaculture systems. Mortality rates of up to 80 % account for substantial economic losses. Most substances used for disease prevention have been banned because of environmental and consumer risks. Stimulating the systemic immune response for disease prevention has become a promising approach to protecting fish from pathogens. However, the main entrance portals for external pathogens are the gills and skin, and little research has been performed on stimulating the mucosal defense mechanisms.

Methodology: Juvenile rainbow trout (Oncorhynchus mykiss) were exposed in a recirculating aquaculture system (RAS) to 5 mg C/L and 50 mg C/L of a commercially available natural fulvic acid (FA). After four weeks of exposure, mucus was collected from the skin by gently massaging the fish for 2 min in 50 mM NaCl. Lysozyme and alkaline phosphatase activities were measured turbidimetric using Micrococcus lysodeikticus and para-nitrophenol as substrates, respectively. The difference in protein concentration before and after precipitation with polyethylene glycol was considered the immunoglobulin content. Antibacterial properties were determined by counting the colony-forming units after inoculating mucus with Yersinia ruckeri.

Results and Conclusion: Adding FA significantly stimulated fish mucus's innate and adaptive immune response. Lysozyme and alkaline phosphatase activity were significantly increased in both exposure groups. Furthermore, the concentration of immunoglobulins was higher in fish exposed to 50 mg C/L. Mucus from control fish repressed the growth of Y. ruckeri by almost 80 %, showing the great importance of mucus in defending against pathogens. The addition of both concentrations of FA to the water increased the reduction in bacterial load even further to 13-14 %. Preliminary experiments furthermore showed that the FA itself did not affect bacterial growth. Our results show that FA increases different aspects of the skin mucosal immunity, thereby increasing the antibacterial property of the mucus. FA as a water additive improves fish's natural defense and well-being in aquaculture and helps ensure safe and sustainable food production.

To immunomodulate or not to immunomodulate: a nematode host defence peptide's dilemma

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Introduction: Host Defence Peptides (HDP), or Antimicrobial Peptides (AMP) are pleiotropic, multifunctional effector molecules of the innate immunity present throughout the tree of life. Their antimicrobial activity spans over a range of pathogens; Gram-negative and Gram-positive bacteria, mycota, viruses, parasites. Their unspecificity allows us to capitalise on HDPs to fight the rising antimicrobial resistance (AMR). Helminthic HDPs have been recently marked as potentially actors against AMR, as they are primarily membranolytic and do not exert cyto- and genotoxicity toward the host cell, unlike HDPs deriving from the free-living organisms. In particular, anisaxins, cecropin-like HDPs deriving from the zoonotic marine nematodes Anisakis simplex and Anisakis pegreffii have been proven very efficient against referent and clinical, even MDR human bacterial isolates.

Methodology: To test the immunomodulatory repertoire of HDP anisaxin-2S (A-2S), the blood of the common carp, Cyprinus carpio has been collected from fish maintained under four conditions; i) specific pathogen free fish (SPF); ii) fish infected by Sphaerospora molnari blood stages (BS); iii) fish infected by S. molnari blood stages and immunosuppressed (IS+BS); and iv) non-infected immunosupressed fish (IS). Blood was collected at four time-points (T0, week 2, week 3, week 4) to scrutinise the kinetics of the immune response against the myxozoan, with or without stimulation by A-2S. Leukocyte and erythrocyte fractions obtained by Ficoll were treated for 1h with A-2S (10 µM) in 24-well plates. Samples were collected to measure cell proliferation and ROS (flow cytometry), and expression of innate immunity targets (il6, il10, il1b, tnfα, and infγ).

Results: Both the cell proliferation and ROS production increased significantly in white and red blood cells stimulated by A-2S, progressing from week 2 toward the week 4, corresponding to the parasitaemia onset. Expression of pro-inflammatory cytokines was in general also significantly upregulated compared to unstimulated blood cells, but the kinetics of the response was not always linear.

Conclusions: Although immunomodulatory properties have been so far attributed mostly to the flatworms' HDPs, the nematode A-2S shows considerable activation of innate cells, which could be a useful asset for designing of aquaculture nutraceuticals.

Sea lice dispersal and vertical migration behaviour for sea lice - a simulation study

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Sea lice are ectoparasites that damage fish health and require significant expenditure for management by aquaculture companies. The high density of suitable adult salmon hosts in salmon farms supports a high number of sea lice. Their planktonic larvae can be released back in the surrounding waters where they can infest sea trout and wild migrating salmon smolts, making sea lice a potential serious pressure on wild salmonids in Scotland and elsewhere. Sustainable aquaculture and conservation of wild Atlantic salmon are both priorities in the context of the Blue Economy vision for Scotland. Consequently, there is a need to identify areas of higher risk to wild salmon populations to facilitate the effective mitigation of sea lice impact.

Dispersion modelling using coupled hydrodynamic and particle tracking models is widely used to understand sea lice distributions in the wild. The transport of a sea louse is affected by physical processes and biological behaviours, such as how much vertical swimming influences depth of the parasite. Quantifying these behaviours in particle tracking models is critical to provide a realistic picture of sea lice dispersion in the marine environment. Here we determine how predicted spatial distributions of sea lice are infleunced by vertical swimming velocity and maximum swimming depth using data from Loch Linnhe (Scotland) as a case study.

We show that wind driven currents and gravitational circulation create a high vertical shear where vertical position of sea lice affect their horizontal trajectory. A review of previous sea lice studies shows no clear consensus on how vertical movements can be incorporated in sea lice distributions models, underpinning the importance of the present study for elucidating the situation in the context of a fjiordic sea loch.

Although sea lice modelling has improved during the last 15 years, this study suggests that refinement of numerical models is still required by using more observational data to reduce uncertainties due to the effect of sea lice vertical swimming behaviour.

Risk factors for increasing salmon lice levels during marine production cycles in Scotland

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Introduction: Sea lice, an ectoparasitic copepod of salmonids, represents one of the most prolific and costly issues in marine salmon aquaculture. Their ability to disperse on ocean currents means they are also a threat to wild salmonid populations and as such are heavily regulated in almost all salmon farming countries. Understanding how the dynamics of lice infestation change over the course of the seawater production cycle on salmon farms and some of the risk factors associated with them is an important step in managing the risk of lice infestation and improving the sustainability of salmon aquaculture.

Method: Here we assess the temporal dynamics of lice numbers and the major risk factors associated with their increase during the seawater production phase on salmon farms in Scotland. Using farm-level data from across Scotland and a mixture of time series and statistical dredge approaches we assess the core factors influenceable through changes in management practices leading to increases in reported lice numbers on farms.

Results: Results show a range of factors that contribute to increasing lice numbers such as time into the production cycle, the consented biomass of the farm and the region the farming takes place in.
Other factors are assessed with plots showing relative levels of core factors needed to exceed legislator reporting thresholds of 2 and 4 lice per fish.

Conclusion: This analysis provides evidence to support improved management practices during seawater production cycles to maintain sea lice to lower levels both reducing the risks to wild salmonids and lower the costs of control measures on the farms themselves.

Assessment of Efficacy and Immunogenicity of an Orally Administered Salmon Louse (Lepeophtheirus salmonis) Extract Incorporated into Silicon-Lipid Nanoparticles

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Introduction: The salmon louse (Lepeophtheirus salmonis) is an ectoparasitic copepod that feeds on the skin and underlying mucous and epithelium of Atlantic salmon (Salmo salar) inflicting massive economic and welfare implications to the salmon industry. Considering the ectoparasitic life strategy, exploiting skin mucosal immunity may prove fruitful at targeting antigen-specific antibody uptake in the parasite. This study evaluated the immunogenicity and efficacy of a sea louse protein extract formulated into silicon-lipid nanoparticles for oral administration in Atlantic salmon.

Methodology: Thiol-sepharose L. salmonis extracts derived from adult L. salmonis homogenates were analysed by Liquid Chromatography Mass spectrometry (LC ESI MS/MS) in conjunction with ProteinScape™ V3.1 (Bruker). Antigens were incorporated into silicon stabilized hybrid lipid nanoparticles (sshLNP) (SiSaf Ltd. Bio-Courier technology) as a L. salmonis vaccine formulation (SLE-NP). The SLE-NP was further formulated with Montanide adjuvant (763A, Seppic) for injection vaccination and as a feed paste (BioMar) for oral (gavage) vaccination. The study compared immune responses (ELISA, qPCR, immunoassays) of fish immunised with 50µg dose-1 SLE-NP by an IP prime followed by oral boost (gavage) (600 dd) compared to fish immunised with a SLE-NP oral gavage only and empty NP controls. Salmon (n= 6 x 20) were then challenged with larval (copepodid) L. salmonis and counts performed 10-14 dpc on attached chalimus lice.

Results: The 90haracterized L. salmonis extract included antigens previously reported as potential vaccine candidates in other parasites. The SLE-NP antigen was stable (4°C over 21 days). Serological immune parameters, including anti-louse antibodies, were significantly increased in oral-boost vaccinated fish compared to IP only or controls. Fish vaccinated with SLE-NP with or without boost did not significantly reduce lice burden (chalimus) post-challenge. Further antibody assessment in the skin mucus and immune gene expression analysis will reveal whether oral boosting has enhanced skin mucosal responsiveness.

Conclusions: Incorporation of sea lice Thiol-sepharose extracts into silicon-lipid nanoparticles enriches for L. salmonis virulence factors that are stable. This cocktail of antigens was not protective as an IP – Oral vaccine strategy against larval lice. Understanding the skin mucosal response will elucidate whether the oral bio-courier approach may be protective with increased target antigen doses.

Ensemble models for salmon lice management under uncertainty

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Introduction: Salmon lice (Lepeophtheirus salmonis) management is key to sustainable salmonid aquaculture, a major component of the Scottish Blue Economy. Salmon lice' planktonic larvae are transmitted over long distances by ocean currents, causing transmission between farms and farmed and wild salmonid populations. This larval transport is widely assessed using coupled hydrodynamic – particle models.
Differences in modelling hydrodynamic and biological processes can result in differences in model outputs. Here we describe the use of an ensemble of different models to provide information on confidence in infection pressure predictions and hence better inform management.

Method: In Scotland, several different hydrodynamic (HD) models coupled with particle tracking models are currently used by various public and private sector organizations to provide inference on salmon lice abundance and distribution at various spatial and temporal scales or connectivity between sites.

These models are informed by salmon lice counts from fish farm data and validated against deployed sentinel cages field data to assess the attached, non-planktonic salmon lice stages.

Here, we evaluated three of these coupled bio-physical models, describing salmon lice dispersion in Loch Linnhe Scotland, carrying out physical model validation and biological inter-model comparison, through comparison with field data.

We then assessed use of ensemble modelling techniques to identify levels of agreement and disagreement and so provide estimates of extent of uncertainty in the simulation of this system.

Results: The coupled bio physical models produced different results within the Loch Linnhe system in terms of local lice abundance, but each fitted well to the patterns of variation in lice on salmon in sentinel cages for Autumn 2011, the time period of the sensitivity analysis focus.

The "best fit" models were used to create an ensemble model, which allows visualisation of how coherence of the models varied across the spatial domain.

Conclusion: The ensemble modelling approach provides a way to improve understanding of areas of least, and greatest, uncertainty in model predictions, which can aid confidence in decision-making. We aim to develop the application incorporating further models into the ensemble approach.

Three lice population density variables that inform management of salmon lice impacts on wild salmonids

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Salmon lice are a parasite that is a critical limiting factor on sustainable marine salmonid aquaculture. Managing impacts on wild fish in Scotland requires indicator measures to assess sea lice control effectiveness. Three are described covering key steps for assessment.

Methods: Three key population density variables are identified using a model structure to inform management strategies. These are: (1) adult female lice on the source host population, (2) the concentration of larval lice in the environment and (3) the number of mobile stage lice infecting wild fish. All three variables are assessed relative to threshold values whose exceedance indicates risks of aquaculture impacts.

Results:

1. Adult female lice (AF) count per fish determines production of larval lice (nauplii). Weekly averaged AF counts are reported by farms. AF are regulated officially using reporting and action thresholds, but are generally kept below these thresholds by industry practice. Fish numbers and viable egg production per ovigerous AF determine nauplii production per farm.
2. Concentrations of infectious stage larval lice (copepodids) depend on production, decay, maturation and dispersal of larvae. Critical concentration thresholds depend on the length of exposure and infection rate. A threshold value is identified dependent on smolt size and speed from analysis of infection rates, survival to mobile development stages and thresholds T1 or T2 under final indicator variable 3.
3. Numbers of mobile stage lice on wild smolt follow infection and maturation. Threshold levels associated with welfare impacts (T1 = 0.08-0.1 lice/g) or that cause mortality (T2 = 0.24 lice/g) are assessed from a metanalysis of laboratory studies.

Conclusions: These three key indicators and associated threshold values are important determinants for managing the sustainability of wild fish and may be obtained from surveillance, with 2 and 3 often assessed from models.

Ceratonova shasta genotypes infection dynamics comparison using temporal RNA-seq data

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Ceratonova shasta is an economically and ecologically important myxozoan parasite of salmonid fishes from Pacific Northwest of North America. This parasite is a complex species that has host-specific genotypes with different virulence levels. Infections in rainbow trout by genotypes 0 and IIR are a contrasting system that is a good model for investigating virulence in myxozoans. Genotype 0 causes a chronic low-virulence infection, with minimal proliferation, delayed spore formation, reduced tissue damage and no mortality. Genotype IIR causes a highly virulent infection, with rapid proliferation, fast spore formation, inflammation and tissue destruction, with up to 100% mortality. We explored the changes and regulation of genes of C. shasta genotype 0 and IIR during their infection course. Using a long-read parasite reference genome and Illumina RNA-seq data, we identified differentially expressed genes (DE) between days 7, 14 and 21 days post exposure (dpe) for genotype 0 and IIR proliferating in intestines of rainbow trout (Oncorhynchus mykiss). Gene counts and DE analyses were obtained using Salmon and DESeq2. A Trinotate pipeline and custom databases were used for functional annotation to discover enriched pathways and disease mechanisms. Our preliminary results indicate that the differences in expression between time points were larger for genotype IIR than for genotype 0. 76 (7 vs 14 dpe), 1444 (7 vs 21 dpe) and 479 (14 vs 21 dpe) DE genes were observed between days 7, 14 and 21 for genotype 0 infection, while 5687, 4625 and 3864 genes were DE between these days for genotype IIR infection. Larger differences in gene expression were observed between earlier time points for genotype IIR (7 vs 14 – 5687 DE) than for type 0, which correlated with the differences in development and proliferation rates observed in each genotype. We identified these genes and discussed their potential role as virulence factors. These findings contribute to understanding the molecular basis of myxozoan virulence.

Dams out on the klamath: predicting how ceratonova shasta disease dynamics will change

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The demolition of four dams on the Klamath River, USA will be the largest dam removal project in history. These dams, constructed between 1912 and 1962, block more than six hundred kilometers of potential habitat for culturally and economically important populations of Chinook (Oncorhynchus tshawytscha) and coho salmon (O. kisutch) and steelhead trout (O. mykiss). In addition to being barriers to migration, construction of these dams and associated reservoirs have substantially modified the riverine ecosystem. These ecosystem alterations, and the steps taken to mitigate for them, also significantly shifted the balance between salmon and their pathogens.

Klamath River salmon encounter a variety of pathogens, both as juveniles migrating downstream to the ocean and as adults returning to spawning grounds. The myxozoan parasite Ceratonova shasta is a primary factor affecting salmon recovery in the Klamath River because of its consistent presence and impacts on juvenile salmon as a result of direct mortality or predation associated with disease morbidity. With dam removal, the opening of new habitat and the coincident closure of a major mitigation hatchery below the dams will result in changes in fish distribution and shifts in fish species and life history composition. The removal will also result in changes to river flow dynamics, geomorphology, temperature regimes and water quality that are expected to benefit ecosystem health. All of these changes will affect fish-pathogen interactions; while many of these changes are likely to be positive in the long term, there will also be some challenges. Long-term monitoring and research on C. shasta has informed current fisheries management and resulted in models that inform predictions on the effects of dam removal on this disease. Here we present an overview of the Klamath River and the changes in disease dynamics that are expected to occur following dam removal.

Environmental DNA - development, comparison and use of non-invasive tools to detect and monitor Tetracapsuloides bryosalmonae

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Environmental DNA (eDNA) is used to non-invasively detect and monitor pathogens, including the water-borne transmission stages of Tetracapsuloides bryosalmonae. T. bryosalmonae is a myxozoan endoparasite causing Proliferative Kidney Disease (PKD), an emerging disease in salmonids. Although several studies showed successful detection of T. bryosalmonae with various eDNA protocols, an appropriate comparison and cross-validation of these methods is missing, as well as knowledge about spore presence and detection probability in the context of seasonality and other environmental parameters.

This study compares T. bryosalmonae eDNA detection methods, to develop an easy-to-apply and sensitive eDNA protocol, and to evaluate the influence of different environmental parameters on the detection probability during non-invasive monitoring of T. bryosalmonae in water samples.

First, three probe-based T. bryosalmonae specific detection assays were compared in parallel, testing their limit of detection (LOD) and limit of quantification (LOQ) using quantitative PCR (qPCR) versus digital droplet PCR (ddPCR). Second, the impact of different filters and water volumes on the detection probability were tested by sampling water directly from riverbanks with a syringe-based protocol. Based on the resulting protocol, presence of T. bryosalmonae DNA was monitored during an entire year. Those eDNA samples are currently analysed and environmental parameters (e.g., water temperature, seasonality, presence and infection status of the main host) are tested by multilevel occupancy models for their influence on cumulative detection probability.

The most sensitive detection protocol was a combination of the probe-based assay by Bettge et al. (2009) and ddPCR with a LOD of 1.65 copies/µl sample input and a LOQ of 3.66 copies/µl sample input. Multilevel occupancy models revealed highest detection probability using three Sterivex™ filter replicates of 600 ml filtered water.

This cross-validation of assays and detection platforms provides a highly sensitive laboratory analysis workflow and combines it with an easy and cost-effective field sampling protocol. Together with the evaluation of ideal sampling time and environmental conditions, which are currently evaluated, this research presents the basis for a sensitive and non-invasive monitoring of T. bryosalmonae.

Environmental DNA-based monitoring of aquatic parasites: a case study of Myxozoa and Microsporidia

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Endoparasites such as Myxozoa and Microsporidia are often overlooked due to their microscopic size and hidden lifestyle within their hosts. They exhibit remarkable diversity and infect a wide range of hosts and invade all known organ and tissue systems. Many of them are host-specific parasites that have evolved and co-speciated with their original hosts, suggesting that the known species diversity represents only a fraction of their true diversity. Traditional research on parasite diversity relies on parasitological studies of hosts, which can be laborious and may not reflect their full diversity. However, eDNA metabarcoding is a promising approach that can overcome this obstacle. This method involves detecting parasite DNA from host or environmental samples, and it can successfully detect both Myxozoa and Microsporidia due to their resistant spores that can persist in the environment.

We evaluated the effectiveness of this method for Myxozoa and adapted it for Microsporidia. We estimated the potential maximum diversity of parasites using extrapolation and so-called Hill numbers. We collected and isolated DNA from both aquatic sediment (eDNA) and invertebrate hosts, performed metabarcoding using specific barcoded primers targeted on SSU rDNA and sequenced the resulting amplicons on an Illumina Mi-Seq (250 bp). We used a bioinformatic pipeline to demultiplex reads and cluster operational taxonomic units (OTUs), and then performed maximum likelihood analysis to reveal relationships of gained OTUs.

We revealed 75 microsporidian OTUs in the invertebrates and 69 microsporidian OTUs in eDNA samples. Several OTUs were assigned to known species, while the majority represented unknown microsporidia. The species accumulation curve showed a steady increase in Myxozoa and Microsporidia species number with an increasing number of eDNA samples. The increment of species richness quickly and steadily decreased with an increasing number of samples, suggesting that further samples will lead only to minor changes in myxozoan and microsporidian diversity.

Our study demonstrated the potential of this method for eDNA-based diversity assessment of aquatic parasites and offers a promising approach for their monitoring and understanding of their diversity and life cycles.

Ecological insight into myxozoan diversity via eDNA analysis: seasonal patterns

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Introduction: Monitoring the parasitic diversity in fish is crucial for fish aquaculture. Myxozoans are microscopic endoparasites that are economically and veterinary important, particularly for fish. While this group includes more than 2,600 known species, the number is greatly underestimated. The classical approach to studying their diversity involves invasive dissection of fish followed by microscopic and molecular screening. The use of environmental DNA (eDNA) containing DNA of myxozoan stages presents a promising solution for their monitoring, detecting their diversity and

ecological trends. Our objective was to apply our metabarcoding approach to selected freshwater ecosystems in order to i) identify and compare myxozoan diversity in different freshwater ecosystems, ii) investigate myxozoan seasonal distribution at selected sites, iii) to compare the diversity of Myxozoa observed in sediment and water.

Methods: We collected sediment and water samples from various freshwater ecosystems in Central Europe at different time points in accordance with the objectives of the study. DNA from collected samples was extracted using a commercial soil kit. The V4 region of the SSU rDNA was amplified by PCR with barcoded primer sets (covering freshwater myxozoan diversity) and prepared for Illumina sequencing. The obtained data were analyzed bioinformatically.

Results and conclusions: Our eDNA analysis spanning diverse freshwater habitats successfully identified various OTUs that clustered in all myxozoan freshwater lineages, covering a significant portion of the hidden diversity of Myxozoa. In particular, we found a high level of species diversity within Myxobolus subclades, Paramyxidium, and Chloromyxum sensu lato clades. In ponds, we observed seasonal trends in the occurrence of Myxobolus subclades and the Myxidium lieberkuehni clade in the water. Malacosporea were constantly observed throughout the entire season. In reservoirs, we identified a significant difference in the number of myxozoan OTUs detected in water and sediment. At the same time, the diversity composition in water did not differ much between the years.

Conclusions: We have shown that myxozoans are prevalent in freshwater ecosystems, and their diversity remains largely unexplored. Our methodological approach utilizing eDNA is suitable for assessing and studying various ecological aspects, such as seasonal patterns and for myxozoan diversity monitoring.

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Molecular detection of Tetracapsuloides bryosalmonae in migratory salmonids in the Great Lakes

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Introduction: Migratory salmonids represent an important recreational fishery resource in the Great Lakes ecosystem. Sexually mature salmonids harvested during seasonal spawning migrations are used to produce juveniles to enhance natural populations. The myxozoan parasite Tetracapsuloides bryosalmonae (Malacosporea) may cause Proliferative Kidney Disease (PKD), a severe immunopathology predisposing susceptible hosts to polymicrobial infections, therefore threatens salmonids in Europe and Northwest America. We investigated T. bryosalmonae occurrence in the Great Lakes watershed, where host availability and environmental conditions may be suitable to complete their two-host life cycle.

Methodology: Migratory Chinook Salmon (Oncorhynchus tshawytscha), Coho Salmon (O. kisutch), and Steelhead Trout O. mykiss) were opportunistically sampled at fish weirs during seasonal spawning migrations. In Michigan, 359 Chinook and 367 Coho were sampled from 4 weirs in October 2020-2022. In Indiana, 102 Rainbow Trout (Steelhead) were sampled from captive broodstock that were collected from a weir in Trail Creek during the Summer 2022 migration, and bryozoan colonies were collected from St. Joseph River fish ladder. DNA extracted from posterior kidneys and bryozoans was used for qPCR specifically targeting T. bryosalmonae SSU rDNA. Positive samples were processed by conventional PCR to retrieve larger genomic sequences from the malacosporean SSU rDNA and CO1 for confirmatory analysis.

Results and Conclusions: Molecular biology examination showed positives between large adults and precocious males, returning from Lakes Michigan and Huron. In Michigan, the infection prevalence varied between 3-18% across samples stocks and locations, although most of the specimens were asymptomatic and with a low parasite burden. Cytology examinations of kidney and spleen added scarce evidence, while histopathology sporadically revealed sporogonic and extrasporogonic T. bryosalmonae stages, but typical PKD pathology was never seen. In Indiana, Steelhead Trout were found infected with a prevalence of 14% (Summer sampling) and 38% (Winter sampling), and the bryozoans were also positive to T. bryosalmonae. Sequence analysis confirmed the detection of T. bryosalmonae DNA from these salmonid species. These findings represent the first report of T. bryosalmonae in Great Lakes fish species, providing preliminary evidence that the parasite might be already endemic in this large area of North America but was previously undetected from asymptomatic carriers.

Tracking Tetracapsuloides bryosalmonae infection in juvenile Coho Salmon

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Introduction: The myxozoan parasite Tetracapsuloides bryosalmonae (Malacosporea) was recently discovered to infect Pacific Salmon species as north as in Alaska, while we are gathering new evidence about its detection in adult migratory salmonids in the Great Lakes watershed. In the Northwestern America and Europe susceptible hosts may

develop Proliferative Kidney Disease (PKD), a severe immunopathology that predispose fish to polymicrobial infections. Coho Salmon (Oncorhynchus tshawytscha) are farmed in Michigan to restock naturalized populations that support the sport fishing industry and contribute to the ecosystem in the Great Lakes. This study aims at assessing T. bryosalmonae prevalence, and any PKD occurrence, in hatchery-reared juveniles throughout a production cycle.

Methodology: Sixty fish were sampled each month from the same stock of hatchery farmed juvenile Coho Salmon, after they were moved in unfiltered river water and held at constant water temperature of ~10°C. DNA extracted from posterior kidneys was used for qPCR to specifically target T. bryosalmonae SSU rDNA. Positive samples were further processed by conventional PCR to retrieve larger genomic sequences from the malacosporean SSU rDNA and CO1 for confirmatory analysis. A panel of organs was collected for histopathological assessment.

Results and Conclusions: During the production cycle no clinical signs, nor remarkable mortality were recorded, neither any of the sampled fish showed signs of PKD. Although, the PCR screening revealed that juvenile Coho were positive for T. bryosalmonae through the entire production cycle with low infection prevalence (3-27%). A lower prevalence was recorded through the fall to spring (13-3%). The analysis of retrieved sequences confirmed the detection of T. bryosalmonae, indeed matching sequences deposited in GenBank. Histopathology sporadically revealed sporogonic and extrasporogonic stages of T. bryosalmonae, but the typical PKD pathology was not seen.

This is the first detection of T. bryosalmonae from juvenile of Coho Salmon in the Great Lakes. Fish exposed to unfiltered river water might gradually become tolerant to the parasite infection, and thereafter acting as asymptomatic carriers. This result will provide important information for hatchery and fisheries management in the Great lakes.

Are wild brown trout better adapted to infection with T.bryosalmonae than stocked trout?

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After decades of yearly stocking measurements with thousands of farm-raised brown trout to counteract brown trout declines in swiss rivers, this human action stopped in some regions of the alpine country. It seems that stocked trout did not increase the native brown trout population nor did reach sexual maturity in the wild. Since then, in those regions, it is now nature that regulates the biological processes in the wild, without the effects of stocking.

In fact, brown trout are facing massive declines worldwide largely due to Proliferative Kidney Disease (PKD), caused by the parasite Tetracapsuloides bryosalmonae. Infection and disease dynamics are strongly modulated by temperature increases, giving rise to mortality events and to big trout losses in aquaculture but also in natural habitats.

Our study wants to analyse the immune responses of farm-raised, wild-raised and mixed-origin-raised brown trout towards the infection with the causative agent of PKD, T.bryosalmonae. The goal was to examine possible differences in their immune system responses that potentially lead to different survival chances in the wild or to different adaptation strategies to the disease. In a controlled lab-experiment, we exposed brown trout of these three different rearing conditions to the parasite and monitored periodically the mortality, the pathological changes, presence/ absence of the parasite (via qPCR) and the immune cell responses through FACS measurements over a period of 2 months after infection. Additionally, single cell RNAseq was performed once.

All exposed groups were positive for PKD and the results show that in the presence of subclinical PKD, there is a higher immune response involving monocytes in the exposed wild trout group, compared with the wild non-exposed groups and with the farm- and with the mixed-origin-raised exposed groups. Whereas B cells are slightly downregulated in all groups in exposed vs. non-exposed animals. scRNAseq revealed multiple differently expressed genes in exposed animals vs. non-exposed.

We could find differences in the immune response of the differently reared trout that can potentially explain why wild fish exposed to T. bryosalmoae survive better in the wild than farmed-raised trout.

Early warning through video monitoring: dissolved hydrogen sulphide (H2S) affects Atlantic salmon swimming behavior in recirculating aquaculture systems

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Introduction: Hydrogen sulphide (H2S) poses a major threat in marine land-based recirculating aquaculture systems (RAS) leading to acute mortality in sensitive fish species such as Atlantic salmon. To date, little is known about the effects of sub-lethal H2S on the physiology and behaviour of the species.

Methodology: The present study analysed swimming behaviour in Atlantic salmon in response to H2S in a controlled trial. The setup consisted of two RAS in parallel. The control RAS comprised of one fish tank (800L, 10 Kg fish/m3 (≈ 70 fish)) and one biofilter, while the exposure RAS included two fish tanks (800L; 10 and 30 Kg fish/m3 (≈ 70 and 200 fish)) connected to one biofilter. Swimming behaviour was monitored via both a submerged custom built stereo camera system and an overhead surveillance camera system filming from above. Fish (smolt, ≈ 114g) were exposed once a day for 10 consecutive days to increasing H2S concentrations, from ≈ 1- up to ≈ 68-µg/L (2 µM). Dissolved H2S, O2 and CO2 were measured continuously using Aquasense real-time monitoring system (SeaRAS Bergen, Norway). Three parameters were extrapolated from video recordings using artificial intelligence: i) average swimming speed, ii) swimming pattern (representing whether the fish swim in a straight or zigzagging direction) and iii) asynchronization (indicating to what degree the fish maintain a schooling behaviour).

Results: The results demonstrated that fish quickly react to H2S, showing a panic response characterised by faster swimming speed, erratic pattern, and loss of schooling behaviour. The response was concentration-dependent, increasing linearly up to 30-40 µg/L, above which a clear threshold was present. At concentrations around 40-50 µg/L, the change in behavior was significantly higher compared to lower concentrations, and additionally raising H2S did not result in further changes in behavior. Swimming parameters quickly returned to basal levels once H2S was no longer present in the water.

Conclusions: This study provides new insights on the sensitivity of Atlantic salmon to acute H2S exposure and highlights the potential behind the use of artificial intelligence and video-monitoring as early warning tools for poor water quality in RAS.

Characterization and possible mechanisms behind growth retardation (stuns) in atlantic salmon (salmo salar) under intensive farming contotions

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Characterization and possible mechanisms behind growth retardation (stunts) in Atlantic salmon (salmo salar) during intensive farming conditions

Introduction: Production-related disorders are non-pathogenic factors that impact the survival and welfare of farmed Atlantic salmon, with increased mortality rates, particularly in the early stages after sea transfer. Stunting, a disease-free condition characterized by impaired growth and high mortality, affects post-stocking fish and has been linked to sub-optimal smoltification including impaired hypoosmoregulatory capacity. To understand the underlying mechanisms of stunting, analyzing key processes associated with smoltification can identify critical factors contributing to this phenomenon.

Methodology: A longitudinal field study followed one fish group through six samplings from pre-smolt to post-stocking, the final sampling specifically targeting unaffected and stunted individuals. Important processes connected to smoltification were analyzed including gill gene expression related to osmoregulation and plasma osmolality. Regulatory mechanisms for smoltification, specifically gene expression of the pituitary-thyroid axis, were analyzed by real-time PCR. Plasma concentrations of cortisol, thyroxin (T4) and triiodothyronine (T3) were analyzed by a multiplex immunoassay.

Results: In the sea phase, stunted individuals showed significantly reduced weight and condition factor, a relationship between length and weight, while having significantly increased hepatosomatic index. Expression levels of two of three analyzed genes for Na/K-pump subunits (nka) in the gills, nkaα1a and nkaα3, were significantly different between stunts and normal growing fish. Interestingly, plasma osmolality was lower in stunts. Plasma T4 steadily increased through the freshwater phase, whereas T3 decreased. No correlation was found between T4 increase and the release of tshβb in the pituitary and no differences in plasma cortisol. Stunts showed significantly reduced plasma levels of T4 and T3 compared to unaffected fish, but a significant increase in gene expression for tshβb.

Conclusions: Stunted individuals showed significant differences in gene expression in two of the analyzed nka subunits. The freshwater subunit nkaα1a was upregulated, while the less known nkaα3 was downregulated. However, the stunted individuals showed lower plasma osmolality than unaffected fish. Stunts showed significantly lower plasma thyroid hormone levels. These differences might result from the reprioritization of energy expenditure in stunts from growth to maintaining homeostasis.

Progressive changes in Atlantic salmon gill health over time under culture in recirculating aquaculture systems

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There is a general understanding that gill health is compromised in fish reared under intenstive recirculating aquaculture systems (RAS). In two 3 month long studies of both commercial RAS post-smolt facilities as well as post-smolt production in RASLab under controlled conditions at commercial densities, the 97cceptable97ation and quantification of gill pathologies was undertaken using histopathology. In both studies water quality and 97cceptabl was also tracked. The severity of lesions, based on number of affected lamellae on affected filaments was generally low. However, there was a progressive increase in the amount of pathology 97cceptabl and number of affected filaments. The majority of lesions included lamellar clubbing and inflammatory infiltration of the lamellar and filament epithlelium. Hyperplasia of the lamellar and filamental epitheium occurred while extensive hyperplasia and lamellar fusion (synechia) was rare. Over the duration of the studies water water quality was within 97cceptable limits (ammonium, nitrite, nitrate, pH oxygen and carbon dioxide) and total suspended solids levels were low. There was a progressive increase in the total organic carbon load in both studies and gill pathology appeared to correlate with the increased load of organic carbon. There did not appear to be any clear patterns with regard to inflammatory or immunological responses in the gill as a result of the increased levels of inflammatory cell infiltration over time. It therefore appears that total organic carbon load may play a significant role in the progressive development of gill pathology in RAS.

Presence of salmon impact behaviour and survival of cleaner fish in sea-cages

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Introduction: Cleaner fish are used for delousing of farmed salmonids in sea water. However, mortality rates are high (40-100%). It is therefore relevant to ask whether cohabitation with salmon, is a problem. We have therefore studied the behaviour and welfare of wild-caught goldsinny, corkwing and both farmed and wild-caught ballan wrasse held in cages with and without Atlantic salmon.

Methodology: Six sea cages (12×12×12 m) were stocked with 9000 Atlantic salmon, 540 wild-caught wrasse (three cages) and farmed ballan wrasse (three cages) during summer 2022. An additional three cages were stocked with 540 wild-caught wrasse without salmon. Cages were equipped with artificial kelp and wrasse were fed feed pellets, feeding blocks and shrimp at 3, 6 and 9 m.
Behaviour and depth observations were done with underwater cameras three times a week for 10 weeks during daylight. Every 4 weeks, 10 fish from each cage were examined. Welfare score and stomach content were noted. Inner organs were sampled for histological and PCR analysis, in addition to bacteriological sampling from head kidney.

Results and conclusions: No clear differences in welfare scores were observed between wrasse held in cages with or without salmon. However, mortality rates differed between cages. Total loss (registered mortality + fish missing at end) where 54 ± 4 % (mean ± SE) for wrasse held together with salmon, while wrasse without salmon had a loss of 38 ± 5 %.

There was a clear difference in behavior. Wrasse in cages without salmon tended to use the whole volume to a greater extent and exhibited a greater variety of depth preferences than wrasse habiting with salmon.
Aeromonas salmonicida infection was confirmed by bacteriology and histopathology in diseased individuals with white granulomas in the kidney.

Examination of seemingly healthy individuals showed no signs of disease. However, histopathological examination of gills commonly showed presence of Tricodina sp., Some individuals showed signs of inflammation in the heart. In other individuals, examination of kidney, spleen and liver showed aggregates of melanomacrophages (melano-macrophage centres).

Salmon seemed to have an impact on behaviour and to some extent survival of wrasse, potentially affecting their lice-eating abilities.

The effect of repeated Hydrolicer treatments on Atlantic salmon (Salmo salar L.) in a commercial production cycle

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With the expansion of Atlantic salmon aquaculture, the economic impacts of salmon lice (Lepeophtheirus salmonis) have increased in Norwegian farms. With widespread resistance of salmon louse towards all available medicinal treatments, the industry focused on non-medicinal operations to control salmon lice. In this study, Atlantic salmon (Salmo salar L.) were reared in sea-cages at Skogshamn in Dyrøy (Troms, Norway), and they were treated consecutively three times with the mechanical de-lice method Hydrolicer. This technology uses high pressure water and require the fish to be crowded and then pumped up into a treatment system where the lice are mechanically removed from fish. Samples were taken from the examined fish pre- and post each treatment. Growth and mortality data were used to assess fish welfare status, and collected tissue samples were analyzed using histology, immunohistochemistry and Real-Time PCR methods.

Increased mortality and reduced specific growth rate (SGR) were shown following the Hydrolicer treatments. Findings from histological examinations of HE-stained gills, thymus and nasal cavity tissues showed the presence of bleeding, hyperplasia and metaplasia of mucous cells, and epithelial hyperplasia. Pathological alterations in different tissue structures were acerbated with the number of treatments, except of lamellar clubbing and bleeding in the thymus and nasal cavity. IHC-stained thymus and nasal cavity tissues showed the presence of both IgM+ and PCNA+ cells throughout the treatment period. The number of IgM+ cells did not change in both organs during the treatment period; however, PCNA+ cells increased during the treatment period. Relative gene expression data showed a decrease in both CD4 and CD8 expression in the thymus and an increase of both genes in the head kidney. Expression of IL-β and TNF-α was less affected by the Hydrolicer treatments, and this might be explained by sampling time interval.

To conclude, repeated Hydrolicer treatments negatively affect Atlantic salmon welfare and induce pathological changes in the exposed tissues such as gills, thymus, and nasal cavity.

Keywords: Atlantic salmon, Lepeophtheirus salmonis, Salmo salar, sea lice, Hydrolicer treatment

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Acute and chronic stress response of lumpfish following air exposure: effects on cortisol, health, cataract and plasma free amino acids

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Introduction: Lumpfish (Cyclopterus lumpus), a novel aquaculture species, emerged from the urgent need for alternative delousing methods. However, knowledge on lumpfish stress physiology have not accompanied the pace of expansion of this species. This has become apparent with increasing evidence reporting high mortality rates and disease outbreaks. In order to contribute to this endeavour of improving the lumpfish welfare situation, two studies were conducted to investigate the stress response following 1 min acute air exposure over a 24h period, and the effects of repeated acute air exposure over 12 weeks on health, cataract development, immune parameters, stress response and plasma free amino acids.

Methods: Acute study: lumpfish were air-exposed for 1 minute and sampled at specific timepoints (30 min, 1 h, 2 h, 4 h and 24 h) following stress (n=8). Stress and energy biomarkers were analysed in plasma and liver using spectrophotometric assays.

Chronic study: 4 groups (0C; 2S; 1S and 4S) were set up and exposed to air (1 min) along 12 weeks. Group 4S was subjected to air exposure 4 times per week, group 2S twice a week, 1s once a week, and group 0C, was the control, left undisturbed. Stress and immune biomarkers were analysed with spectrophotometry. Health was assessed using a lumpfish welfare scoring system and plasma free amino acids were investigated using high-performance liquid chromatography.

Results: Acute study: Cortisol increased significantly on stressed fish, peaking at 30 min post stress and returning to resting levels 4 hours post stress. Triglycerides in plasma and liver were affected by stress, indicating a potential change in energy substrate preference.

Chronic study: Repeated air exposure revealed significant changes in a group of essential amino acids. Immune modulation was also identified, by significant changes in plasma bactericidal activity and other immune parameters. Cataract developed in virtually all fish, regardless of group.

Conclusion: Overall, neither the acute nor the chronic air exposure reveal profound effects on lumpfish physiology, indicating that lumpfish tolerate rather well 1-min air exposure.

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Determination of Operational Welfare indicators as a tool for a sustainable aquaculture of the common octopus Octopus vulgaris

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Animal welfare is an issue of growing public concern, and of great importance for the incipient development of octopus aquaculture. The common octopus, Octopus vulgaris, is a species of great commercial interest, and an excellent candidate for aquaculture diversification. Overexploitation of natural stocks and high market demand mean that sustainable octopus farming has a positive impact on natural stocks, thus being a tool to alleviate fishing pressure and preserve the species. Once the zootechnical deficiencies have been overcome, the optimization and standardisation of specific approaches and practices to monitor and safeguard animal welfare are needed to achieve a sustainable culture. Criteria for assessing welfare should focus primarily on diet, appropriate housing conditions and living environment as well as behaviour and health.

In this study, specific octopus welfare markers defined as biological Operational Welfare Indicators (OWIs) have been identified in order to be measured and integrated into good aquaculture practices monitoring plans. Here is presented the first determination of biological OWI for pathogen identification, disease diagnosis and animal health monitoring in captivity. Non invasive diagnostic tools for pathology prevention and control includes microbiological analysis of tank water and skin, and parasitological analysis of fecal smears. Quantitative PCR protocols have been optimized for early identification and absolute quantification of pathogens such as Vibrio sp. in mucus and tank water, or Aggegata octopiana in fecal samples. In addition, specific parameters involved in defense mechanisms, such as antibacterial or antiviral antivities of skin mucus have been studied as indicators of natural defense capability.

The results presented on the identification and selection of biological OWIs will be a key tool for the incipient development of octopus aquaculture.

Beating primary heart cell cultures from salmonids resemble expression profiles similar to their tissue origin

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The aquaculture sector is threatened by various challenges ranging from climate change to disease outbreaks, while the demand for seafood continues to grow, requiring an increase and optimisation of fish production. To address these emerging issues, fish cell cultures can serve as predictive platforms to study different areas, while reducing the need for experimental animals. In Europe, salmonids are the most consumed fish species and are increasingly challenged by high midsummer water temperatures and pathogens. These external influences can be challenging, particularly for the cardiovascular system. To mimic stressors such as viral infections or oxygen deprivation, long-term cardiac cell cultures from salmonids can be used to investigate therapeutic interventions. They exhibit high proliferation, while losing their specific properties and cell tropism required for proper modelling.

The aim of our study was to establish a protocol for the isolation, maintenance and characterisation of primary cardiomyocytes as an improved model for long-term cultures. We used hearts from adult Atlantic salmon, brown trout and rainbow trout. During the development of the protocol, the cells were regularly monitored for their beating patterns, responsiveness to adrenergic drugs, cell growth and the formation of cell-cell junctions. Furthermore, different culture conditions such as various cell culture media and the influence of culture time on cell characteristics were investigated by immunocytochemistry, microscopy and quantitative real-time PCR.

The main outcome of our study is an adapted protocol leading to beating primary cell cultures from salmonid hearts. In all species, contraction was observed for up to 6 weeks, with some cultures beating for more than 100 days. This observation is consistent with immunocytochemical staining, which reveals a massive functional network of tropomyosin. Furthermore, mRNA expressions of cardiac transcription factors such as gata4 or mef2c, as well as functional markers such as serca2 and myh7, are comparable to their tissue origin and increased compared to long-term cardiac cell cultures.

To our knowledge, this is the first study describing beating primary salmonid heart cells grown as a monolayer. This study adds to the portfolio of reliable in vitro models for the study of fish physiology and health, and for vaccine development.

RNAi-directed knockdown in micro-jellyfish blood parasite Sphaerospora molnari

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Introduction: The method of RNA interference (RNAi) is recognized as an effective approach for the suppression of gene expression and monitoring gene regulation in various organisms. Despite the wide application of RNAi in free-living cnidarians, research on its use in endoparasitic cnidarians Myxozoa is currently limited.

Methodology: We implemented the RNA interference (RNAi) method through the use of dsRNA soaking and successfully suppressed gene expression in vitro cultures of Sphaerospora molnari, a parasite infecting common carp. Specifically, we knocked down two unusual actin and motor myosin 10 homologs. We tested a dsRNA soaking strategy to induce RNAi knockdown and established a protocol for the synthesis and purification of dsRNA. To investigate the cell entry of dsRNA, we labeled the dsRNA and performed confocal microscopy. We quantified the gene expression downregulation upon gene knockdown using real-time PCR, confocal microscopy, and western blotting.

Results: We propose an optimal workflow for the generation of dsRNA and induction of RNAi interference in vitro myxozoan culture of S. molnari. We observed intracellular uptake of dsRNA after 30 minutes which accumulated within the cell-in-cell structure of blood stages. We successfully performed knockdown in vitro cultured blood stages of S. molnari for 48 hours. Gene silencing of selected target genes resulted in different phenotypes. Actin showed loss of rotational cell tumbling motility of blood stages whereas knockdown of myosin 10-like altered the speed of cell motility of S. molnari.

Conclusions: The dsRNA soaking approach to cell entry represents a low-cost, effective, and constitutive method. We present the pilot application of RNAi on myxozoan S. molnari blood stages. Our study supports previous findings on the crucial role of atypical actin in S. molnari cell motility. This approach not only advances functional research in Myxozoa but also provides new opportunities for the investigation of potential therapeutic targets and drug discovery in economically important fish parasites. This work was supported by the Czech Science Foundation [20-30321Y, 19-28399X].

Phylogenetic analysis, tissue distribution and response of fish cathepsin Ls to myxozoan infection in common carp

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Introduction: Fish cathepsins play important roles in the proteolysis, regulation of immune functions and tissue remodeling. In common carp Cyprinus carpio, L-like cathepsins have been characterized biochemically, however, their homology to other fish cathepsins with known functions, tissue distribution, and response to pathogen infections are unknown.

Methodology: To explore the functional diversity of carp fish cathepsins, we have mined the target genes in all publicly available carp genomic datasets and investigated their genome location, gene synteny, and phylogeny in relation to other fish species. qPCR-based expressional profiling of cathepsin Ls was performed in various carp tissues to determine the gene spatial distribution. To further elucidate the roles of these enzymes in parasitic infections, challenges to Sphaerospora molnari, a common carp pathogen, were perfomed and gene spatial and temporal gene expression was identified.

Results and Conclusions: Our bioinformatic analyses demonstrate that clustering of fish cathepsin Ls mirrors the vertebrate evolution and their expansion is linked to the whole-genome duplications in fish. In agnathans, the earliest known vertebrates, three ancestral homologues are present, and this number increases in cartilaginous and teleost fish to reach eight homologues in tetraploid cyprinids. An extra gene exists in C. carpio genome due to a gene duplication. Cathepsin Ls of common carp cluster in four major phylogenetic clades which we assign to ctsl.1, cts.12, ctsll and ctsla based on the homology to zebrafish genes. Expression of carp cathepsin Ls is tissue-specific with highest levels in gills and intestine for ctsl.1, testes and brain for ctsl.12 and ctsll, and blood and liver for ctsla homologues. Gene expressional profiling following S. molnari-infection revealed tissue-specific and time-dependent pattern of ctsl.1 and ctsla homologues with upregulation encountered in the gills, skin, spleen, liver, intestine, and leukocytes mainly during the acute phase of infection. As evidenced by their diverse spatial and temporal gene expression in tissues and during infection, carp cathepsin Ls appear to have developed distinct functional roles throughout evolution. Our findings hold significance for comparative immunological research and functional investigation of cathepsins in various fish species.

Myxozoans modulate early immune responses in rainbow trout: Quantitative shotgun proteomics at the portals of entry

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Background: To date, little is known about the proteomic changes in rainbow trout during whirling disease and proliferative kidney disease at the portals of entry after infection with the two myxozoans, Myxobolus cerebralis, and Tetracapsuloides bryosalmonae that cause these diseases. Although we have a general view of the cellular basis of the immune response of rainbow trout, the proteomic alterations underlying the immune responses of fish to myxozoan parasites remain elusive. Therefore, the aim of this study was to provide the first proteomic profiles of the host in the search for evasion strategies against coinfection with M. cerebralis and T. bryosalmonae.

Methods: One group of fish was initially infected with M. cerebralis and the other group with T. bryosalmonae. After 30 days, fish in each group were co-infected with the other parasite. At days 1 and 4 post exposure as well as day 1 post co-infection, we examined proteomic changes in the caudal fins and gills of rainbow trout as portals of entry for M. cerebralis and T. pryosalmonae, respectively before and after co-infection, using a quantitative proteomic approach.

Results: The data obtained allowed an insight into the post-transcriptional and post-translational regulation of host proteins to the two myxozoan parasites at the portals of entry in the infected rainbow trout at the above sampling times. The proteins were analysed to provide dynamic information on host-parasite interactions, biological processes and pathways activated by co-infection and revealed infection-dependant defence strategies of the host fish. Depending on the order of infection with the two myxozoans and the portal of entry, mucosal responses varied, indicating differential priming of mucosal tissue responses by the two species.

Conclusion: The results of this study increase our knowledge on co-infections by myxozoan parasites, fill the gaps of rainbow trout immune responses against myxozoans at the portals of entry and support a better understanding of host-parasite interactions.

Understanding the role of immuno-reactive proteins may be useful for drug development and expand tools for disease management. Future comparative proteomic studies may help uncover the mechanisms of disease development and the impact of myxozoans on the outcome of rainbow trout co-infections.

Survival strategy of Tetracapsuloides bryosalmonae: Adapting gene expression to fish host environment?

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Tetracapsuloides bryosalmonae causes proliferative kidney disease (PKD) in many freshwater salmonids. Among the different salmonids, brown trout is a carrier host while rainbow trout is a dead-end host for this myxozoan parasite. The differential behaviour of the parasite between these two fish hosts could be a determinant of either host or parasite associated factors. Hence, we attempted to investigate the role of parasite associated factors in the differential progression of parasite development and their fate in these two closely related hosts. Both brown trout and rainbow trout were exposed to the spores of T. bryosalmonae collected from the infected bryozoan host Fredericella sultana. Using fluorescence activated cell sorting (FACS), we isolated parasite cells from the infected kidneys of both fish hosts using anti-Tetracapsuloides bryosalmonae (PKX) monoclonal antibody. RNA extracted from sorted parasite cells were further processed for RNA sequencing. Adopting this strategy, we identified 1120 transcripts to be expressed differentially in parasites derived from brown trout and rainbow trout. We found the differentially expressed transcripts were involved in important biological pathways such as cytoskeleton organisation, cell polarity, translation, ribonucleoprotein complex biogenesis and subunit organisation. These findings show that T. bryosalmonae has distinct molecular adaptations, which may account for its different outcomes in the two hosts. The identification of these differentially expressed transcripts may offer opportunities for the discovery of novel drug targets that could be used to treat PKD. By employing FACS-based isolation of T. bryosalmonae cells from infected kidney of fish, we offer a novel approach to advance research in virulence and host-pathogen interactions.

Myxozoan laboratory life cycle models: recent progress and pending requirements

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Several myxozoan parasites are important pathogens of fish species relevant to freshwater and marine aquaculture, but also to natural stocks. Research on parasite development and localization in fish, host-parasite interaction and trials using treatments and vaccines are being performed in a handful of myxozoan model systems which are being perpetuated in the lab in vivo, often including both, vertebrate and invertebrate hosts. This offers advantages to studying the related diseases in the wild, mainly independency from seasonal cycles and co-infections with other pathogens.

However, laboratory maintenance is both labor and time intensive, and the lack of in vitro models creates unwanted dependencies on the use of animals for the maintenance of the parasites.

Here we present a summary of the progress we have made towards the reduction of fish for the maintenance of our laboratory model, Sphaerospora molnari, a parasite of common carp. We developed parasite isolation methods, optimized short-term in vitro culture and cryopreservation by comparative approach, hence allowing us to ‘freeze’ the parasite life cycle until stages are required for infection experiments. For another recently adopted myxozoan laboratory life cycle, that of Tetracapsuloides bryosalmonae, the etiological agent of proliferative kidney disease, we developed semi-automated feeding systems for bryozoan host colonies using small bioreactors for brown and blue algae.

Improving myxozoan laboratory cultures is key to producing infective spore stages year-round, to test parasite mitigation and preventive strategies on demand. We propose further steps towards host-independent in vitro approaches including bioprints.

Parvicapsula pseudobranchicola infections in Iceland - lifecycle and trends

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Parvicapsulosis is a problematic disease caused by the myxozoan parasite Parvicapsula pseudobranchicola (P.p). The parasite was described by Karlsbakk et al. (2002), in farmed Atlantic salmon (Salmo salar) in Norway. The main target organ of P.p are the pseudobranchs and clinical signs include surface dwelling, disorganized swimming, lethargy and signs of blindness. P.p. was first reported in Iceland in 2019 and has been a recurring problem in the Icelandic salmon farming industry ever since. The aim of this project is to map the distribution and prevalence of P.p in Icelandic salmonids, both farmed and wild, as well as identifying the final host. Fish from several locations around the island were screened for P.p. by using PCR, histology and in situ hybridization. Preliminary results reveal that the prevalence of P.p. in wild salmonids is very low, both in proximity to farming sites and further away. In contrast, prevalence in farmed salmon is high, although variable depending on the location. The parasite was successfully amplified in a polychaete host species. Hence, this is the first report of the final host of Parvicapsula pseudobranchicola.

Histopathological assessment of Atlantic salmon exposed to calcium oxide particles. A controlled clinical study

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Introduction: The use of quicklime, or calcium oxide (CaO), as a controlling agent for pests is documented back to the beginning of the 20th century. CaO particles have been successfully applied in Nova Scotia and California for the management of sea urchins and starfish in commercial kelp and oyster beds and in Norwegian fjords for the control of sea urchin populations. Recent studies have proven the efficacy of CaO to kill planktonic copepods, making it a promising and cost-effective candidate for lice control in Atlantic salmon farming industry.

Methodology: To investigate the safety of quicklime treatment on farmed fish, the present study exposed Atlantic salmon (Salmo salar) post smolts to fine CaO particles (0.1-0.3 mm; 0.3 g/L) twice a week for three consecutive weeks at 5˚C and 12˚C in a flow-through system. Mortality and histopathology of skin, eyes, gills, and intestine were evaluated and compared with an untreated control group.

Results: CaO exposure did not induced fish mortality nor histopathological damages in skin, eyes, or intestine. At the gill level, CaO exposure was not correlated with the occurrence of inflammation and hyperplasia. A significant positive correlation was detected between CaO exposure and the occurrence of vascular damages and necrosis, extending however to less than 10% of each affected tissue. The effect was not independent from water temperature.

Conclusions: A good anti-parasitic treatment should not only be effective against the intended target but also harmless to the farmed species. This study showed that CaO, while caused no mortality and no harm to most tissues, it induced mild to moderate gills damages under laboratory conditions. Follow-up studies will be performed to further evaluate its safety under standard farming conditions at sea.

Typical effects of stress on Cyprinids

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Introduction: Cyprinid fish are commonly used for research and in aquaculture. Raising cyprinids is often connected with exposure to stressors, e.g., handling stress. The stress can be acute stress or chronic stress. Both may influence the appetite and the well-being of fish. But stress does not include only negative influences on fish, since events such as feed application or manipulations in the tank may cause stress but not necessarily have negative consequence for the fish. Obviously, fish are able to differentiate between negative and positive influences which is necessary for an optimal adaptation to environmental changes. A number of experiments was conducted to increase our understanding of the different stress responses.

Methodology: Experiments including acutely and chronic stressed carp (Cyprinus carpio) and zebrafish (Danio rerio) have been performed. The different settings for the experiments will be explained in the presentation. Physiological parameters and gene expression studies have been performed on different brain parts. For the chronic experiment, also skin colour has been assessed.

Results: Negative stressors, i.e., air exposure, chasing and confinement resulted in typical stress responses observed in the blood and activation of stress axis-related genes in the brain. In addition, the regulation of appetite genes in the brain was also affected by application of negative stressors. The experiments that have been conducted also revealed that rearing fish in groups with low numbers of individuals causes changes of stress-related parameters. Furthermore, feed rewarding resulted in different effects on some of the measured parameters. Chronic stress also affected body coloration of carp but not their fin condition.

Conclusion: Typical effects of air exposure were directly compared to effects of confinement and chasing. It is noteworthy that a stressful event for one minute changes the body physiology and brain gene regulation for further 60 - 90 min. Different effects of negative stressors compared with positive stimuli could be identified. Furthermore, evidence that stressful events affect appetite regulation in the fish brain is given. Finally, signs of chronic stress are summarized in order to identify parameters that may be helpful for the identification of effects of prolonged stress exposure of farmed fish.

What effect does biofilter volume have on water quality and fish welfare in a RAS system?

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Recirculating aquaculture systems (RAS) rely on a stable and established biofilter to provide optimal rearing conditions for Atlantic salmon. The aim of the biofilter is to convert the ammonia into nitrite, which is then converted to the less harmful nitrate. In order to do this, the biofilter chips must be colonised by a range of bacteria, including ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB). The accepted method to set up a biofilter is to use as many chips as possible, in order to be as efficient as possible.

This project wanted to challenge the norm and test whether changing the volume of biofilter chips would affect the ability of the AOBs and NOBs to maintain optimal rearing conditions.

This study included two 2500L RAS units with a starting stocking density of ±8kg/m³, with mature biochips from a single source. One system was left as a control, while 3/4 of the biochips in the second system were removed. Both systems were then allowed to continue to develop through a brackish water phase to a seawater phase, maintaining the altered biochip volume throughout.

Parameters such as oxygen, salinity, pH, NH_4, NO_2, and NO_3 were measured throughout the study for both systems. In addition, monthly welfare checks were conducted to map the fish health and growth.

The systems functioned almost identically with the primary parameters remaining stable, even the NH_4 which was expected to increase. This project suggests that biofilters are able to adjust fairly well under unfavourable conditions.

Validation of automated camera based assignment of marked salmon for field trials in commercial marine farming

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Introduction: Camera based monitoring of marine farmed salmon is rapidly moving into automated applications, such as louse counts, weight estimation, and cutaneous wound classification. In a recently started "mark-and-mix" design field trial where adipose fin marked individuals (test group) will be held together with unmarked (control) fish in the same commercial size net pen. Subject of the trial is prevention of cutaneous wounds using a probiotic bacterial culture. As the first step, we validate the camera-based assignment of fish to the marked or the unmarked group established using machine learning techniques.

Methodology: The image analysis software is based on “deep learning” where photographic images showing a clear lateral outline of each fish and detecting the presence and position of eye and main fins are required for acceptance. Machine learning was initiated by visual annotation of images showing or lacking an intact adipose fin between the dorsal fin and the tail fin. Should the algorithm show a below-threshold deviation from a straight line between the dorsal and tail fin, the fish is being categorized as lacking its adipose fin. For validation of group assignment, images showing close to the threshold value will be summarized as possible false positives or false negatives and the impact of such possible false assignments will be quantified.

Results: Initial modelling suggests that the model can effectively assign fish to the adipose fin-clipped or -intact group. During initial assignment runs using an available population with adipose fin clipped salmon, 3-4% erroneous assignments to either group was observed. Further training and refinement of the model is under way, aiming to categorize most such erroneous assignments “inconclusive”. Results showing the impact of the refined assignments will be presented.

Conclusions: Camera based assignment of individual, visibly marked salmon being reared in “common garden”/cohabitant setup can give exceptionally high number of observations per group in real-time during the study period. An early model for adipose fin clip assignment suggests the model is valid for use in commercial-size salmon farming.

Highly-specialised training programs for veterinarians devoted to aquatic animal health: Progress since the EAFP 2021 Aquatic Veterinary Education Special Session

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Providing veterinarians and paraveterinarians with opportunities to achieve a specific skill set to support recognition of a high level professional qualification (Veterinary Specialist) was discussed during previous EAFP meeting in a special session on aquatic veterinary education. However, it became apparent that despite reasonable success in promoting specific aquatic animal health training programs to veterinary practitioners, wider stakeholder audience has only limited awareness about them. European College of Aquatic Animal Health (ECAAH) has recently received full recognition by the European Board of Veterinary Specialisation (EBVS), and currently offers four residency training programs through twelve main and satellite training centers in three countries. Upon completion of the requirements, residents become EBVS® Specialists in Aquatic Animal Health. ECAAH has worked with EBVS to achieve standardization of residency programs under the 8th level of European Qualification Framework. ECAAH residents are frequently combining AAH specialty training with the academic PhD, or professional employment in government or companies. Such diversification helps the stakeholders to become aware of highly trained and credentialled workforce, leading to a formal recognition by the national health services. ECAAH has increased efforts to communicate existence of the AAH specialty educational programs, targeting audiences both at national and international conferences (e.g., SIPI, EAS and EAFP) and through intensifying collaboration with World Aquatic Veterinary Medical Association (WAVMA) and EAFP. It is expected that continued interaction with private feed companies, aquaculture producers, diagnostic services, or veterinary practices, could actively contribute to increased “hireability” of residents and improvement of continuous education process itself, offering opportunities for hosting the residents within respective stakeholders sectors as part of their training. Therefore, from an educational point of view, the progress is satisfactory thanks to the developed synergies, however, stakeholder awareness needs further improvements. In this regard, making aquatic animal health education programs and specialist role more visible through Federation of European Aquaculture Producers (FEAP), Aquaculture Advisory Council (AAC) and other entities, as a joined effort of ECAAH, EAFP and WAVMA, could act as a powerful interface between stakeholders and educational organizations, with a goal to benefit the industry through increased specialist involvement, life-long learning, and job placement opportunities.

Fostering international opportunities towards aquatic animal medical competence certification

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Introduction: Worldwide there is an increasing demand for professional services and research related to a broad range of aquatic animal species, stimulated by an increasing demand of sustainably farmed fish and shellfish. Aquatic veterinarians and para-veterinarians play a key role to provide diagnostic services and therapeutic solutions to support the fast-growing sectors of aquaculture. Professional support is needed in zoo attractions, for ornamental aquatic pets, to help with wildlife species management, and to support the exponential use of aquatic species in biomedical research. WAVMA supports the global development of a veterinary task force with expertise in a wide range of aquatic animals and specialties.

Methodology: The Education and Students Committee (ESC) fosters opportunities to dispense complementary training in aquatic animal health worldwide. Composed of volunteers, ESC is currently organized into three subcommittees, each chaired by a dedicated WAVMA member. WAVMA Student Chapters are established at veterinary universities to stimulate extracurricular learning and to promote student networking with professionals. The Education Resources subcommittee exposes students to training opportunities, thus they can better appreciate the roles of an aquatic veterinarian in aquatic animal wellbeing, within the framework of the UN Sustainable Development Goals and One Health. The WebCEPD subcommittee coordinates the Continuing Education and Professional Development (CEPD) program, monthly offering virtual lectures given by experts and often in partnership with other professional associations. Continuous Education (CE) credits can be obtained after completing the knowledge and skills assessment (KSA). The Education Support subcommittee manages small funding schemes, both for Student Chapters (Mini Grant) and for individuals (John Pitts Aquatic Veterinary Education Awards), enabling awardees to gain expertise with any aquatic specialty. Through the Credential Committee WAVMA offers the only day one competence certification programs that are recognized worldwide: the well-established Certified Aquatic Veterinarian (CertAqV), and the recently started Certified Aquatic Veterinary Nurse/Technician (CertAqVNT).

Results and Conclusions: WAVMA gradually became a global reference for professionals approaching aquatic species and specialties. The success of the WAVMA training opportunities and the numerous applicants seeking for the

WAVMA certifications indicate as new generations of veterinarians and para-veterinarians are attracted in the field of the aquatic animal health.

Continuing education, specialization and worldwide certification of professional veterinarians in aquaculture

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In Aquaculture, the offer of post-graduate training for veterinarians and non-veterinarians is abundant. Veterinary or agronomic faculties offer Ph.D. or other specific programs. Associations such as WAVMA, WAS and others organize numerous quality congresses, seminars, and workshops... and deliver diplomas.

Many specialized Webinars offer training, but attendants have gaps in their knowledge. In many countries, only those courses are available to professionals, some of whom have good skills but no official recognition.
American / European Colleges members only are considered specialists among veterinarians, but PhD, Certificates... are other recognitions of competencies.

When organizations such as WOAH, WTO, FAO, WB, insurance companies, etc., are looking for experts, the list is long, but "who" is really an expert? So, in 2010, WOAH supported the creation of the World Veterinary Education in Production Animal Health (NPO). The WVEPAH was mandated to build a body of international experts in Health and Production in: Aviculture, Aquaculture, Swine and Laboratory Animals, all having in common the high level required by this expert status. The WVEPAH offers additional training programs to professionals working in those fields to master all aspects of their field and thus manage severe crises and audit farms. They also can be the trainer of trainers for national programs.

The examination requires this highly homogeneous knowledge and is the same for all nationalities. The graduation is "Professional Certificate in Animal Health: *Aquaculture Production" (PCAHAP). The University of Montreal delivers the diploma that the WOAH validates worldwide for its regulatory part, which gives the graduates de facto the status of an international expert.

In Aviculture, this new experts' body now establishes. Its members have an official recognition important in their practice; their employer companies a certainty of competence; their country, through the National Veterinary Services Authority, gets by preventing crisis a continuity assurance of production in quantity and quality, a training capacity but also the independence of the expertise in aquaculture including in commercial exchanges. The WVEPAH is open to cooperation with other Aquaculture training institutions since its goal is mainly the expert's certification only based on their competencies.

Keywords: Training, Certification, Validation, International experts.

A new era for Aquatic Veterinary Education: MicroMOOC Courses

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Introduction: Virtual continuing education can provide valuable learning opportunities on cutting-edge technologies and allows to maintain up-to-date knowledge. MicroMOOCs (Massive Open Online Courses) have become increasingly important in higher education, thanks to their ability to provide flexible and widely accessible learning opportunities for students and professionals worldwide. With the increasing global need for aquatic veterinarian services, traditional educational methods face challenges in providing accessible, timely, and comprehensive learning experiences. MicroMOOCs are suitable to enhance the aquatic veterinary education, providing compact, targeted, and high-quality educational modules that can be accessed remotely by learners.

Methodology: We evaluated the effectiveness of MicroMOOCs and evaluated their potential in aquatic veterinary education and toward competence certification programs. When designing these programs, advantages and limitations of this approach should be kept into consideration, evaluating the pedagogical models and the updated contents delivered. The WAVMA Education and Students Committee (ESC) actively works to dispense complementary training to aquatic students, veterinarians, and para-veterinarians around the world. We analyzed the example of the successful WAVMA WebCEPD (Continuing Education and Professional Development) webinar series, in which lectures are given by invited experts from various fields of expertise. Live webinars can be attended upon free registration and the recordings remain available through the WAVMA website. Upon the completion of a short knowledge and skills assessment (KSA), Continuous Education (CE) credits can be obtained by each participant.

Conclusions: Virtual learning opportunities have become important tools in higher education thanks to their ability to provide flexible and accessible learning opportunities, and to democratize education. MicroMOOCs offer a promising opportunity to revolutionize the veterinary education and we do recommend further exploration of this model to meet the evolving needs of the aquaculture industry.

Offering fresh aquatic veterinary training to students in Michigan

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Introduction: Veterinary students receive little training on health and medicine of aquatic species. Many North American veterinary universities often focus their training on traditional companion pets and agriculture species. Professional veterinary careers are becoming more appealing under the One Health perspective, due to an increasing demand for professional services in industrial aquaculture, seafood inspection, ornamental aquatic pets care, wildlife management, and biomedical research. In 2020, the 3-week elective Clerkship in Aquatic Animal Medicine (PDI-636) was reinvented to provide Michigan State University DVM students with fundamental knowledge on core subjects, to orientate them towards clinical reasoning to integrate aquatics into their career practices.

Methodology: During the morning the class is introduced to the topic of the day with specialized lectures, given either by the course coordinator or by many local and international guest lectures. During the afternoon students get involved in practical learning through individual, in pair, or group activities designed to foster adaptive learning while focusing on assigned topics. Simultaneously student practice writing, with assignments about summarizing articles, webinars, or solving real case studies using scientific English to prepare presentations, abstracts, and diagnostic reports. Laboratory activities train students in fish anatomy, necropsy techniques with internal organs sampling, anesthesia and biopsy procedures completed by microscopy evaluation of the specimens collected. Several excursions allow students visiting public aquaria, research lab, fish hatchery, and a local large aquatic pet shop. Through the entire course each student retrieves biological, production, and health information about an assigned fish species, delivering a final presentation. During the Spring of 2023, an entire week of this course was organized in partnership with University of Pretoria, South Africa, providing veterinary students from both universities with additional learning opportunities.

Results and Conclusions: The clerkship is highly appreciated by students and course collaborators. The broad diversity of lecturers and students, experiences, traits, and backgrounds are valuable in providing a positive learning environment, rich with many perspectives. Despite the direction within veterinary medicine that each student will individually pursue, the updated knowledge provided by this unique course serves as a valuable source of information to orientate young veterinarians in their career decisions.

A Sparicotyle chrysophrii in vivo model reproducing the pathological outcomes of sparicotylosis in gilthead seabream (Sparus aurata)

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Sparicotyle chrysophrii (Microcotylidae) is considered the most threatening parasite affecting the gilthead seabream (Sparus aurata, GSB) in sea cage farming. There are no standardised in vivo models fully described in the scientific literature. This study aims to explore the best experimental conditions to set up an in vivo infection model capable of mimicking the sparicotylosis signs observed in diseased farmed fish.

A recipient (R) fish tank (200L) with naïve GSB (N=28) received water from two S. chrysophrii-infected donor tanks (N=52; 200L) in a recirculating aquaculture system (RAS). Parasite egg collectors, consisting of a polyester mesh in a supporting plastic frame, were placed in the R tank in order to monitor the progression of the parasitosis. An additional tank with control unexposed naïve fish (C) was maintained in parallel (N=20) disconnected from the RAS. Five C were sampled at 0 days post exposure (dpe), whereas 3 C and 5 R were sampled at 14, 28, 42, 50, 58 dpe. Haemoglobin values were immediately measured and haematocrit values determined by microhematocrit capillary centrifugation. All right gill arches were dissected and infection intensities of juvenile and adult S. chrysophrii determined under a stereomicroscope. The left gill arches, head kidney, spleen and liver samples were fixed in Bouin's solution and processed for routine paraffin histology.

R fish were successfully infected (100% prevalence after 14 dpe) and the parasite cycle was completed in the experimental setup. Adult parasite load was positively correlated with host biometric factors, temperature, dpe and tank biomass, whereas it negatively correlated with haemoglobin, haematocrit and condition factor. Egg counts peaked at 35 dpe and haemoglobin and haematocrit significantly dropped around 40 dpe. Furthermore, the abundance of eosinophilic granular cells and goblet cells in gill filaments, and splenic melanomacrophagic centres increased, whereas hepatic fat was depleted in infected GSB. These signs of infection mimic those of clinical sparicotylosis observed in enzootic culture facilities.

This study provides an advancement not only for studying S. chrysophrii's biology and its interaction with its host, but also for further studying the disease under experimental conditions in search of treatment alternatives and prophylactic measures.

Reproductive strategies of the parasitic flatworm Thaparocleidus vistulensis (Platyhelminthes, Monogenea) infecting the European catfish (Silurus glanis)

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Introduction: In this study, the life cycle of Thaparocleidus vistulensis, a host-specific monogenean parasite of the European catfish (Silurus glanis), was investigated by detailed observation of infection dynamics, hatching rates, egg development and in vitro survival rates of the parasite at different life stages.

Methodology: A total of 30 naïve fingerlings cohabiting with infectious donor fish were examined in triplicate. Two fish were dissected every two days during the 10-day experimental period to explore the infection dynamics of the parasite on their gills. Freshly laid eggs by adult monogeneans were collected and observed daily under a light microscope until they hatched. A total of 445 eggs were collected using in-house devices or glass petri dishes and distributed into wells of 96-well microtiter plate containing filtered fish tank water to determine their hatching rates. A similar method was used to investigate the survival rates of the parasite without a host in the different life stages (larvae, developing, and adult).

Results: The infection dynamics in the fish tanks revealed significant potential for T. vistulensis to propagate in European catfish within ten days, depending on the severity of the initial infection of the donor fish. The first eggs hatched three to four days after oviposition, and the hatching rate peaked on the fifth day (89.7%). The highest survival rate for oncomiracidia without a host was found to be 7.4% after five days, with developing and adult parasites only having survival rates of 0.9% and 1.6% for three days on average, respectively.

Conclusion: The knowledge acquired from this study on the reproductive strategies of T. vistulensis can provide a valuable basis for controlling gill-fluke infections in farmed European catfish stocks in fish farms.

Keywords: Thaparocleidus vistulensis, specific monogenean, Silurus glanis, European catfish, infection dynamics, life cycle, survival rates

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Target protein expression on Tetrahymena cell-surface using the signal peptide and GPI-anchor sequences of a immobilization antigen of Cryptocaryon irritans

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Introduction: Cryptocaryoniasis, caused by Cryptocaryon irritans, is a significant threat to marine fish cultures in tropical and subtropical waters. However, controlling this disease is still challenging. Fish infected with C. irritans acquire immunity; however, C. irritans is difficult to culture in large quantities, obstructing vaccine development using parasite cells. In this study, to develop a mimetic C. irritans, we established a method for expressing an arbitrary protein on the surface of Tetrahymena thermophila, a culturable ciliate.

Methodology: The signal peptide and GPI anchor sequences of the immobilization antigen (i-antigen) of C. irritans were fused with a green fluorescent protein gene (GFP) by PCR. The resulting PCR product was inserted into a Tetrahymena rDNA vector (pD5H8) to construct an expression vector. The expression vector was introduced into Tetrahymena cells by electroporation to obtain transgenic cells, and the localization of GFP was examined by live cell observation, immunofluorescence antibody test, and western blotting.

Results: GFP signals were detected on the membrane of the transgenic Tetrahymena cells. The protein was detected in the membrane and ciliary fractions in western blotting. In addition, immobilized and/or aggregated cells were observed when transgenic Tetrahymena cells were incubated with an anti-GFP antibody.

Conclusions: Fusing the signal peptide and GPI anchor sequences of the i-antigen of C. irritans allows the protein expression on the surface and cilia of transgenic Tetrahymena cells. Although GFP was used as a model in this study, this method can be applied to express various proteins on the Tetrahymena cell surface. This technique may help develop transgenic Tetrahymena that display parasite antigens on their cell surface, potentially contributing to the development of vaccines using mimetic parasites.

Evidence of a negative effect of Tetracapsuloides bryosalmonae infection on brown trout (Salmo trutta) populations in Tyrol, Austria

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Introduction: The decline of brown trout (Salmo trutta) populations in many parts of Europe is a result of various abiotic and biotic factors. Among relevant diseases contributing to this development, the proliferative kidney disease (PKD) of salmonid fish, caused by the myxozoan parasite Tetracapsuliodes bryosamonae, is considered to play a crucial role. Due to the complex interactions between fish, parasite and environment, the impact of prevalence and infection on brown trout populations is difficult to assess.
The observation of declining brown trout populations in several rivers of the state of Tyrol prompted an investigation of the prevalence of T. bryosalmonae in brown- and rainbow trout populations of several rivers, together with additional data. The aim of this study was to demonstrate the distribution of the parasite and a possible negative effect of the infection on the brown trout populations.

Methodology: During late summer of 2021, 213 brown- and 124 rainbow trout were caught by electro-fishing in 12 sections of the river Inn, as well as in the catchments of the rivers Inn, Lech, Große Isel, and Drau. Patho-anatomical findings, total length, weight, and condition factor of the fish were recorded and tissue from the trunk kidney of each fish was used for molecular detection of T. bryosalmonae. Additionally, water temperatures were collected for interpreting the results.

Results and Conclusions: Samples of fish from 7/12 rivers tested positive for T. bryosalmonae by PCR. Body length distribution of brown trout from five rivers indicated a self-sustaining population; in four of these rivers, T. bryosalmonae was not detected, and in the fifth, only one fish was tested positive. Otherwise, in five of the positive rivers, only few brown trout were caught. Enlarged kidneys were only observed in trouts from one river, which also showed the highest prevalence of the parasite and provided only two brown trout for examination. All investigated rivers reach temperatures of at least 15°C during summer. Although these are preliminary results, due to river management and sample sizes, our results indicate an adverse effect of T. Bryosalmonae infection on the brown trout population.

Combining morphological and molecular characteristics for the identification of muscle metacercariae in tench (tinca tinca)

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Introduction: Given the zoonotic potential of metacercariae, the aim of this study was to determine the diversity of muscle metacercariae in tench from fish farms and lakes in Germany. Since metacercariae are often difficult or impossible to identify based solely on morphological characteristics, and molecular markers could be associated with potentially incorrect morphological identification, we combined morphological and molecular methods to determine muscle metacercariae.

Methodology: Between March and July 2022, a total of 88 tench from two lakes in the Berlin-Brandenburg region and three pond farms in Brandenburg, Saxony and Bavaria were examined. Fillets of the fish were inspected by the compression method. Size and shape of the metacercarial cysts were recorded, the metacercariae were freed from their cysts with dissecting needles and measured in native condition. For molecular biological analysis, the metacercariae were fixed in ethanol. DNA extraction was followed by PCR to amplify the mitochondrial cytochrome c oxidase subunit I (COI) of opisthorchid metacercariae and the COI region and internal transcribed spacer (ITS) of diplostomoid trematodes. The PCR products were purified, sequenced, and sequences obtained were aligned with sequences from the GenBank database.

Results: The prevalence of muscle metacercariae in lakes was 92%, while in fish farms it ranged from 9% to 100%. Three trematode species were idendified based on morphological and molecular characteristics: Pseudamphistomum truncatum (Opisthorchiidae), Hysteromorpha triloba (Diplostomidae), and Paracoenogonimus ovatus (Cyathocotylidae). With the exception of the fish farm in Bavaria, where only H. triloba was present, all three species were detected at all study sites. Metacercariae of more than one trematode species were frequently found in the muscles of tench.

Conclusion: In addition to piscivorous mammals, P. truncatum infects humans as a final host, and P. ovatus is suspected to have zoonotic potential. The high prevalence of these parasites in tench combined with altered dietary habits (consumption of raw or inadequately heated tench fillets) poses a risk for fish-borne trematode infections in humans. The combination of morphological and molecular identification should become the standard for hard-to-identify groups.

Size-dependent resistance to amoebic gill disease in naïve Atlantic salmon (Salmo salar)

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Amoebic gill disease, caused by the protozoan ectoparasite Neoparamoeba perurans, remains a significant threat to commercial Atlantic salmon aquaculture operations worldwide, despite partial control afforded by selective breeding and therapeutic intervention. Anecdotal reports from commercial producers suggest that historically, smaller Atlantic salmon smolts are more susceptible to AGD than larger smolts. Here, large (>350 g) and small (<200 g) commercially sourced, AGD-naïve Atlantic salmon cohorts were experimentally exposed to 50 N. perurans L-1 without intervention. Progression and severity of AGD in challenged cohorts was evaluated through gill pathology, using gill score and histological examination, and quantification of gill-associated amoebae burden using qPCR. To determine the potential basis for differences in AGD susceptibility between cohorts, transcriptome analysis was conducted using RNA extracted from whole gill arches. Overall, the large Atlantic salmon cohort had significantly lower gill parasite burdens and reduced AGD-related gross pathology compared to the small cohort. Relative gill burden of N. perurans appeared to be proportional to gill score in both size classes, with larger smolts typically observed to have comparatively reduced parasite burdens at a given gill score. Moreover, comparison between gene expression profiles of large and small smolts highlighted upregulation of genes consistent with elevated immune activity in large smolts. Combined, the results presented here provide strong evidence of size-dependent resistance to AGD in AGD-naïve Atlantic salmon.

Amoeba species colonizing the gills of rainbow trout (Oncorhynchus mykiss) in Swiss aquaculture

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Nodular gill disease (NGD) is an infectious condition characterized by proliferative gill lesions leading to respiratory problems, oxygen deficiency, and mortality in fish. Globally, NGD primarily impacts freshwater salmonids in intensive aquaculture systems. In recent years, numerous outbreaks of severe gill disease have affected more than half of the larger rainbow trout (Oncorhynchus mykiss) farms in Switzerland, mainly during spring and early summer. Mortality has reached up to 50% in cases where no treatment was administered. Freshwater amoebae are the presumed etiologic agent of NGD. The gross gill score (GS) categories severity of gill pathology and is a valuable first-line diagnostic tool aiding fish farmers in identifying and quantifying amoebic gill disease (AGD) in farmed marine salmonids. In this study, the GS was adapted to the NGD outbreak in farmed trout in Switzerland. In addition to scoring disease severity, gill swabs from NGD-affected rainbow trout were sampled and amoebae were cultured from these swabs. Morphologic and molecular methods identified six amoeba strains: Cochliopodium sp., Naegleria sp., Vannella sp., Ripella sp., Saccamoeba sp., and Mycamoeba sp. However, the importance of the different amoeba species for the onset and progression of NGD still have to be evaluated. This paper presents the first description of NGD with associated amoebae infection in farmed rainbow trout in Switzerland.

Dietary supplementation in fish affected by Complex Gill Disease: in vivo and in vitro studies

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Introduction: Complex Gill Disease (CGD) is a pathological condition with multiple aetiologies that affects the gills of farmed Atlantic salmon (Salmo salar), being responsible for high mortality rates, growth arrest, increased respiratory rate, and decreased food conversion efficiency. As no vaccines nor specific treatments are licensed to treat CGD, the optimization of diet formulation is a good strategy for improving fish health during natural outbreaks.

Methodology: The effects of a commercial feed (Protec Gill, Skretting) were assessed in vivo (macroscopic and histopathological gill assessment, pathogen detection, and analysis of plasma parameters), and in vitro (amoebicidal activity of Protec Gill and three of its functional ingredients, cell line assays using RTgill-W1 to evaluate the effects of several functional ingredients on viability and reactive oxygen species (ROS) production).

Results: For the in vivo study, a three-week period of feeding with Protec Gill slowed CGD progression by reducing pathogen load, and significantly improving gill tissue condition, as revealed by histological evaluation. In vitro testing on

amoebae viability demonstrated a significant amoebicidal activity of the tested ingredients (0.01% for phytogenic 1, and 0.1% for arginine and phytogenic 2) and Protec Gill (as low as 0.01%). According to cell line assays using RTgill-W1 cells, arginine and β-glucan promoted ROS production, and were cytotoxic at 500 and 1000 µg/mL, respectively. Vitamin C showed an increase in cell viability at 0.1 µM/mL and antioxidant properties within the range 0.1 – 5000 µM/mL. Two phytogenic compounds were also tested, and even they showed a significant amoebicidal activity at low concentrations, although they reduce RTgill-W1 viability.

Conclusion: The combination of in vivo and in vivo approaches resulted in a successful strategy to assess the beneficial effects of Protec Gill in fish affected by CGD. The feed formulation was effective in slowing disease progression and improving the health of fish.

Three functional ingredients and Protec Gill had a powerful amoebicidal activity at low dosage. The fish cell line RTgill-W1 is regarded as useful screening tools for screening functional feed ingredients and for the selection of the best candidates to include in fish diet.

Genomic and ultrastructural insights into virulence attributes of Ca. Ichthyocystis seriolae sp. nov., a new epitheliocystis-causing agent in greater amberjack

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Introduction: Betaproteobacteria are an emerging group of epitheliocystis agents responsible for mortality in fishes. In this phylum, the novel genus Candidatus Ichthyocystis currently includes the only two species of betaproteobacteria with sequenced genomes. These genomes, obtained from gilthead seabream, Sparus aurata, have revealed the presence of known bacterial virulence factors such as type II, III, IV secretion system, type four pilus, and have moreover uncovered a large array of bacterial effectors of unknown function.

In a recent study, we characterized the presence of a novel betaproteobacterial agent belonging to the same genus in the gills of greater amberjack, Seriola durmerili, in Greece. Histology and PCR were used to track the disease and characterize the proliferative focal lesions displayed during the outbreaks. The present study comes as a continuation of the monitoring study with the aim of describing the lesions by ultrastructure analysis and exploring and discussing novel genomic findings following whole genome sequencing (WGS).

Methods: Gill samples were obtained from a one-year monitoring study of greater amberjack cultured in Greece. Samples obtained from different time points of infection were selected and processed for transmission electron microscopy. WGS was performed directly on micro-dissected cysts. Comparative genomic analysis was done using available genomes of the different epitheliocystis agents. Functional analysis of proteins of interest was performed using INTERPROSCAN and their structure modelled using SWISS-MODEL.

Results: Ultrastructural observations indicated genetic damage in both infected cells and surrounding proliferative tissue cells. We moreover observed congregation of filament bundles outside the bacterial vacuoles and presence of a fibroblast ring in the area surrounding the infected cells. Using comparative genomics, we identified an area unique in the novel genome where a protein containing a eukaryotic-like domain was present, whose putative function is discussed. Moreover, we uncovered a family of large multidomain toxins with potential major role in the infective process.

Conclusions: Comparative genomic analysis coupled with ultrastructure observations offer novel insights into unique virulence traits of betaproteobacterial epitheliocystis agents.

Effect of light exposure on the circadian pattern in theront excystment of Cryptocaryon irritans

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Introduction: Cryptocaryon irritans, a parasitic ciliate causing cryptocaryoniasis in marine teleosts, encysts during its proliferative stage, making it difficult to eliminate from aquaculture facilities. The timing of excysting of theronts, the parasitic stage, can be controlled by adjusting the photoperiod given to the cyst or trophonts, the stage feeding within the epithelial tissues of host fish. Understanding this mechanism would enable the prediction of disease outbreak, and possibly help develop new control methods based on parasite's biological features. In this study, we developed an apparatus to provide specific photoperiods to cysts and collect excysted theronts automatically to examine the effect of light on the circadian pattern.

Materials and methods: By subjecting the parasite to direct light or light-exposed seawater, we investigated its light responsiveness. Cysts were exposed to 12 hours of light once on the first, second, or third day of incubation to study changes in photosensitivity during cyst development. Since cysts appears at seabed where the light is low, the effect of low light exposure was examined. We also attempted to search for photoreceptors using the transcriptomic data previously obtained in our laboratory and genome data newly obtained in this experiment.

Results: Direct light exposure developed a new circadian pattern, achievable with a single 12-hour exposure with some variations at times. Remarkably, even a low light intensity of 1 lx triggered a new circadian pattern of excystment. Also, from the transcriptomic data, we found a possible photoreceptor, and its structure was predicted in silico.

Conclusion: We found that the parasites receive light and establish the circadian pattern during the entire cystic stage, with some variations likely due to cyst development differences. Additionally, the result of low light exposure experiment suggests the entrainment of the circadian pattern in the seabed near aquaculture facilities. Further studies are required to investigate the mechanism underlying the development of the circadian pattern after photoreception occurs.

Understanding natural resistance to white spot disease

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White spot disease (WSD) caused by the parasite Ichthyophthirius multifiliis (I. multifiliis) is associated with high mortality and morbidity and subsequent economic losses for both the aquaculture and ornamental industry. Severe outbreaks of WSD have been reported in a wide range of freshwater fish species. Zebrafish (Danio rerio), on the other hand, have shown high natural resistance towards the parasite. The immunological mechanisms causing the resistance in zebrafish are however still unknown. Identification of genes responsible for resistance could be highly beneficial in understanding protective immunity as well as in mitigating the disease. Here we performed comparative transcriptomic analyses on naturally resistant zebrafish and susceptible rainbow trout (Oncorhynchus mykiss). Both species were exposed to an equal number of I. multifiliis per unit surface area, and gills were sampled at 2, 24, 48 and 72 hours post infection (hpi). Differential gene expression analyses showed that zebrafish elicits an immediate response with a peak at 24 hpi with 50 significantly upregulated and 1182 downregulated genes, which then had normalized almost completely at 72 hpi. The transcriptomic data also showed continued decline in transcripts mapping to the I. multifiliis genome, which could indicate a clearing of the infection. Differential gene expression in rainbow trout showed a similar pattern as in zebrafish, however, on a much smaller scale. The transcriptomic data, however, showed increased transcription mapping to the I. multifiliis genome for in rainbow trout, indicating a continued growth and establishment of the parasite. Increase in transcription in zebrafish genes associated with, amongst others, Toll-like receptor signaling pathway and MAPK signaling pathway were found. The implications of these findings are discussed, candidates for resistance have been identified, and the functionality of these genes will be tested by knockout studies using the CRISPR/Cas technology. Knockout fish will be exposed to I. multifiliis to evaluate susceptibility in future studies.

Phylogenetic diversity of water moulds, Saprolegnia spp. (Oomycota) in fish farms in Hungary

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Introduction: Saprolegnia spp. (Oomycota: Saprolegniales) are endemic in most freshwater habitats worldwide. Water moulds cause remarkable losses to fish farms every year, by damaging fish eggs, fry or even adult fish. In a comprehensive research project, we aim to obtain a global picture of the genetic diversity of Saprolegnia spp., besides the nature and environmental preference of the pathogen species in fish hatcheries.

Methodology: From more than 200 samples, 160 Saprolegnia isolates were collected in twelve fish farms across Hungary. Molecular characterization and species identification was based on the internal transcribed spacer regions (ITS 1 & 2). Phylogenetic trees were reconstructed using maximum likelihood and Bayesian inference methods with the software RAxML and MrBayes, respectively.

Results: Saprolegnia parasitica, S. ferax and S. australis were the most common species isolated. In the water of the brood house outflows, S. torulosa, S. aenigmatica, S. delica and S. eccentrica were detected occasionally, whereas S. anisospora was isolated from a few affected fish. S. parasitica was detected rather from fish or egg samples, than from surface or water samples. This trend was reversed for S. ferax and S. australis species, which were more abundant in water samples. The species composition appeared to vary between sampling sites, although this might be due to the uneven number of samples. The high degree of DNA sequence identity (between species 88.3-98.9%; within species 97.8-100%) confirmed that the ITS region allows species-level identification only.

Conclusions: Our findings suggest that differences in habitat and hatchery characteristics may influence the species composition of water moulds, and the exposure of fish farms to saprolegniosis.

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First detection of Ichthyophonus sp. In invasive pink salmon (Oncorhynchus gorbuscha) in Norway

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Introduction: Pink salmon (Oncorhynchus gorbuscha) are native to the Pacific Ocean but were deliberately introduced and released to rivers surrounding the White Sea for sea ranching purposes during the periods 1956-1979, and 1985-2000. Secondary spread has resulted in pink salmon runs in rivers in Norway and several other countries surrounding the North Atlantic Ocean. Several risk assessments and authors have identified introduction and spread of pathogens as potential risks associated with pink salmon invasions. Here we report the detection of Ichthyophonus sp. in invasive pink salmon captured in a Norwegian river.

Methodology: The study sample comprise a pink salmon captured in River Lakselv in Northern Norway in August 2021. The fish was one of two pink salmon that due to abnormal behavior attracted attention from the local river manager. It was apathetic, found close to the riverbanks, had pale gills and appeared to be of normal to good nutritional status. The pink salmon was killed and stored frozen at -20℃ until they were shipped to the Norwegian Veterinary Institute in 2022, where we performed a post-mortem examination with subsequent histopathological, microbial and molecular analysis.

Results: The infected pink salmon was an adult female with mature eggs in the abdominal cavity, ready to spawn. Apart from ongoing cadaverosis, no other external signs were observed. However, during histological examination of organ samples several fungal-like structures were noted, especially in the heart and skeletal musculature. The structures were round, basophilic, double-walled and of varying sizes, either singular or several arranged into clusters. Some also displayed signs of budding, a form of asexual reproduction, and several were PAS positive. Local tissue responses were none to very mild. These findings were indicative of systemic ichthyophonosis, a well-known fungus-like parasitic disease described from many different species of fish. This was later confirmed through identification of Ichthyophonus sp. with PCR and ribosomal 18S sequencing. No other microbial agents were found.

Conclusion: To our knowledge, this is the first report of Ichthyophonus sp. from pink salmon in Norway. We recommend that surveillance and disease screening programs include Ichthyophonus as a possible disease agent for the fish species.

Metagenomic analysis and traditional laboratory methods to investigate systemic granulomatosis in cultured meagre argyrosomus regius

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Introduction: Systemic granulomatosis (SG) is a disease of unknown etiology characterized by multiple granulomas in fish organs; it typically affects meagre (Argyrosomus regius), a promising fish species for Mediterranean aquaculture. The disease is not directly linked to mass mortalities, however, contributes to reduced rearing performance of the fish. So far, infectious (such as Mycobacteria and Nocardia sp.) and non-infectious (nutritional) aetiologies have been proposed for SG

Methodology: Fifty-four (24 juveniles and 30 adults) reared fish and samples of feed were submitted to the Department of Veterinary Medicine of Sassari. Healthy (with no granulomas) fish were included as negative controls. Tissue samples were processed for histology, microbiology, PCR targeting the hsp65 gene, and Sanger sequencing. Metagenomic analysis, targeting the 16S rRNA gene, was also performed.

Results: Multifocal granulomas Ziehl-Neelsen negative were detected in the organs of 30/30 adults and 4/24 juveniles. In the adults, the kidney was the most affected organ (29/30) followed by the heart (13/30), liver (11/30), intestine (5/30), and brain (1/30); 2/24 juveniles presented granulomas in the brain and one each in the heart and in the liver. Microbiology detected the presence of Ziehl-Neelsen-positive colonies from 20/30 adults, phenotypically and biochemically identified as Mycobacterium spp. The microbiological examination of all samples did not show the presence of other bacteria. The presence of mycobacteria was not found in the feed. The results of hsp65 PCR showed the presence of 441 bp amplicons in 20/30 adults and 4/24 juvenile samples and the sequences showed the highest similarity with mycobacteria. The metagenomic analysis, along with unclassified species, showed that the phyla with the most reads were Bacteroidetes in the spleen and Proteobacteria in other tested organs, followed by Firmicutes. Moreover, the phylum Actinobacteria, which includes Mycobacterium spp., was detected in low prevalence in the kidney, the most affected granuloma-bearing organ in adults.

Conclusions: Our preliminary results concerning microbiome investigation identified several microorganisms in tissues of meagre affected by SG. However, sensitive traditional methodologies such as microbiology and PCR and sequences identified mycobacteria as microorganisms associated with SG. Further studies are needed to reinforce the hypothesis of the infectious cause of SG

Identification of a novel toti-like virus and its potential association with Red Skin Disease in Atlantic salmon (Salmo salar)

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A new skin condition characterised by haemorrhaging on the ventral surface of returning wild Atlantic salmon (Salmo salar) was identified in Sweden around 2015 and termed Red Skin Disease[1]. In the last 6 years it has apparently spread, and is now observed in Sweden, Denmark, Norway, Scotland, Ireland, and England[2]. To date traditional diagnostic methods have failed to indicate an aetiological agent. We undertook a meta-transcriptomics approach on skin biopsies from Atlantic salmon and sea trout from Scotland and England (sampled 2020, 2021 and 2022) displaying clinical signs. The approach aimed to identify potential pathogens and characterise host response providing indicators to the type of insult experienced.

Total RNA was extracted from 28 skin and muscle biopsies from affected and apparently unaffected skin regions. mRNA sequencing libraries were prepared using the Illumina Stranded mRNA Prep Kit and sequenced on an Illumina NextSeq550. Multiple bioinformatic pipeline tools were used to remove adapters, low-quality bases, host and rRNA sequences. Remaining sequences were assembled using Megahit v1.2.9, keeping only contigs that were ≥200bp.

Contigs were screened for plasmid and/or viral sequences using Genomad v1.5.0. and candidates subjected to similarity searches using blastn, blastp and NCBI databases.

No obvious significant candidate bacterial or eukaryotic pathogens were identified. The proposed near full genome of a virus not previously described was obtained from six of 28 samples across four of the ten fish analysed. Gene prediction analysis of a 8,128 bp contig identified two putative protein coding regions of 5046 bp (1681 aa) and 2307 bp (768 aa) in length. Sequence similarity searches indicated 68% and 56% similarity respectively to the RNA polymerase and polyprotein of Golden shiner totivirus 2 (7788 bp). The similar length and structure further support that this novel virus sequence is related to toti-like viruses. Two of three Scottish salmon and two of five English salmon contained detectible sequences (ranging from 174 to 40,779 mapped sequences). Virus sequences were found in apparently normal, mild and moderately affected tissues, but not in severely affected tissues nor in the sea trout.

This finding warrants further investigation which is ongoing.

[1]Weichert, et al. 2021(https://doi.org/10.1111/jfd.13288);
[2]https://ifm.org.uk/wp-content/uploads/2022/08/RSD_severity_guide_fin.pdf

Experimental transmission and pathogenicity of ulcerative dermal necrosis (UDN) in sea trout from Baltic Sea

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Introduction: Ulcerative dermal necrosis (UDN) is a contagious fish disease which has been observed in European aquatic environment from late 19th century. Since then, outbreaks affecting spawning salmonids were reported in many countries, also in Baltic region. UDN is an infectious skin disease of unknown etiology affecting adult, wild, anadromous salmonids migrating from open seas to fresh water during the spawning season.

Methodology: The disease's transmission model in our study was based on the cohabitation experiment of healthy sea trout obtained from a fish farm and spawners returning in November 2021 from the Baltic Sea to the Słupia River. During the course of the experiment, progressive changes of the skin were observed in farm sea trout. Light and electron microscopy examination of skin samples of affected trout confirmed the progressive degeneration of the skin surface. Healthy farm fish were also placed in a separate tank as a control group.

Results and conclusions: The skin cells showed degenerative features, lost their tight connections and became necrotic. Unlike normal skin, infected cells were not replaced by new cells from the layer below. The intercellular spaces widened which led to shedding of the cells. The round, pale circles were created by necrosis of pigment cells. The association with any virus resembling structure has not yet been confirmed, and in the later stages of the disease, secondary fungal and bacterial infections have become increasingly evident. None of the control fish exhibited lesions of UDN.

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Poster Presentations

Abstracts listed alphabetical by surname of first presenting author

Surname: A

Comparing viral replication kinetics and pathogenicity of IHNV and VHSV in single and in co-infection in rainbow trout (O. mykiss)

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Introduction: In the last years, Italian rainbow trout farming industry has experienced frequent co-infections of both IHNV (Infectious Hematopoietic Necrosis virus) and VHSV (Viral Haemorrhagic Septicemia virus) within the same farm or in individual fish, resulting in severe disease outbreaks and significant production losses. In the present study, we performed VHSV and IHNV experimental infections in rainbow trout (O. mykiss) in order to evaluate mortality kinetics and intra-host viral replication during single and co-infections.

Methodology: Two Italian VHSV and IHNV strains were used to perform in vivo experimental infections using 15 grams rainbow trout juveniles. Fish were divided into four experimental groups i) mock-infection; ii) VHSV and IHNV co-infection; iii) VHSV infection and iv) IHNV infection. Each group was housed in a 80 L tank, challenged with 105 TCID50/ml of virus and monitored for 30 days. Daily mortality was recorded and survival probability was estimated by Kaplan-Meier curves, while intra-host viral replication was assessed in target organs (spleen and head kidney) of all the dead animals using a quantitative real-time PCR (qRT-PCR).

Results: Cumulative mortality estimates confirmed the different pathogenicity induced by single IHNV and VHSV infections that caused 68.42% and 97.50% mortality, respectively. A 100% mortality was observed in co-infected fish. qRT-PCR analyses allowed to confirm the presence of the virus and quantify the viral load in all specimens deriving from single and co-infection. Interestingly, all the co-infected dead fish tested positive for both viruses. Moreover, intra-host viral quantification results evidenced greater viral loads in spleen rather than in head kidney and in all the infected dead fish. While comparing viral replication of the viruses in all experiments, a higher replication trend of VHSV in both single and co-infected fish compared to IHNV was observed.

Conclusions: Although it was not possible to determine the contribution of each virus to the mortality, fish infected simultaneously with VHSV and IHNV showed positivity to both viruses, with viral loads similar to those observed in single infections.
Further analyses are required to better investigate the interaction of VHSV and IHNV during co-infection.

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Phylogenetic reconstruction, histopathological characterization, and virulence determination of a novel fish pathogen, Nocardia brasiliensis

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Nocardiosis caused by diverse, cosmopolitan aerobic actinomycetes species of the genus Nocardia is a common disease reported on several aquatic species. Recently, variated internal granulomas resembling typical nocardiosis were unexpectedly observed in cultured European meagre (Argyrosomus regius) at Las Palmas de Gran Canaria, Spain. Hence, we aimed to identify the causative agent and experimentally reproduce the disease using specific pathogen-free (SPF) meagre specimens. Following classical methods, the bacterial isolates from liver granulomas of several specimens were obtained and identified by combining MALDI-TOF mass spectrometry (MS), genus-specific nested PCR, and 16S ribosomal RNA gene sequencing. Comparative in silico alignments with known 16S-rRNA sequences and their unique mass spectrum revealed for the first time in fish the target identity as Nocardia brasiliensis. The virulence of this species was experimentally characterized in vivo. Juvenile meagre intraperitoneally infected with N. brasilensis develop internal micro granulomas without gross external signs or significant mortalities. Surprisingly, fish exposed to concentrations lower than 106 CFU ml-1 did not die within the two months trial. Five types of microscopic granulomas with classic necrotic centers and macrophage arranges were present in a dose-dependency fashion. Besides, immunofluorescence revealed the presence of live bacteria within some granulomas. Together, these results show that the bacterial species N. brasilensis successfully colonizes internal piscine organs and is sufficient to develop chronic granulomatosis in fish reared in marine waters.

Study of prophages in bacterial populations isolated from aquaculture facilities

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Bacteriophages or "phages" with therapeutic potential are a promising alternative to antibiotics on the management of bacterial infections. Bacteriophages have long been used against bacterial pathogens of fish and shellfish, however, the extent of phage diversity in aquaculture systems and its prevalence in the fish or shellfish tissues remains largely unexplored. Lytic phages infect a bacterial host, replicate themselves and kill their host to release their progeny. Alternatively, they can reproduce as a lysogen integrated into the bacterial genome, monitoring bacterial health and reverting to lytic growth to exit the host. Identifying prophages remains challenging despite their importance to microbial survival, diversity and evolution. In the present work we have initiated a prospective study for the detection and characterization of prophages related to the species of native bacteria that live or are present in facilities related to aquaculture. Our main objective was to verify the presence of prophages in the isolated strains by means of induction tests with Mitomycin C. One hundred and eighty strains of bacteria isolated in aquaculture facilities (mainly in areas where sea bream and sea bass are farmed) were cultured for testing. A total of 80 (44%) presented a sharp deviation of exponential growth during cultures in liquid medium when induced with Mitomycin C. Four strains that showed positive results for induction with mitomycin C were sequenced and 6 complete phages characterized using RAST and PHASTER computer packages, and by transmission electron microscopy, showing that they belong primarily to families Myoviridae y Siphoviridae. Although lytic phages are being widely studied because they have significant potential to combat infections by fish pathogens, we must also pay attention to lysogenic phages living in bacterial populations present in aquaculture facilities. This will help us understand the complexity of interactions between lytic and lysogenic phages and their bacterial hosts.

Bacillus velezensis D-18 inhibits quorum sensing and biofilm formation of Vibrio anguillarum

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Nowadays, Vibrio spp. is one of the main threats in aquaculture production. Vibrio spp. ability to colonize the medium and disease farmed fish is well known. The use of probiotics is currently presented as a solution to the application of antibiotics, which generate multi-resistant bacteria. Bacillus spp. supplementation as probiotics in cultured fish has a long history of safe and effective use. Previously, B. velezensis D-18 showed great promise in fine-tuning the European sea bass disease resistance against the pathogenicity caused by several members of the Vibrio family. This resistance to diseases was contrasted by determining the innate immunological parameters after probiotic administration. However, there are more mechanism of action used by probiotic that inhibit pathogen, such as quorum sensing inhibition, also called quorum quenching (QQ), which potential in Bacillus velezensis D-18 is questioned. Quorum quenching assay through a life biomarker, the capacity to degrade long and short acyl homoserine lactone (AHL) as the main autoinducer peptides in gram-negative bacteria, the genotypic corroboration by PCR and its effect on Vibrio anguillarum 507 pathogen were tested. Overall, our results provide clear vertical evidence that Bacillus velezensis D-18 produce acyl homoserine lactonases and have the capacity to inhibit growth and avoid the biofilm formation of Vibrio anguillarum 507.

Isolation and characterization of a new Paenibacillus strain with inhibitory effect against fish pathogens

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Probiotics use in aquaculture has gained attention as microbial candidates to maintain the health and the wellbeing of many aquaculture animals. A Gram positive, motile, spore former bacillus strain, called CA237, was isolated from aquaculture facilities. This strain showed antibacterial activity by direct contact or through its extracellular products on diffusion agar plates against several Gram positive and Gram negative fish pathogens including Aeromonas hydrophila, Yersinia ruckeri, Streptococcus iniae, and Photobacterium damselae subsp. piscicida. The whole genome of strain CA237 was sequenced using the Illumina technology. After assembly and optimization, the resulting genome sequence had a size of 5.819.503 bp and an overall GC content of 45,5%. The comparison of the sequence of 16 sRNA showed a similarity of 76% with strains of the species Paenibacillus polymyxa, but the comparison of the whole genome showed that it could even be a new species located between P. polymyxa and P. peoriae. The bacterium does not contain plasmids or phages, two desirable characteristics for use as a probiotic. The whole genome analysis showed that strain CA237 possesses several gene clusters, including lanthipeptides and lipopeptides and other secondary metabolites

which can be explored as antimicrobial peptides against fish pathogens. These results indicated that strain CA237 has the potential to be developed as a probiotic candidate for the control of bacterial diseases in aquaculture.

Glass half full or half empty? - Salt treatment delivers both: persistence of carp edema virus and protection against reinfection

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Introduction: Carp edema virus (CEV) is a highly virulent fish poxvirus that frequently causes a very severe koi sleeping disease (KSD), leading to high mortality in carp populations. Its worldwide spread is due to the international trade in koi and carp. In Germany, the virus has repeatedly been found in consignments of completely asymptomatic fish. In addition, some fish farmers and ornamental fish keepers have experienced repeated outbreaks of the disease without introducing new fish. This raised several questions about the ability of the virus to persist in populations following salt treatment and the effectiveness of natural immunity following this treatment.

Methods: To answer these questions, a combination of case studies documenting the outcome of salt treatments in koi and farmed carp and experimental infections were used. Virus load was measured by qPCR and the development of clinical signs was measured by observing behaviour, gill pathology and blood Na+ and ammonia concentrations. Immune responses were evaluated with qPCR and immunocytochemistry.

Results: Salt treatment was found to be highly effective in preventing most of the morbidity and mortality associated with KSD. The main mechanism of action of salt treatment was confirmed to be the prevention of osmotic disorders by stabilising blood sodium concentration and the prevention of ammonia intoxication by facilitating gill excretion of ammonia. Results from the monitoring of the KSD outbreak showed that over 25% of the fish sampled remained CEV positive for five months without clinical signs. Further studies were carried out on carp rescued from a fish farm in Saxony, Germany. Experimental reinfections of these fish three months after recovery showed that they were protected against reinfection. Compared to naive fish infected with CEV, the naturally immunised fish did not develop the clinical signs of KSD, including pathophysiology and immunosuppression. Conversely, reinfection resulted in increased expression of gzma, zap70, cd4 and igm, which are normally downregulated during KDS.

Conclusion: The long persistence of CEV may explain its successful global spread. On the other hand, the protection against reinfection conferred by increased T-cell and B-cell responses gives hope for the development of effective vaccines against CEV.

New insights into the immunosuppression observed during carp edema virus-induced gill disease provided by proteomics and flow cytometry.

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Introduction: Gill disorders cause substantial losses in the aquaculture industry. Koi sleepy disease (KSD), caused by the gill infecting poxvirus carp edema virus (CEV), is a unique model to study an impact of branchial diseases on the immune response in fish.

Methods: In the present study, we performed 2D-DIGE based proteomics of gills to evaluate biological pathways affected by the disease. Furthermore, using flow cytometry we measured the level of IgM+, CD4+, and CD8+ lymphocytes in the blood, gills, head kidney and spleen. Both results were supplemented by the analysis of the gene expression measured by RT-qPCR.

Results: CEV-infected fish developed classical clinical signs of infection related to gill dysfunction, manifested as ion dysregulation: decrease of sodium level under 100 mmol L-1 and an accumulation of ammonia to over 600 µmol L-1, which can induce immunotoxic effect. The proteomic analysis indicated that the abundance of 86 proteins was significantly changed in gills during the infection. The IPA-analysis indicated an activation of regulatory networks

responsible for the response to cellular compromise, infectious diseases, inflammatory response and connective tissues disorders. Furthermore, CEV-infection affected: regulatory networks for drug metabolism and glutathione depletion as well as activation of the xenobiotic metabolism CAR signaling pathway. The changes in these networks indicate a response to ammonia intoxication and point to the effort of gill cells to handle and eliminate this immunotoxic metabolic by-product. Increased concentrations of several heat shock proteins and activation of the upstream heat shock factor protein 2 suggest increased stress response in infected gills. Moreover, observed in infected animals, down-regulation of the antimicrobial peptide NK-lysin-like suggests a lower activity of NK cells. This corroborates with flow cytometer analyzes in which we observed a drastic drop in IgM+, CD4+, and CD8+ lymphocyte counts in the blood and the spleen. All these changes are accompanied by the increased expression of viral genes responsible for immunosupression like multiple paralogues of B22R and the viral HSP70.

Conclusions: Taken together, the results allowed to show connection beween CEV-induced pathology and immunosuppression leading to a severe gill disease.

Use of environmental DNA-based diagnostics to detect multi-pathogen gill disease associated with carp edema virus infection in common carp

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Introduction: Recently, environmental DNA (eDNA) analysis has been recognised as a tool for monitoring the presence of pathogens in aquaculture. eDNA-based diagnostic appears to be particularly suitable for the detection of mucosal pathogens in fish. Gill and skin diseases are often multi-pathogenic including co-infections with viruses, bacteria and parasites. For example, carp edema virus, an immunosuppressive poxvirus that infects the gills and causes koi sleepy disease (KSD), often occurs with co-infections with ectoparasites such as Ichthyobodo necator and the bacterium Flavobacterium branchiophilum, making diagnosis and treatment difficult. Furthermore, the disease occurs in common carp and koi that are less accessible and cannot always be sacrificed for sampling (e.g. high-value ornamental koi or common carp broodstock). We therefore tested the applicability and robustness of eDNA-based methods for the detection of pathogens associated with KDS.

Methods: To test eDNA-based methods for rapid detection of KDS, water samples, gill swabs and gill biopsies were collected during disease outbreaks and experimental infections and stored frozen at -20°C. Several centrifugation speeds and different pore size filters were used to select the best method for concentrating pathogens from water. Detection of carp edema virus, Ichthyobodo necator and Flavobacterium sp. was performed by qPCR after DNA extraction using Qiagen DNA mini kit.

Results: Filtration (0.20 µm and 0.45 µm) appeared to be the most reliable method for concentrating the pathogens associated with KDS outbreaks. The detection of CEV, I. necator and Flavobacterium sp. was possible at very early stages of infection and the CEV concentration increased rapidly on day 4 onwards when the first clinical signs were visible. Furthermore, the DNA of all pathogens could be detected in the water for at least 8 days after removal of infected fish.

Conclusions: Concentration of all pathogens involved in multi-pathogen gill disease associated with carp edema virus infection was possible with a single water filtration procedure using a e.g. 0.20 µm syringe filter. eDNA-based diagnosis could therefore be a very efficient method for detecting outbreaks of KSD, flavobacteriosis and ichthyobodiasis, at least in relatively small water bodies such as small ponds or tanks.

Evidence for biomineralization in myxozoan parasite spores

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Myxozoan parasites have two-host life cycles with two waterborne spore stages. These spores share common functions of being resistant, durable vessels that transport living parasite material from one host to the next. Yet for life cycles in which both spore stages have been observed, the spores - actinospores and myxospores - differ in morphology, buoyancy and degree of rigidity in their outer valve cells, suggesting compositional variation. The few studies on myxozoan spore compositions have revealed evidence that biomineralization may be a feature - as is present in their stony coral relatives.

To determine if myxozoan spores utilize the relatively rare trait of biomineralization, and if alternate spore stages of the same organisms have different spore wall compositions, we are using multiple techniques to investigate spore compositions. Specifically, we are analyzing "oligochaete host lineage" myxozoan species including Henneguya

salminicola, Myxobolus cerebralis and Myxobolus squamalis, and comparing these with the "polychaete host lineage" species Ceratonova shasta.

We are using bioinformatics to search existing 'omics databases that we have generated for these species, to look for known components of the "biomineralization toolkit" and related genes. Scanning (SEM) and Transmission electron microscopy (TEM) energy-dispersed x-ray spectroscopy was used to determine presence and localization of elements, with inductively coupled plasma mass spectroscopy (ICP-MS) of entire spores to determine primary inorganic components.

Our initial data show that the myxozoan genomes do not have homologs to "biomineralization" associated genes, particularly no genes known to be associated with silicon-mineralized species. ICP-MS showed that myxospores of different species have magnesium and calcium present in differing ratios, though magnesium was typically higher than calcium, which is atypical for biomineralization in other organisms. Presence of silicon was evident in SEM and ICP-MS, but not TEM. A primarily protein composition was indicated by TEM, which resolved layers in the spore walls differentially enriched in phosphorus, sulphur or nitrogen - suggesting different protein compositions, but no distinct layers of mineralization.

These ambiguous data are being further investigated to clarify and corroborate the signatures of inorganic elements in the spores.

Characterization of extracellular products for Tenacibaculum dicentrarchi: composition and biological activity

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Introduction: Extracellular products with a polymeric matrix released by marine microorganisms are formed by polysaccharides, proteins, lipids, and some nucleic acids. The function of these products is diverse but commonly associated with a pathogenic role, ranging from the adhesion of the pathogen to surfaces to forming bacterial aggregates, and, even, participating in bacterial quorum sensing. Knowledge available on this topic is null for Tenacibaculum dicentrarchi. Therefore, we focused research on the following strains: TdCh05 from Atlantic salmon; QCR29 from red conger eel; and the type strain CECT 7612T. Included as controls were Vibrio anguillarum ATCC 43307 and Tenacibaculum maritimum CECT 4276.

Methodology: Extracellular products were extracted from the bacteria at 72 h post-growth and were collected through centrifugation. Tests were conducted with the concentrated, crude extract. Composition was determined using the phenol-sulfuric acid method (sugars), BCA (proteins), and spectroscopy (nucleic acids). Protease activity was determined by the degradation of azocasein, gelatin, and collagen. The ability to degrade collagen, glycosaminoglycans, and elastin were discovered using the degradation of the FALPA peptide, chondroitin sulphate/heparan sulphate, and elastin, respectively. Finally, the ability to lyse erythrocytes following the release of hemoglobin was evaluated through spectroscopy.

Results: The extracellular products of T. dicentrarchi were mostly composed of five-carbon sugars (µg of fructose), followed by proteins to a maximum of 2.6 µg µL-1, and, finally, remnants of nucleic acids. All had a proteolytic effect on gelatin. This effect was not inhibited by temperature but was inhibited by exposure to inhibitors. This suggests the existence of proteases with different cofactors, depending on the strain. The degradation of GAG was similar for all T. dicentrarchi, with the most susceptible being heparan sulphate. Regarding collagen, all isolates presented collagenase activity to FALGPA, although greater affinity was found for types I and II. Elastase activity was negative, and T. dicentrarchi provoked the lysis of erythrocytes, with maximum lysis during the first hour of interaction.

Conclusion: The composition of extracellular products has been determined, demonstrating that these actively participate in the degradation of constitutive components in fish and can lyse erythrocytes in fish.

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Exploration of knowledge available for tenacibaculosis and causative agents in Chilean salmon farming

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Introduction: Salmon farming is the second leading export for Chile. Production is increasingly threatened by emergent infectious diseases, including tenacibaculosis, which was recognized by local authorities in July 2018 in List 3 of high-risk diseases. Tenacibaculosis is today the second cause for Atlantic-salmon mortalities in Chile, going from 4.3% in 2018 to 36.4% in 2021. The present study describes currently available knowledge on tenacibaculosis and causative

bacterial species. In-depth information is presented for virulence mechanisms, the development of diagnostic tools, and treatment efficacies.

Methods: Over five years, causative isolates of tenacibaculosis were recovered from farming centers located from the Los Lagos to the Chilean Patagonia Regions. These isolates were subjected to polyphasic studies for identification and microbiological, biological, and genomic tools for species-level characterization. Further susceptibility research to antimicrobials was established and experimental vaccines against Tenacibaculum dicentrarchi were evaluated.

Results: Outbreaks in Atlantic salmon (Salmo salar) are mostly caused by T. dicentrarchi, although infectious outbreaks were found in farmed rainbow trout (Oncorhynchus mykiss) and Coho salmon (Oncorhynchus kisutch). We recommend the use of MLSA for the genotyping and identification of different Tenacibaculum species, which has helped broaden descriptions of tenacibaculosis and in demonstrating the existence of other Tenacibaculum species, including T. maritimum, T. finnmarkense, and T. ovolyticum. A new species that does not only affect Atlantic salmon was also described – T. piscium. Genome analyses of representative T. dicentrarchi isolates confirmed the presence of genes that explain the genetic and antigenic heterogeneity of this bacteria, which complicates fabricating vaccines with an RPS greater than 60%. Dependent on isolate, the discovered diversity includes genes associated with iron-acquisition mechanisms, coding for copper homeostasis, resistance to tetracycline and fluoroquinolones, pathogen genomic islands, and phages. Regarding treatment, a new in vitro protocol for antimicrobial susceptibility demonstrated that florfenicol treatment should be replaced by tiamulin, but the maximum residual limits need establishing.

Conclusion: The present study elucidates the health impacts of tenacibaculosis for Chilean salmon farming, providing knowledge and facilitating information for the development of control and prevention tools, as well as contributing to the establishment of public policies.

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Antimicrobial dose optimization in Aquaculture

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Introduction: Aquaculture is a rapidly growing industry that provides a significant proportion of the world's fish supply. The use of antimicrobials in aquaculture is essential for controlling bacterial infections and maintaining fish health. However, the overuse and misuse of antimicrobials in aquaculture can lead to the emergence and spread of antimicrobial resistance (AMR), posing a significant threat to public health and the sustainability of the industry. Antimicrobial dose optimization is a key strategy for reducing the risk of AMR while maintaining treatment efficacy in aquaculture.

Methodology: This review paper provides a comprehensive overview of the current state of knowledge regarding antimicrobial dose optimization in aquaculture. The paper synthesizes the existing literature on the topic and discusses the various factors that affect antimicrobial dose optimization in aquaculture, including the species, size, and life stage of the animals, as well as environmental factors such as water quality and temperature. The review highlights the importance of considering both the benefits and risks of antimicrobial use in aquaculture and provides guidance on how to balance treatment efficacy and AMR risk through dose optimization.

The paper presents the different methods for determining optimal antimicrobial doses, including in vitro assays and in vivo experiments, as well as pharmacokinetic and pharmacodynamic modeling. The review also discusses the challenges and limitations of antimicrobial dose optimization in aquaculture, such as the lack of standardized protocols and the need for further research to address data gaps.

Conclusions: Overall, this review paper provides a valuable resource for researchers, policymakers, and practitioners involved in the development of sustainable and effective disease management strategies in aquaculture. The paper emphasizes the importance of antimicrobial dose optimization in reducing the risk of AMR and provides practical recommendations for optimizing antimicrobial use in aquaculture while maintaining treatment efficacy.

Tryptophan accelerates anti-inflammatory mechanisms in a teleost chronic inflammation model

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Introduction: Despite the limited number of studies testing the effects of tryptophan on fish immune responses, data generally points out to immunosuppressive properties. Therefore, tryptophan poses as a potential regulator of an

ongoing inflammatory process. This study main goal was to evaluate the effects of tryptophan dietary supplementation on immune and neuroendocrine responses of the European seabass, Dicentrarchus labrax, undergoing a chronic inflammatory response.

Methodology: European seabass juveniles (34.55 ± 7.84 g) were distributed into twelve tanks of a recirculating seawater system. At first, fish were intraperitoneally injected with either Freund's Incomplete Adjuvant (FIA, inflamed group), or Hanks' Balanced Salt Solution (HBSS, control group). Within each group, fish were fed a control diet (CTRL) and a CTRL-based diet supplemented with tryptophan (0.3% DM basis; TRP) for 28 days. Samples of head-kidney were taken every week for neuroendocrine- and immune-related gene expression analysis.

Results: The expression levels of gr1 at the end of 1 week were lower in FIA-injected fish than in HBSS-injected fish, irrespective of dietary treatment. When TRP was provided to FIA-injected fish, mcsfr increased from 1 to 2 weeks and remained high until the end of the experiment. Differently, CTRL-fed fish mcsfr mRNA levels increased later, from 3 to 4 weeks. Moreover, il34 expression at 1-week post FIA injection were higher in TRP-fed than in CTRL-fed fish.

Conclusions: The feeding period seems to be critical in what tryptophan supplementation is concerned, since at the end of the feeding trial fish fed TRP displayed a molecular profile similar to that of the CTRL group. In turn, after one week, anti-inflammatory processes seemed to be favored by TRP (higher mcsfr, gr1, il34 and tgfβ), highlighting its potential in accelerating inflammation resolution.

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Genomic modification of a wild NNV reassortant strain: a potential live vaccine for sole

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Introduction: Viral Encephalopathy and Retinopathy (VER) is a neurological fish disease that affects mainly larvae and juveniles of Mediterranean species such as sea bass, gilthead sea bream or sole. The etiological agent is the Nervous Necrosis Virus (NNV), a non-enveloped virus containing bisegmented ssRNA (+) genome which codes for the viral polymerase (RNA1) and the coat protein (RNA2). During the infection, vacuolization and necrosis in the central nervous system and the retina leads to swimming abnormalities and, generally, host death. Vaccination is a promising strategy for VER prevention, although only two inactivated vaccines are licensed for sea bass immunization. Therefore, the objective of this study was to assess the potential of a mutated NNV strain as a live vaccine for sole.

Methodology: The NNV attenuated strain, namely mut93/08-12, was obtained by site-directed mutagenesis and harbors point mutations in 3'-UTR regions of both RNA segments of a wild type (wt) strain highly pathogenic to sole. Efficacy of this potential vaccine was assessed by experimental infections in sole, challenged by bath or intramuscular injection. Results were expressed as survival curves and viral replication in brain. Immune response was evaluated by IgM production and immune-related gene expression.

Results: Experimental infections revealed a higher survival of sole challenged with mut93/08-12 with respect to the wt-infected fish, although both strains replicated in a similar extent. Immune-related genes (tnfα, mx, rtp3, herc4, cd4 and cd8a) expression in head kidney of vaccinated fish showed a significant enhancement during the immunization period, while in brain a subtle up-regulation was observed. However, IgM production in fish immunized with mut93/08-12 was significantly lower to that of fish infected the wt strain.

Conclusions: The overall results of this study suggest that the point mutations generated in the UTR regions of both NNV genomic segments do not affect viral replicative and immunogenic fitness but favor sole survival. Further research is needed to better determine the potential of this live attenuated vaccine.

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Detection of different betanodavirus genotypes in wild fish from Galician coastal waters (NW Spain)

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Introduction: Viral Encephalopathy and Retinopathy (VER) is a severe neurological infectious disease affecting marine fish caused by the Nervous Necrosis Virus (NNV, G. Betanodavirus). NNV has a positive sense single stranded RNA genome composed of two segments, RNA1 and RNA2. The T4 region, a conserved nucleotide sequence in the RNA2 molecule, allows the classification of the betanodaviruses into four genotypes: barfin flounder-, redspotted grouper-, striped jack-, and tiger puffer nervous necrosis virus (BFNNV, RGNNV, SJNNV, and TPNNV, respectively). VER outbreaks in Southern Europe led to the detection of genetic reassortment between RGNNV and SJNNV genotypes. Since the 2000s, several epidemiological surveys on NNV prevalence have reported the presence of the virus in an increasing number of marine fish, other marine vertebrates, and invertebrates. In this study a NNV survey was conducted in wild fish caught along the northwestern Atlantic coast of Spain from 2019 to 2021.

Methodology: A total of 1,277 fish samples belonging to 16 species were virologically analyzed (cell culture and RT-qPCR). Betanodavirus genotype was determined using TaqMan probes in the samples which tested positive by RT-qPCR, and NNV isolates were also characterized at nucleotide and amino acid level.

Results: This study reveals NNV presence in 1.72% of fish analyzed. The percentage varied between the different species, but in all of them, the positivity rate was less than 5%. The two genotypes found were the reassortant RGNNV/SJNNV and the SJNNV in a similar proportion. Both NNV types were present in blue whiting, hake, and European pilchard, although no coinfections were observed. Moreover, four reassortant RGNNV/SJNNV strains were isolated in cell culture: three from pilchard and one from mackerel. These isolates contained amino acid mutations in both the viral polymerase and the capsid protein when compared with the parental RGNNV and SJNNV strains, in regions related to temperature adaptation, host specificity or virulence.

Conclusion: This study provides new data on NNV presence in the northwestern Atlantic coast of Spain at water temperatures not higher than 16ºC and highlights the importance of monitoring wild fish populations to foresee potential outbreaks in susceptible fish farmed in the area.

An oral inactivated vaccine against nervous necrosis virus (NNV) elicits immune response in Senegalese sole juveniles

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Introduction: Nervous necrosis virus (NNV) is one of the most threatening viruses affecting Mediterranean aquaculture. Several experimental NNV vaccines have been tested for different fish species in recent years. However, commercial vaccines, administered by intraperitoneal injection, are only available for European sea bass. The oral route is one of the most appropriate delivery methods for vaccines because no fish handling is required. In the present study we have orally vaccinated Senegalese sole (Solea senegalensis) juveniles with an inactivated vaccine encapsulated into alginate microspheres and mixed with food pellets. The production of specific antibodies and the stimulation of immune-related genes was assessed at different time points after vaccination.

Methodology: Juvenile sole (2.5 g average weight) individuals were fed 3 times/day with 1 g food pellets containing a binary ethylenimine (BEI)- inactivated vaccine for 3 days. The vaccine concentration was $3x10^4$ TCID50/g. The mock-vaccinated group received commercial pellets. After 30 days post-vaccination (dpv) fish were challenged by intramuscular injection with the homologous strain (10^5 TCID50/fish). At 3-, 5-, 7-, and 30-days post-vaccination (dpv) serum, gut, and kidney were sampled. Antibody production in serum and modulation of genes coding for toll like receptor 7 (TLR7), tumor necrosis alfa (TNF-α), and Mx proteins, as well as the cytotoxic and T-helper lymphocytes markers (CD8 and CD4) in gut and kidney samples was assessed. In addition, ight induction was tested only in gut.

Results: During the immune induction period no antibody production was detected in the fish. However, at 3 dpv tlr-7, tnf-α, mx, cd4, and cd8a genes were significantly up-regulated in kidney whereas no further modulation was observed. In the gut it was necessary to wait until 7 dpv to observe a significant up-regulation of ight.

Conclusions: The gene stimulation profile of the vaccinated fish suggests that both an inflammatory and a cellular response are induced after the oral vaccination in kidney, whereas in the gut only ight up-regulation was recorded. Further studies will be necessary to determine the protection conferred by the vaccine.

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Antocyanins and grapevine shoots potential antivirals against the viral haemorrhagic septicaemia virus

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Introduction: Over the past few decades, there has been a growing interest in using phytochemicals as a safer and more environmentally-friendly alternative to conventional chemical agents for controlling viral infections. This is especially relevant given consumers' concerns about the presence of chemicals in food. However, the agricultural industry generates a significant amount of waste that is often underutilized, despite its potential value. In Europe, viral haemorrhagic septicaemia virus (VHSV) infections cause an estimated annual economic loss of around 50 million euros, highlighting the significance of this rhabdovirus which is also notifiable to the EU and the OIE. VHSV typically causes systemic disease and haemorrhagic lesions, particularly in young fish, with mortality rates that can reach up to 90%. In this study, we aimed to explore the potential use of different waste products from the agricultural industry in Spain against VHSV. Specifically, we sought to identify antiviral compounds while also revaluing natural waste products.

Methodology: We tested the virucidal activity of nine different natural extracts derived from waste generated by saffron and grapevine cultivation against VHSV in vitro. We then evaluated the impact of the extracts showing virucidal activity at different stages of the virus life cycle in EPC cells by TCID50 and PFU methods. Finally, pre-treatment of cells with the extracts was also assessed to test a potential prophylactic activity

Results: The antocyanins and grapevine shoots demonstrated virucidal activity, apparently affecting only the replication step but not adsorption to the EPC cells. However, pre-treatment of cells did not affect viral replication. These results suggest that the antocyanins and grapevine shoots may block viral replication once into the cells or even stimulate the immune system of cells, which deserves further investigation.

Conclusions: These results suggest that antocyanine and grapewine shoots may be used to control VHSV infections and offer a promising approach to revaluing agricultural waste.

Impact of Pseudoalteromonas strains on the flat oyster Ostrea edulis

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Aquaculture encounters numerous infection event linked to different factors such as stress, dysbiosis or host genetics. The misuse and/or overuse of antibiotics have led to the emergence of antimicrobial resistance, which has become a major concern in aquaculture and a global public health threat. (Martínez Cruz et al., 2012; Preena et al., 2020; Quintanilla-Villanueva et al., 2023).

Probiotics are considered as a relevant tool to reduce the use of antibiotics. They are defined as "[..] a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community" (Verschuere et al., 2000).

Five strains of Pseudoalteromonas bacteria were isolated from the hemolymph of healthy bivalves. They produce original antimicrobial cationic cyclolipopeptides named alterins which are active against Gram-negative bacteria. The structural diversity of those compounds and their antimicrobial activity spectrum have been studied (Defer et al., 2013; Desriac et al., 2020; Offret et al., 2022). Those strains exerted a beneficial effect on marine bivalves and gastropods by improving their growth or helping the survival of those species against infections (Offret et al., 2019).

The FEAMP project PAQMAN aims to evaluate those strains as potential probiotics for marine aquaculture. To do so, the effects of Pseudoalteromonas strains during the sexual maturation of the flat oyster Ostrea edulis have been assessed. During 4 months, oysters were weekly exposed to Pseudoalteromonas strains, inoculated at 10^6 UFC/mL.

The effects on the biofilm formation in the tanks have been studied with a confocal laser microscope. The microbial communities of the seawater, the biofilm and the hemolymph have been observed via a metabarcoding analysis (ADNr 16S V3-V4 regions). The impact of the Pseudoalteromonas strains on oysters' larvae emissions was also assessed.

Significant reduction of the biofilm was demonstrated as well as an improvement of the larval emission. Analysis of the microbial communities of the hemolymph of the flat oysters and the environment (seawater and biofilm) are in progress.

Antibiotic resistance genes distribution among Vibrio spp. Isolates collected from Brazilian shrimp farms

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Vibrio spp. is a genus of ubiquitous bacteria found in a wide variety of aquatic and marine habitats. Some species are pathogenic for humans and/or aquatic animals like shrimps.

The aims of this study were to investigate transferable resistance genes and their impact on the phenotypic profile of antimicrobial resistance in Vibrio isolates.

Eighty-three Vibrio isolates belonging to the V. harveyi clade (Maldi-Tof and PCR identification) were collected in 2018 from 50 Brazilian shrimp ponds. Their antimicrobial susceptibility profiles have been determined for six antimicrobial classes (14 agents): beta-lactams (ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, imipenem), phenicols (chloramphenicol, florfenicol), quinolones (oxolinic acid, enrofloxacin), macrolides (erythromycin), aminoglycosides (gentamicin), tetracyclines (oxytetracycline) and folate pathways inhibitors (trimethoprim-sulfamethoxazole) using the agar diffusion method.

The presence of 44 transferable resistance genes (including resistance to the six classes tested phenotypically) and 48 genetic mobile elements (known to mobilize these antimicrobial resistance genes) was investigated using high-throughput qPCR.

A mobile genetic element (IS6100) was detected in a single isolate.

Antimicrobial resistance genes were detected in 12 isolates (14.4%), coding for quinolone and/or tetracycline resistance. Tet genes were the more frequent (12.0%, n=10), nine harbored tetB and one tetM. QnrS was detected in three isolates. One single isolate carried both qnrS and tetB genes.

In agreement with phenotypic data, no extended beta-lactamases and carbapenemases spectrum were detected. Tet genes were found in all 10 isolates exhibiting a reduced susceptibility to tetracycline. Based on phenotypic results, 10 isolates were resistant to oxolinic acid with inhibition zones inferior or equal to 10 mm, but no qnr genes were detected in these isolates, suggesting a different mechanism for this resistance (such as another qnr gene or mutations in the gyrA, gyrB or parC genes). On the contrary, the three isolates harboring qnr genes were found susceptible to oxolinic acid and enrofloxacin.

The V. harveyi strains investigated here cannot be considered as reservoir of antimicrobial resistance genes that could lead to the dissemination of AMR to the food chain. However, whole genome sequencing of the 10 quinolone-susceptible isolates should allow us to investigate more precisely the genetic support of AMR.

Fecundity of in vitro cultivated Anisakis pegreffii and its production of extracellular vesicles

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Introduction: In vitro life cycle protocols for helminths are important tools that enable ideal conditions for rearing of parasites in large numbers, and the repeatability of the experiments. Marine nematodes from genus Anisakis are gaining more research spotlight following a recent and noticeable increase in the number of anisakiasis cases (i.e., the clinical condition in humnas cused by Anisakis spp.) in Europe. Under the natural conditions, free-swimming second-stage larvae (L2) hatch from the eggs expelled in the seawater via feces of the final hosts (marine mammal). Larvae are preyed upon by crustaceans (mainly euphausiids) and possibly small fish (intermediate hosts), moulting into third-stage larvae (L3). Intermediate hosts are consumed by larger fish (paratenic hosts) in which L3 migrate in the visceral cavity and remain in parathenesis until final hosts pray and digest the infected paratenic host. In the final hosts, L3 moult through fourth and fifth (juvenile) stage in reproductively active adults of separate sex.

Methodology: Anisakis spp. type I larvae (identified as A. pegreffii following genotyping) were collected from naturally infected blue whiting Micromesistius poutassou in the Adriatic Sea (Croatia). L3 larvae were cultured in autoclaved glass bottles to reach the adult stage (Mladineo et al., 2023). After observing the first eggs expelled in the medium, the eggs were collected for quantification 3x per week. The fecundity was expressed as the number of eggs expelled per day, accounting for the number, sex and date of adults removed from the culture. Media from cultivated adults and L2 resulting from collected and hatched eggs was collected for isolation of the extracellular vesicles (EVs) following published protocol (Mazanec et al., 2021; 2023). L2 were also collected for transmission electron microscopy (TEM).

Results: The first number of eggs was observed already 17 days post-incubation of L3 isolated from the blue whiting viscera. The number progressed reaching over 1 million eggs produced/day, after 2 months post-incubation. EVs and cell-free mitochondria were observed discharged between the two cuticle sheets of L2. Proteomic profile of the larval and adult secretome revealed elements known to be affect the virulence, and host-/ host-associated microbiome interaction.

In vitro study with viperin as an antiviral effector in rainbow trout (Oncorhynchus mykiss) cells

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Introduction: Viperin (VIP) is a protein from the interferon family produced as an antiviral response in many organisms. Recently its role as a chain terminator impairing RNA polymerization was described. By catalysing the conversion of CTP to ddhCTP, the modified nucleotide, when fused into the nascent chain of the viral RNA mechanism, VIP inhibitits viral replication. On the other hand, Viral hemorrhagic septicemia virus (VHSV) affects many fishes in aquaculture being responsible for major biologic and economic losses, affecting more then 50 species including freshwater and saltwater fishes.

Methodology: To evaluate VIP role in fish antiviral response, gene knockdown was performed in an in vitro trial was designed in Rainbow trout (Oncorhynchus mykiss) macrophage cell lines (RTS11). VIP gene (vig1, XM_021582972.2)

knockdown was first tested by using four different combinations of three distinct small interfering RNA (siRNAs); Vig1, Vig2 and Vig3) of 21–23 nucleotides in length.

The assays were performed applying 2 and 3 days of siRNA inoculation at three different concentrations (10pmol, 50pmol and 100pmol) in RTS11 cells at a concentration of 1-2x106cells.mL-1. Afterwards, Poly I:C was used in a concentration of 25 mg/uL to induce cells antiviral mechanisms after siRNA inoculation. After 24 hours, RNA from each treatment was extracted and cDNA obtained to determinate the best set, amount and incubation period of siRNA, by Real-time PCR (RT-PCR). Once the treatment settings are defined, VIP-knockout cells and non-silencing siRNA cells (control) will be infected with inactivated VHSV to understand the pathways related to the transcriptional regulation of VIP expression, the pathways induced by VIP itself.

Results and Conclusions: It is expected a downsize in levels of gene transcription related with cell's inflammatory response (rtIFN1, rtIFN2, rtIFN3, rtIFN4, rtIFN-g1 and rtIFN-g2) that were treated with iRNAs. Ultimately, this study will allow a better understanding of VIP role in fish antiviral immune response and can help to create new prophylactics measures or treatments in the aquaculture environment.

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Updating of iridovirus prevalence within sturgeon farms in northern italy in the years 2021-2023

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Sturgeon fishing has all but disappeared and the sale of caviar from caught fish is now prohibited due to the depletion of wild populations. So, aquaculture has taken the place of fishing. The main threat to farmed sturgeon is infection with Iridovirus, which mainly affects fry and juveniles.

In this study, 303 sturgeons from a farms in Northern Italy, belonging to five different species of the genus Acipenser and to the species Huso huso and aged 6 months or less, were tested between January 2021 and January 2023. Necroscopic, bacteriological, parasitological and virological control was subsequently carried out to certify its state of health.

To perform the virological analysis, the gills were taken. Once the extract was obtained, it was subjected to a PCR-real time according to the protocol described by Bigarrè et al., 2017.

Once the molecular analyses were performed, 116 samples tested positive, 38.2% of the total. Almost all the fish that tested positive at molecular analysis showed a tense and swollen abdomen, hepatic steatosis, splenomegaly and increased gill volume on an anatopathological level.

No statistically significant differences in the number of positives from the different species could be found, suggesting possible horizontal transmission within the tanks. However, it was possible to find a statistically significant difference between infections in the different seasons of the year. In fact, in the winter months the positivity rate stands at 57%, whereas it does not exceed 37% in the other months of the year.

This type of breeding has become increasingly important over the years, so it is extremely important to constantly monitor incoming batches and breeding stock, as well as batches already on the farm even if they are older than the most at risk, in order to prevent the introduction of the virus and to avoid losses in such a significant sector.

First indentificagtion of lactococcus petauri in pumpinkseed sunfish (lepomis gibbosus) in Italy

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Lactococcus petauri was identified as a new species in 2017, distinguishing it from Lactococcus garvieae. In 2022, it was isolated as the agent responsible for an outbreak of lactococcosis in Turkey and has been isolated in several outbreaks in various areas of the Mediterranean basin. Despite this, this pathogen had never been identified in Italy. The distinction between the two species is complex, as the most common microbiological identification methods. Distinction is only possible by sequencing the ITS tract of the 16s-23s rRNA, or the entire bacterial genome.

During a sampling in the summer of 2022 in Lake Candia (Piedmont, Italy), sun perches (lepiomis gibbosus) were caught. The fishes were subjected to anatomopathological, parasitological and culture examinations of the eye, kidney and brain. A gram-positive coccus was isolated from the eye of the fish examined, which was then identified using

MALDI-TOF. Spectrophotometric analysis resulted in Lactococcus garvieae. Subsequently, the method proposed by Colussi et al (2023) was used to distinguish whether the isolated strain was actually L. garvieae or was Lactococcus petauri. The genetic analysis resulted in the attribution of the strain found in the eye to L. petauri. To obtain a biochemical characterisation of the isolated strain, three API galleries were performed, namely 20E, 20STREP and 32STREP. In addition, the susceptibility to antibiotics of the strain was determined, which revealed that it was sensitive to β-lactams and had an intermediate sensitivity to enrofloxacin.

The isolation of Lactococcus petauri on Italian territory constitutes an important piece in the picture of the spread of this pathogen. Although it has not yet been identified as the aetiological agent of the outbreaks of lactococcosis occurring in Italy, it is safe to assume that this pathogen, currently found in wild fish, can reach trout farms. There is therefore a need to map the spread of L. petauri on the territory, to predict possibile epidemic outbreaks of this pathogen.

The myxozoan parasite Tetracapsuloides bryosalmonae is infecting wild salmonid populations in Lake Huron

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Introduction: *Tetracapsuloides bryosalmonae* is a myxozoan parasite (Malacosporea) acting a two-host life cycle and causing Proliferative Kidney Disease (PKD) when infecting susceptible fishes under favorable environmental conditions. Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo trutta*) are well-known hosts, suffering PKD in regions of Europe and Northwestern America. Currently, little is known about myxozoan parasites infecting fishes in the Great Lakes basin. The objective of this study was to assess *T. bryosalmonae* infection in Great Lakes salmonid species, focusing on those with relevance for local fisheries and ecosystems, and with an unknown susceptibility to this parasite infection.

Methodology: Kidney samples from Lake Whitefish (*Coregonus clupeaformis*), Lake Trout (*Salvelinus namaycush*) and Bloater (*C. hoyi*) were opportunistically retrieved. USGS researchers facilitated the sampling of Lake Huron fish populations, including of fish accidentally dead or that had to be sacrificed during wild population monitoring programs, or after Sea Lamprey (*Pteromyzon marinus*) parasitism assessments. DNA was extracted from the posterior kidney samples and used for PCR detection of generic myxozoan parasites and targeting *T. bryosalmonae* 18S rDNA. Positive samples upon qPCR were further processed by conventional PCR to retrieve genomic sequences from the parasite small subunit ribosomal DNA (SSU rDNA) and of the mitochondrially encoded cytochrome c oxidase I (CO1).

Results: Based on the qPCR assessment we estimated an infection prevalence of 19% in Lake Trout (out of 59 sampled), 19% in Lake Whitefish (out of 26 sampled), and 45% in Bloater (out of 55 sampled). The highest parasite burden was retrieved from Bloater with a geometric mean of 8.53×10^5 copies gene/g of kidney. Sequences analysis confirmed the detection of *T. bryosalmonae* from all these salmonid species, respectively with 100% and 99.76% similarity to *T. bryosalmonae* CO1 and SSU rDNA sequences deposited in GenBank.

Conclusions: This is the first report of *T. bryosalmonae* from Lake Trout, Lake Whitefish, and a Cisco species from the Great Lakes. Towards understanding the relevance that *T. bryosalmonae* may have for fisheries management in the Great Lakes, further study is needed to characterize the specific susceptibility of each of these hosts, *viz* the occurrence of coelozoic/histozoic parasite stages and of PKD pathology.

Genomic comparison of Francisella halioticida : focus on French isolates

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Unusual mortality events affecting mussels (Mytilus spp.) are recently observed in France, in UK and in the Netherlands. The DNA of the known Yesso scallops' and giant abalone's pathogen Francisella halioticida was recently detected in mussels collected in area suffering mortalities. A total of five isolates belonging to F. halioticida were obtained from mussels in France. Two were found to show high virulence toward adult and juvenile mussels in the tested conditions. This study aims to compare F. halioticida's genomes and highlight genomic differences that could explain the virulence variability among the isolates.

All French isolates were submitted to whole genome sequencing using both short-read (Illumina) and long-read (PacBio Sequel II) technologies. After pre-treatment, complete genome and plasmids sequences were obtained with Unicycler software and annotation performed with PGAP. Genomic sequences from the two previously sequenced F. halioticida strains (DSM23729 and UTH170823) were retrieved from NCBI as well as 25 other Francisella species. Phylogenetic and comparative analyses were performed to highlight differences and similarities among the different strains. Virulence factors were searched from prediction tools and with homology to known virulence factors from mammal and fish

pathogenic Francisella species. Finally, F. halioticida genomes were compared to find specific virulence factors and metabolism differences.

Genomic comparisons among Francisella halioticida supported the hypothesis that four of the isolates belonged to a unique type strain, previously named FR22, closely related to previously described strains. The isolate FR21, unique isolate belonging to this type strain showed distinct differences. Its denomination should be closely analysed to determine if it can be characterized as subspecies. Virulence factor search shed light on the presence of virulence gene not previously described in this species. In addition, it showed that the different French isolates showed particularities such as isolate FR22b (low pathogenicity) not presenting the genes iglA and iglB, belonging to the Francisella pathogenicity island.

This study increase knowledge among genomic of Francisella genus and more specifically Francisella halioticida species. A deeper knowledge of virulence factors is essential to understand francisellosis in bivalves.

Efficacy of autogenous vaccine administrated by three routes against furonculosis by aeromonas salmonicida in large trout (Oncorhynchus mykiss)

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Introduction: Furunculosis caused by Aeromonas salmonicida sub salmonicida is responsible for heavy economic losses in salmonid farms. This disease was rather well controlled in France but a resurgence of clinical cases has been observed in recent years. This could be linked to a lengthening of the production cycle in order to produce trout of 2 to 3 kg for processing into smoked fillets and eggs production for consumation. The objective of our study is to evaluate the level of vaccine efficacy and the duration of protection in large trout.

Material and Methods: The vaccine efficacy of the protocols was studied experimentally on rainbow trout weighing more than 250 g in an experimental facility. An autogenous vaccine (Biovac, CEVA) was used and the efficacy of three different routes of administration, intraperitoneal injection, immersion and oral, was studied. Anti-ASS antibodies were monitored over time and an infectious challenge by immersion with Aeromonas salmonicida salmonicida was performed.

Results: Statistical analysis showed significant vaccine efficacy only in animals vaccinated at least once by intraperitoneal injection, with effective protection against mortality and clinical signs expression. The duration of efficacy after vaccination by intraperitoneal injection is at least 2500 degree.days. This efficacy is, however, temporarily accompanied by reduced growth of the rainbow trout, as these animals show numerous intraperitoneal adhesions.

Conclusions: The duration of efficacy of the vaccination could be clarified in order to determine the value of a booster vaccination, the optimal timing and the modality of administration for its implementation.

Comparison of 4 slaughter methods for rainbow trout (oncorhynchkus mykiss) with regard to animal protection

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Introduction: At present, fish consumption in the human diet has increased and is becoming an important part of protein intake. Given the increase in aquaculture production, it is important to examine the conditions of slaughter methods in order to guarantee animal protection. In this study, we examined the effect of 4 methods of killing rainbow trout, ikejime, percussion, electricity and ice asphyxiation, and in particular on the levels of stress induced.

Methodology: Female triploid rainbow trout (O. mykiss) were acclimatized in 3 fiberglass tanks (200 L) with 25 fish per tank. At 250g, 16 fish were used per slaughter method. A culling order with a rotation of killing methods was determined with always order 1 for the asphyxiation-on-ice killing method due to its duration. After died of fish, blood sample (1 ml) was collected for analysis of blood parameters (cortisol, glucose, lactate), muscle pH and flesh quality were also evaluated.

Results: Statistical analysis showed significant differences between slaughter methods used for some indicators such as glucose with AS > IK ≈ EL > CO; lactacte with AS ≈ EL > CO ≈ IK. For cortisol, trends was observed with cortisol AS > IK ≈ CO ≈ EL. For TPA (Texture profile analysis), statistical analysis showed significant difference at J0: IK > AS ≈ EL ≈ CO.

Conclusions: Slaughter methods have an impact on stress and food quality. Ikejime seems to be as respectful as commotion and electronarcosis, two methods recommended in order two diminish suffering in European ethical guidelines for aquaculture. While asphyxiation on ice is the least suitable method, we need to improve our knowledge in order to propose a method adapted to the protection of fish.

Effects of physical or sensory enrichments on rainbow trout (oncorhynchus mykiss) welfare

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Introduction: Today, fish consumption in the human diet has increased and is becoming an important part of protein intake. Given the increase of aquaculture production, it is important to consider the rearing conditions of fish and especially their welfare. In this study, we investigated the influence of enrichment of the rearing environment on the welfare of rainbow trout, and in particular on stress levels reduced or not in the presence of structural and sensory enrichment.

Methodology: Female triploid rainbow trout (O. mykiss) were acclimatized in 24 fiberglass tanks (200 L) with 30 fish per tank. The experimental conditions were as follows: (1) neutral condition with PVC pipe, (2) positive stress condition without enrichment and with a stress protocol conducted weekly with water reduction and dip-net fishing, (3) structural enrichment with rubber tubbing, (4) structural enrichment with a rubber wall in the tank, (5) structural enrichment with a floating grass carpet, (6) structural enrichment with various thin pipes imitating luminaria seaweed, (7) sensorial enrichment with gas bubbles and (8) sensory enrichment with a blue filter on the tank. Each condition was run in triplicate with 30 fish per tank, i.e. 90 fish per condition at the beginning. Each week, all conditions were observed and scored using various behavioural indicators. Blood, scales, fin and brain were collected at different time to assess some stress indicators (cortisol, glucose and lactate).

At the end, robustness of the fish was evaluated by a bacteria challenge (Aeromonas salmonicida salmonicida) conducted by immersion.

Results: Statistical analysis showed significant differences between enrichment conditions for growth performance and behavioural indicators such as swimming, space occupation and aggressive behaviour. Response to the infectious challenge showed different trends depending on the enrichment tested.

Conclusions: Some enrichments seem be more efficient on welfare indicators and fish robustness to pathogens: rubber tubbing, blue filter, and “luminaria seaweed”. The duration of enrichment exposure could be extended to determine more benefits, but this seems to be a good way of improving fish welfare.

Specificity of in situ Hybridisation assays used for the diagnosis of mollusc parasites of the genus Marteilia and Bonamia

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Organisms of the genus Marteilia and Bonamia are protistan parasites of bivalve molluscs. Some of them are regulated at the European and international level, namely Marteilia refringens, Bonamia ostreae, and Bonamia exitiosa, due to their pathogenicity on several flat oyster species.

In situ Hybridisation (ISH) is part of the recommended methods for the detection of mollusc pathogens. Thanks to the use of a specific labelled DNA probe, ISH allows locating a pathogen in tissues and is useful for confirming histological diagnosis. Different ISH assays targeting Bonamia and Marteilia parasites are available in the literature; however their specificity has not been fully assessed, especially regarding closely related pathogens. A review of the literature allowed identifying 3 and 6 probes respectively that could be interesting for the diagnosis of Marteilia and Bonamia parasites. Specificity of selected probes was assessed by performing ISH assays on different species of mollusc infected with different species of the genus Marteilia or Bonamia. Additionally, Bonamia probes were also tested on samples infected with closely related parasites of the Mikrocytos genus.

Results allowed identifying some genus specific probes (detection of all known species within the genus Marteilia and Bonamia), as well as probes allowing the specific detection of Bonamia regulated species. However, probes allowing the detection of M. refringens also detected of M. pararefringens (previously named M. refringens type M). Specific probes for M. refringens and M. pararefringens are missing and would be useful to study host species range between both species. Critical parameters for optimal ISH assay performance were also identified, allowing us to provide technical recommendations, as for example, regarding probe quantity and lysis time.

Altogether, this work allows better interpreting data from ISH assays used for the characterisation of Marteilia and Bonamia parasites, and providing recommendations and perspectives to improve ISH-based diagnosis.

PCR diagnosis of regulated mollusc diseases present in europe

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Bonamia ostreae, Bonamia exitiosa and Marteilia refringens are protozoan parasites responsible for the bonamiosis and the marteiliosis, two diseases which have caused significant mortalities in European populations of flat oysters Ostrea edulis. Those parasites are endemic in Europe; however some countries/ zones are free of one and/or the other. Bonamiosis and marteiliosis are both listed as notifiable diseases at the European and the international levels.

In order to facilitate the diagnosis of these two listed diseases, the EU reference laboratory for mollusc diseases has developed two new multiplex Taqman® PCR assays; the first one allowing the concomitant detection of B. ostreae and B. exitiosa, the second one allowing the detection and typing of M. refringens. Both PCR assays underwent a full validation including an evaluation in the context of an Inter Laboratory Comparison test.

Information on the performances of diagnostic tools as well as the use of quality controls are essential to properly interpret results. Performance parameters of both PCR assays will be presented. Quality controls for PCR assays and the impact of potential PCR inhibitors in mollusc samples will be discussed. Those two new PCR assays would be very helpful to monitor the presence of these EU regulated parasites at the species level in the context of EU national surveillance or other programs such as flat oyster restoration.

Evaluation of pathological effects of mycotoxins and protective effect of Hericium erinaceus in zebrafish larvae

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Introduction: Mycotoxins are secondary metabolites produced by various types of fungi, often reported as food contaminants. Moreover, many mycotoxins have been detected in veterinary feed worldwide, as in those intended for use in aquaculture facilities, providing a tangible risk for both fish and human health. In this study, the effects of Aflatoxin B1 (AFB1) and Fumonisin B1 (FB1), have been investigated on zebrafish embryo development to understand its pathological mechanism.

Materials and Methods: The FET (fish embryo toxicity) test was carried out in accordance with OECD guidelines n°236. Wild type (WT) mature zebrafish were used to produce embryos. Fertilized eggs were exposed to AFB1 (0.01, 0.05, 0.1 mg/kg) and FB1 (0.1, 0.5, and 1 mg/kg) up to 96 hours post fertilization (hpf) into 24-well plates with test solutions and incubated at 28 C°. The entire survival rate, hatching and developmental abnormalities of embryos were monitored and photo-recorded every 24h. We evaluated the potentially protective effect of Hericium erinaceus extract, one of the most characterized fungal extracts, focusing on the nervous system. Furthermore, determination of oxidative stress markers was investigated.

Results: We observed morphological alterations in zebrafish embryos after exposure to different AFB1 and FB1concentrations as well as the oxidative stress pathway involved. These metabolites induced negative effects on the lethal endpoints analysed such as survival, hatching, and heart rate. The combination of AFB1 and FB1 caused an imbalance in the antioxidant defences system with an increase both in antioxidative stress enzymes levels such as superoxide dismutase (SOD) and catalase (CAT), and in detoxification enzymes levels glutathione s-transferases (GST) and cytochromes P450 (CYP450). The induction of apoptosis in zebrafish embryos was observed in the trunk, yolk, and tail after AFB1 exposure. H. erinaceus inhibited the morphological alterations of the embryos as well as the increase of reactive oxygen species (ROS), interacting and modulating the molecular mechanism involved in oxidative stress and lipid peroxidation and in addiction counteracting apoptosis.

Conclusion: The presence of mycotoxins in fish feeds can be a huge risk factor for animal health and therefore an important object of interest for food safety.

Phagocytosis based assay for an in vitro assessment of immunocompetence of the pen shell P. nobilis during Mass Mortality Events

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Mass Mortality Events (MMEs) close to 100% of the populations affecting the noble pen shell Pinna nobilis have been reported since 2016. Currently, residual populations are present only in enclosed bays and lagoons in few countries and conservation efforts have been made to maintain individuals in indoor facilities. The study was performed in July 2021 and May 2022 in two sites in a natural population from the Alfacs Bay in the Ebro Delta (Catalonia, Spain) and animals maintained in captivity at IMEDMAR-UCV (Catholic University of Valencia) system and Murcia Aquarium. Hemolymph was collected from animals in the field and in captivity as a non-destructive sampling method. The animals were immediately relocated into the field/tank substrate and monitored. Hemolymph aliquots were counted to obtain the Total Haemocyte Count (THC). PH rodo green BioParticles (Invitrogen) conjugated with Staphylococcus aureus and Zymosan A were incubated with hemocytes at 25°C at two different timing (30 min and 3 h). After the stimulation, cells were read at a FACScalibus Flow Cytometer. The number of positive cells was measured as the percentage of cells showing MFI (Mean Fluorescence Intensity) higher than the negative control. The phago-lysosome fusion efficiency was also evaluated. The results showed that pen shell in captivity had significantly lower THCs compared with those of Alfacs Bay population (mean number of 7-9 x 104 vs 2-5 x 105 cells/mL, respectively) although with marked variation among individuals. MFI of natural population ranged between 10-30% after 30 minutes in both the stimuli and increased to 30-50% after 3 h. In July 2021 in the two sites of Alfacs Bay showed an increase in the phagocytic activity of 70% after 3h in one individual. In both seasons captive animals, had scarce or absent ability to phagocyte the two stimuli. Pearson correlation of THC values was positive and strong (p-value = 0.7) to the MFI of the 3h experimental conditions.

This represents the first in vitro study of P. nobilis immunity during MMEs events that revealed a strong immunodepression of captive animals and a scarce capacity of the natural population to respond to pathogenic stimuli.

Effects of dietary methionine supplementation on European seabass (*Dicentrarchus labrax*) mucosal immunity and disease resistance against *Tenacibaculum maritimum*

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Introduction: The emergence of infectious diseases and fish feed management are among the major challenges of the aquaculture industry. To overcome these hurdles, the enrichment of fish feed with additives that can boost fish immunity, such as certain amino acids, is becoming a common practice. Methionine is among the amino acids with recognized positive effects on fish immunity and disease resistance. However, its role in mucosal immune machinery and in response to pathogens still needs further investigation. Therefore, this study aimed to investigate the effects of dietary methionine supplementation on the European seabass mucosal immunity and disease resistance against *Tenacibaculum maritimum*.

Methodology: For 4 weeks, juvenile European seabass (*Dicentrarchus labrax)* were fed three experimental diets differing in methionine content: a commercial diet (CTRL), a CTRL diet supplemented with 1% (MET1) or 2% methionine (MET2). At the end of the feeding trial, blood and skin mucus were collected and fish were bath-challenged with *T. maritimum*. The same samples were taken at 4, 24 and 48h post-infection. Several hematological parameters were evaluated and blood smears were prepared for leucocytes counting and classification. The skin mucus was used to assess innate immune parameters.

Results: At the end of the feeding trial, none of the analyzed parameters was significantly different among dietary groups, except for the haemoglobin values, which decreased with increasing dietary methionine supplementation levels. Additionally, regardless of dietary group, the bacterial infection triggered a fast increase in circulating leucocytes. The skin mucus humoral parameters also revealed a mucosal immune response activation by the bacteria, despite delayed compared to the observed in the blood. Although not statistically significant, fish fed the MET2 diet appeared to be more susceptible to the bacterial infection than the other two dietary groups. These results are further supported by the discriminant analysis, which grouped CTRL and MET1 treatments very close together.

Conclusion: Data from haematological and skin mucus humoral parameters suggests that methionine, at the tested levels, did not significantly influence the fish mucosal immunity, nor the resistance against *T. maritimum*. Further analyses are currently underway to delve deeper into this data.

Morphological, ultrastructural and phylogenetic description of two myxosporean parasites from the teleostean fish trisopterus luscus in the Portuguese Atlantic coast

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Introduction: Myxosporeans are economically important fish endoparasites. Members of the genus Kudoa Meglitsch, 1947 (Multivalvulida) mostly parasitize the muscular tissues of estuarine and marine fishes, being frequently associated with post-mortem myoliquefaction and huge economic losses for aquaculture and fishery industries [1]. The present study describes the morphological, ultrastructural, and molecular features of two myxosporeans found parasitizing commercial stocks of pouting Trisopterus luscus caught off the Portuguese Atlantic coast.

Methodology: A myxozoan survey was conducted on the external and internal tissues of several specimens of T. luscus. Isolated cysts or fragments of parasitized tissues were analysed under a light microscope, and subsequently prepared for transmission electron microscopy, histology and molecular studies targeting the SSU rRNA gene. Sequence construction, alignments, and phylogenetic analyses were performed using MEGA10.

Results: Myxosporean infections were detected in the skeletal muscle and eye. Polysporic pseudocysts belonging to a Kudoa sp. were observed developing in the skeletal muscle. Myxospores were sub-quadrangular in apical view, with each of the four valves displaying a fin cytoplasmic projection. One polar capsule was bigger than the remaining three, but all contained a polar tubule coiled in 3 turns. Histological and ultrastructural observations evidenced disorganization of the myofibrils surrounding the pseudocysts. BLASTn search showed highest similarity to K. trifolia with 97.4 % similarity. In turn, the cysts observed in the eye belonged to the genus Myxobolus, with morphology and molecular data identifying this isolate as Myxobolus aeglefini Auerbach, 1906.

Conclusions: This study presents novel data regarding myxosporean infections occurring in a commercially valuable species for Portuguese fishery industries. The myxospore dimensions and host/organ specificity of the Kudoa isolate in study differ from all previously described records, suggesting it as a novel species. Phylogenetic analyses revealed uncertain positioning of the novel sequence within the monophyletic clade of Kudoa, but reinforced tissue tropism as a relevant evolutionary driver for this myxosporean genus. The finding of Myxobolus aeglefini in the eye of T. luscus represents a new host record for this species.

[1] Lom and Dyková, 2006. Folia Parasitol. 53:1-36.

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Preliminary study of a zschokkella sp. (myxozoa) infecting the gallbladder of the marine teleostean micropogonias furnieri in the Brazilian coast

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Introduction: Myxozoa Grassé, 1970 constitutes a diverse and widespread group of endoparasites that mainly use fish as intermediate hosts and are frequently associated with increased mortality [1]. Members of Zschokkella mostly develop in the biliary tract and urinary system of freshwater and marine fishes. Their myxospores are morphologically similar to Myxidium, making distinction between these genera extremely difficult [2]. The present study describes a Zschokkella sp. found infecting the gallbladder of an economically important fish for Southwest Atlantic Ocean fisheries.

Methodology: Fifty-six specimens of whitemouth croaker Micropogonias furnieri caught from the Brazilian Atlantic coast were dissected for the myxozoan survey of internal organs. Infected tissues were examined using a light microscope equipped with differential interference contrast optics and processed for transmission and scanning electron microscopy, as well as molecular analysis of the SSU rDNA. Sequence construction, alignments, and phylogenetic analyses were performed using MEGA10.

Results: Plasmodia and mature myxospores were observed in the gallbladder. Plasmodia were polysporic, with a highly irregular cell membrane covered by fine projections and containing disporic pansporoblasts. Myxospores were elliptical in sutural view and semicircular in valvular view, with rounded extremities. The shell valves bared several surface ridges parallel to a slightly curved suture line. Two equal subspherical polar capsules were located subterminally and opened to opposite sides. BLASTn search showed highest similarity to Z. soleae (97%). Phylogenetic analyses revealed the new sequence clustering within the coelozoic gallbladder clade of the oligochaete-infecting lineage, alongside other Zschokkella and Myxidium.

Conclusions: Comprehensive morphological, ultrastructural, and molecular comparisons to know Zschokkella spp. suggest the isolate in study as a new species. Although molecular analyses have proved fundamental for distinguishing between species which morphological-based differentiation is difficult or impracticable, the phylogenetic analyses performed reinforce the artificiality of the division between Zschokkella and Myxidium, while strengthening tissue tropism as an important evolutionary signal for Zschokkella. Marine oligochaetes are suggested as the most probable definitive hosts of the species in study.

[1] Lom and Dyková, 2006. Folia Parasitol. 53:1-36.
[2] Fiala, 2006. Int. J. Parasitol. 36:1521–1534.

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NeoGiant, a natural alternative for Amoebic Gill Disease treatment

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Polyphenols are plant “non-toxic” components well known for their antioxidant properties. However, these components (high polyphenolic concentration) also show anti-bacterial, anti-parasitic and fungicidal activities. The aim of this study was to evaluate Grape extracts, based on white grape marc obtained from the NeoGiANT-Horizon 2020 Framework Programme project (101036768), for their amoebicidal capacity against Neoparamoeba perurans, the amoeba responsible for amoebic gill disease (AGD) in Atlantic salmon (Salmon salar L.) aquaculture. In vitro, studies were performed with these extracts, evaluated at different concentrations, using pre-formed N. perurans cultures and incubating for 72 h. Viable cells were counted using inverted microscopy. Extracts showed activity against N. perurans at concentrations higher than 10 % v/v. Although this test only focused on amoebicidal activity, it is worth highlighting that the polyphenols also show antioxidant activities. Therefore, the amoebicidal activity seen in this study and the possible improvement in fish health due to their antioxidant activity may enable these NeoGiANT extracts to be used as a viable, non-toxic alternative for treating AGD. In vivo studies are currently under way to confirm this.

Evaluation of the immune response in betanodavirus-vaccinated gilthead seabream

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Introduction: Reassortants between red spotted grouper- and stripped jack nervous necrosis virus (RGNNV and SJNNV, respectively) have been reported as a threat for Senegalese sole (Solea senegalensis) and gilthead seabream (Sparus aurata) aquaculture in Southern Europe. An inactivated vaccine has been developed using a RGNNV/SJNNV reassortant strain isolated from Senegalese sole, which confers moderate protection against the homologous viral strain in sole juveniles. The aim of this study is to evaluate the differential expression of immune-related genes in gilthead seabream after vaccination, and in vaccinated fish after NNV infection. This research was funded by MCIUI and FEDER under Grant RTI2018-094687-B-C22.

Methodology: Gilthead seabream juveniles were vaccinated by intraperitoneal injection with an inactivated NNV vaccine, and control fish were injected with PBS. Thirty days after vaccination, fish were intramuscularly injected with the homologous viral strain (10^5 $TCID_{50}$/g fish). Head-kidney and brain samples were extracted at 2, 3 and 7 d post-vaccinaton (dpv), and at 1, 3, and 5 d post-challenge (dpc). The evaluation of the immune response was carried out using an OpenArray® platform with 56 gene targets.

Results: The vaccination triggered the differential expression of 41 of the immune-related genes analysed. All the differentially expressed genes (DEGs) showed an early down-regulation in head-kidney and/or brain, except rag1 that was up-regulated in head-kidney at 2 dpv. The il6 gene was up-regulated in the brain at 7 dpv, being the only DEG detected at this time point. After viral challenge, the response in vaccinated fish was characterized by an early up-regulation in head-kidney, with 21 out of 29 DEGs up-regulated at 1 dpc, whereas in non-vaccinated fish 15 out of 21 DEGs were down-regulated at the same time point. In brain samples from vaccinated fish, a total of 8, 36 and 32 DEGs were detected at 1, 3 and 5 dpc, respectively, all of them being up-regulated. However, a delay in the up-regulation response was observed in control fish, with most of the DEGs down-regulated at 1 dpc.

Conclusion: The immune response against NNV infection in gilthead seabream seems to be modulated by the previous response to the inactivated NNV vaccine.

The immunomodulatory activities of the rainbow trout cathelicidin antimicrobial peptides in proliferative kidney disease

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Tetracapsuloides bryosalmonae is a myxozoan parasite (phylum Cnidaria) of salmonids and the etiological agent of proliferative kidney disease (PKD). PKD manifests behaviorally as lethargy, anatomically as swollen kidneys, and as B lymphocyte hyperplasia at the cellular level, cumulating in extensive suffering and mortality in susceptible fish species such as the farmed rainbow trout. Several host immune factors such as cytokines, markers of lymphocyte differentiation, antibodies, and antimicrobial peptides are differentially expressed during the course of the disease. Notably, the cathelicidin antimicrobial peptides were the most upregulated with worsening disease and increasing parasite load. They are highly pleiotropic molecules that also have immunomodulatory activities; B cells both produce and are activated by cathelicidins; the cathelicidins also induce cytokine expression. Thus, we sought to understand the extent of their activity

in rainbow trout. What other genes do they regulate? Which host cells are responsive to the cathelicidins? Are myxozoan parasites susceptible to the cathelicidins?

To begin answering these questions, we produced recombinant rainbow trout cathelicidins (rtCath1 and rtCath2). We will confirm the proper function of the recombinant cathelicidins in in vitro antimicrobial activity assays. Rainbow trout host cells will be enriched via density centrifugation (e.g., red blood cells) and/or sorted (e.g., head kidney B cells). The different subpopulations will be exposed to the recombinant peptide to measure what immune genes are regulated by the cathelicidins. Finally, we will explore their antiparasitic activity on myxozoan parasites.

Overall, we will provide new information on the broad-spectrum activity of the cathelicidins, elucidate their roles in host responses and determine if they offer protection against diseases such as PKD.

Antimicrobial peptide Dicentracin shows prophylactic and therapeutic actions against NNV infections in European sea bass

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Introduction: Nodavirus (NNV) is one of the most prevalent pathogens in the Mediterranean Sea, causing viral encephalopathy and retinopathy disease to many fish species such as European sea bass (Dicentrarchus labrax), causing great mortality rates in larvae and juvenile stages. Antimicrobial peptides (AMPs) are low molecular weight, cationic and amphipathic peptides with dual functions: direct lytic effects against a wide range of pathogens, including viruses, and modulating the immune response of the host. Very scarce studies have focused on the antiviral role of AMPs in fish, but with positive results. Therefore, we aimed to evaluate the immunological actions of the European sea bass AMP Dicentracin (Dic) as well as its potential application as preventive or therapeutic agent against NNV infections.

Methodology: To reach our aim, we designed three experiments using sea bass juveniles: i) administered with Dic-encoding plasmids and then challenged with NNV; ii) administered with synthetic Dic peptide and then challenged; or iii) challenged with NNV and then administered with synthetic Dic peptide. Control samples were always included. In all cases, samples of head-kidney, muscle and serum were sampled in order to evaluate the immunological response as well as the mortality and clinical signs.

Results: Dic peptide administration resulted in a significant increase of the sea bass survival upon NNV infection either as preventive or as therapeutic treatment. Priming of the immune response was higher with the synthetic peptide than with the Dic-plasmid.

Conclusions: Dic peptide exerts more potent immunomodulatory actions when administered as synthetic peptide than by plasmids. Dic peptide showed partial protection against NNV infection in European sea bass, pointing to this peptide as a potential tool to prevent and control NNV outbreaks in fish farms.

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The susceptibility of shi drum to betanodavirus depends on the rearing densities

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Introduction: The diversification of species in aquaculture requires the continuous study of animal welfare parameters and the susceptibility to pathogens. An inadequate welfare state results in stress, poor health and increased severity of infectious diseases. In fact, several stress conditions have been related with increased mortalities and betanodavirus (NNV) spread. The shi drum (Umbrina cirrosa) has attracted attention due to its good growth rates and great adaptability to culture conditions together with a great flesh quality. However, this species is susceptible to the four genotypes of NNV and displays stress behavior at high rearing densities.

Methodology: In order to determine the response of shi drum upon NNV infection at different stressed conditions, specimens reared at low or high density were experimentally infected. A transcriptomic study was performed comparing the gene expression pattern of several tissues of infected and non-infected specimens from the group showing mortalities. The presence of NNV in the gonad of survivors was analyzed.

Results: The mortalities observed were low but only occurred at the group reared at high density. The transcriptomic study showed that the number of tissue-specific genes expressed increased in head-kidney, liver and brain, and

decreased in spleen. In the four tissues, cell adhesion, leukocyte migration, cytokine interaction, cell proliferation, cell survival and autophagy related pathways were differentially expressed. NNV was detected in the gonad of surviving fish at all rearing densities analyzed.

Conclusions: No differences in the viral capacity of colonizing the gonad were observed between low- and high-density conditions. At this last condition, NNV infection produced a deep systemic alteration in shi drum. The main pathways altered were related with immune response, cell cycle and autophagy, accordingly with studies performed in other fish species.

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Cytotoxicity study of head-kidney leukocytes against nodavirus infection and novel perforins in sea bass (Dicentrarchus labrax)

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Introduction: Cell-mediated cytotoxicity (CMC) is among the most important immune responses to fight viral infections though fish CMC has been scarcely studied. European sea bass (Dicentrarchus labrax) has great economic interest in the Mediterranean aquaculture sector that suffers from high losses due to Nervous Necrosis Virus (NNV) because of its high pathogenicity. Therefore, the main objective of this study was to evaluate the CMC response of sea bass head-kidney leukocytes (HKL) throughout a NNV infection. Additionally, new sea bass perforins, as the main cytolytic effectors of the CMC response, were also characterised by examining their expression in different tissues in in vitro and in vivo assays.

Methodology: CMC response was conducted using the DLB-1 cell line (mock-, RGNNV- or SJNNV/RGNNV reassortant-infected) as targets and incubated with HKLs from infected sea bass at different infection times as effectors. Target cell viability was determined measuring the release of lactate dehydrogenase enzyme. The mRNA level of novel perforins was determined by real-time PCR in the in vitro assays of the CMC response or in head-kidney and brain of NNV-infected specimens.

Results: Our data show that the CMC response of NNV-infected sea bass HKLs against mock targets was very low and kept steady along the infection time. Interestingly, the CMC response against NNV-infected targets, either wild or reassortant, increased along the infection time from 7 days post-infection and peaking at 15 days. These data suggest that NNV infection greatly induces the specific CMC response in sea bass. On the other hand, prf1.2, prf1.3, prf1.5 and prf1.9 transcripts were identified. They were up-regulated during the CMC response as well as in the head-kidney and brain of NNV-infected specimens though they show differential patterns of expression.

Conclusions: Our findings suggest an important role of the adaptive CMC response, and perforins, in the immune response of European sea bass against NNV. Further studies should contribute to the understanding of the CMC response of sea bass against NNV infections.

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Transcriptome analysis of immune-related genes after streptococcus iniae infected threadfin (eleutheronema tetradactylum)

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Streptococcus iniae is a gram-positive cocci and identified in many fish worldwide, including cultured threadfin (Eleutheronema tetradactylum) in Taiwan. In this study, S. iniae strain was isolated from threadfin reared on a farm in Taiwan. To investigate the gene expression responses to S. iniae infection, we performed transcriptome analysis of the head kidney and spleen in threadfin using RNA-seq. Total RNA was extracted from the head kidney and spleen of infected (S. iniae -injected) and uninfected (control) threadfin at 1-day post-infection, and RNA-seq was performed using the Illumina HiSeq™ 4000 platform for RNA-seq to demonstrate the host immune mechanism against S. iniae. A total

of 7333 genes based on the KEGG database were revealed after de novo assembly of transcripts and functional annotation. Differentially expressed genes (DEGs) were significantly (2-fold difference comparing S. iniae and PBS groups) enriched in the immune-related pathways. We identified 1981 and 1584 differentially expressed genes in the spleen and head kidney, respectively. Based on Venn diagrams, 769 DEGs were commonly identified in both the spleen and head kidney, and 815 and 1212 DEGs were specific to the head kidney and spleen, respectively. The spleen-specific and common DEGs were detected to be significantly enriched in immune-related pathways such as phagosome, complement and coagulation cascades, hematopoietic cell lineage, antigen processing and presentation, and cytokine-cytokine receptor interaction, based on the KEGG database. These pathways contribute to immune responses against S. iniae infection. Inflammatory cytokines (IL-1β, IL-35, and TNF) and chemokines (CXCL8) were upregulated in the head kidney and spleen. This study provides natural disease control strategies against S. iniae infection in threadfin fish.

Prevalence of monogenean parasites in bogue (boops boops l., 1758.) On fish farms

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Introduction: Fish farming cages attract a lot of wild fish. Wild fish can threaten cultured fish because they can have the same pathogens as cultured fish. Monogenean parasites are host specific, but there is also a report of atypical monogeneans on fish. The most common species of wild fish that can be found near cages is bogue (Boops boops L., 1758) which belongs to the same family of fish as sea bream (Sparus aurata L., 1758). Belonging to the same family represents the risk of possible transmission of parasites Sparicotyle chrisophrii and Microcotyle iseby between these two species of fish.

Methodology: The research was carried out at a fish farm with sea bream and sea bass throughout March 2022. Bogues were collected from cages with sea bream (cohabitation) infested with S. chrisophrii and near the cages (wild). In total 60 wild bogue and 62 cohabitation bogues examined. Also from the same cage, a total of 72 sea bream were examined. Parasites were identified based on morphology and molecular methods.

Results: A total of 66 monogenean parasites were found on gill arches of bogue, of which 64 were on wild and 2 on cohabitation bogue. Monogenean parasites found on bogue belong to species M. isyebi. Results showed a higher index of condition and mass of bogue from fish farming cages than wild ones, while the length did not differ significantly. Monogeneans parasites found on gill arches of sea bream were identified as S. chrisophrii and 108 parasite were found.

Conclusions: Despite numerous researches, the transfer of pathogens between wild and farmed fish is not clarified enough. In this research, no parasite transfer from bogue to sea bream and vice versa was found, confirming the strong host specificity of parasite M. isyebi and S. chrisophrii.

Understanding the role of Chinook salmon PKR in the antiviral response during Viral Haemorrhagic Septicaemia Virus (VHSV) infection

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The interferon-induced antiviral response is mediated through a wide range of Interferon-Stimulated Genes (ISGs). The double-stranded RNA-activated Protein Kinase R (PKR), one of the most studied proteins encoded by an ISG, is recognized as a multifunctional key factor of innate immunity. In mammals, it is involved in central cellular processes in response to stress signals, including: (1) inhibition of protein translation via phosphorylating eukaryotic initiation factor 2α (eIF2α), (2) regulation of apoptosis through activation of the caspase cascade, (3) activation of proinflammatory response via regulation of NF-κB activation. In order to understand the role of fish PKR in response to viral infections, we developed a Chinook salmon (Oncorhynchus tshawytscha) cell line, with the pkr gene knocked out by CRISPR/Cas9 genome-editing. Three distinct isoforms of PKR were also cloned from the wild-type cell line infected with Viral Haemorrhagic Septicaemia Virus (VHSV). Overexpression of the full-length isoform inhibited the expression of co-transfected reporter genes as well as the de novo synthesis of endogenous proteins, although the phosphorylated form of eIF2α was not detected. During infection with Golden Shiner Virus (GSV), eIF2α phosphorylation was mainly mediated by another kinase, namely PKR-like endoplasmic reticulum kinase (PERK), rather than PKR. Furthermore, PERK activation was needed for efficient viral protein synthesis. Taken together, these results suggest that GSV evades the PKR-eIF2α pathway and hijacks the PERK-eIF2α pathway to favour its replication.

Small-scale variability of the small-spotted catshark (Scyliorhinus canicula) parasite community: a case study along the Catalan coast (NW Mediterranean)

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Introduction: The small-spotted catshark (Scyliorhinus canicula) is a bottom-dwelling elasmobranch with an Atlantic and Mediterranean distribution that represents the most discarded catch in terms of biomass in the Catalan coast.

Parasite assemblages are an important tool for host stock discrimination and provide information on trophic interactions and host biology and ecology. The present work aims to characterize the parasite community of S. canicula in relation to condition indices and local variability in the NW Mediterranean Sea.

Methodology: A total of 61 individuals of *S. canicula* were captured by commercial vessels during summer 2019 in three different areas of the Catalan coast (off Girona, Barcelona and Ebro Delta). Necessary measures were taken for condition indices calculations. All external surfaces, internal organs and musculature were inspected under a stereomicroscope for parasites, which were counted, identified to the lowest possible taxonomic level and stored in 70% ethanol. Univariate and multivariate statistical analysis were performed on parasitological data using the software R studio.

Results: The parasite community of *S. canicula* was characterized by low richness and diversity in all areas. A total of 12 parasite taxa were found of which five were considered common. All sharks were infected by at least one parasite and showed a total mean abundance of 51.2 parasites/shark.

Multivariate analyses revealed significant differences among localities for the composition and structure of parasite communities. Bray-Curtis similarity index ranged between 41% and 77% among zones and between 78% and 81% within zones, with the most contributing parasites being the nematode *Proleptus obtusus* and the cestode *Grillotia adenoplusia*.

No significant correlations were found between sharks' condition indices and parasitological descriptors, except for total abundance in Ebro Delta, which was correlated with total length ($p < 0.005$, $rp=0.63$).

Conclusions: Small-scale variability in *S. canicula* parasite communities is reported in this study. Differences may be attributed to different habitat features that determine prey-availability, as the most contributing parasites are trophically-transmitted.

Correlation of total parasite abundance and length in Ebro Delta could be explained by the significantly higher abundance of larval *G. adenoplusia*, a parasite that accumulates in the muscle during the host lifetime.

Development of IHC as support technique for the study of immune response of Senegalese sole against systemic amoebiasis: preliminary results

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Introduction: Systemic amoebiasis caused by Endolimax piscium is an emerging threaten for farmed Senegalese sole, characterized by the presence of whitish nodules in muscle and other organs. Histologically, nodules correspond to granulomatous inflammatory lesions. Intestinal epithelium plays a relevant role in the initial stages, but the mechanisms of E. piscium to breach the intestinal barrier and reach other organs, as well as the immune response against the parasite are not clearly identified. Immunohistochemistry (IHC) techniques are particularly useful for the on-site assessment of immune response; thus, the aim of this study was to test different antibodies to delve into the pathogenic mechanisms of the disease.

Methodology: Senegalese sole with lesions consistent with systemic amebiasis were necropsied and samples of muscle and visceral organs were fixed in formalin and processed by histological techniques and in situ hybridization (ISH) specific to E. piscium. Those samples confirmed as E. piscium-positive were processed for IHC, using different antibodies against: the active caspase 3 protease (CAS3) to detect apoptotic cells; the inducible nitric oxide synthase (iNOS) to detect pro-inflammatory effect; and the protein E-cadherin (Ecad) to label the adhesion of epithelial cells. In some cases, IHC techniques were combined with ISH.

Results: Immunoreactivity against iNOS and CAS3 were clearly present in the lesions and easily recognise with no significant background in tissues. CAS3 immunoreactive cells were particularly present in the core of the granulomas, whereas iNOS expression were detected especially at the periphery of the granulomas with diffuse distribution among the lesions.

iNOS expression was also detected in the intestinal mucosa and kidney associated to the presence of parasites, probably indicating the release of signals for proliferation and recruitment of leukocytes in response to the parasitisation. Anti-Ecad positively labelled cell junctions of the intestinal epithelium. However, the labelling was weaker in areas with high parasite intensity, pointing to loss of continuity in the epithelium.

Conclusions: The results of this study confirm the potential useful of these markers in the study of the pathogenesis of systemic amoebiasis in Senegalese sole, allowing further and more complete studies on the immune response against E. piscium in Senegalese sole.

First report of an Aeromonas salmonicida related mortality in Largemouth Black bass (Micropterus salmoides)

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Introduction: In late spring 2023, a mortality event occurred in a largemouth black bass (Micropterus salmoides) farm located in the North-east of Italy. The increased mortality was recorded in a grow-out earten pond after a size selection procedure. Affected fish showed skin lesions and lethargic behaviour.

Methodology: Four moribund specimens were collected, humanly sacrificed, and delivered on ice to the Fish Pathology Unit of the Istituto Zooprofilattico Sperimentale delle Venezie for anatomopathological investigation and further analyses. Parasite load of skin and gills was evaluated through wet mount technique. Spleen, kidney, encephalon and skin lesion were sampled on Blood Agar plates for bacteriological examination and incubated at 22°C for 24 – 48 hours. Small portions of affected skin, gills, spleen, liver, head kidney and heart were fixed in formalin and processed for histological examination. Antimicrobial resistance testing of isolated colonies was performed applying the disk diffusion method and MIC determination (micromethod) as described in the CLSI VET03 manual.

Results: All the examined specimens (15 – 18 cm SL) presented multiple superficial skin erosions associated with haemorrhages on the flank surface, mild splenomegaly, pale kidney, mild encephalic congestion. Gills and skin mucous were positive for Trichodina sp. infestation. Bacteriological examination revealed massive and pure growth of dark-brown pigmenting colonies in all the sampled tissues. Conventional biochemical tests, MALDI-tof MS and the amplification and Sanger sequencing of the DNA gyrase subunit B (gyrB gene) identified these colonies as Aeromonas salmonicida. Histological examination of skin lesions highlighted: flaking of the epithelium with exposure of the stratum laxum of dermis and scale pockets; clusters of Gram-negative bacteria in the scale pockets; inflammatory infiltrate in scale pockets, stratum compactum of dermis, hypodermis, and superficial musculature. Bacterial clusters were also observed in the histological preparations of head kidney, liver, spleen and myocardium. Isolated A. salmonicida tested sensitive for amoxicillin, enrofloxacin, erythromycin, flumequine and resistant for florfenicol, oxytetracycline and enhanced sulphonamides.

Conclusions: This is the first report of Aeromonas salmonicida systemic infection in Micropterus salmoides. The extent of the infection is confirmed by bacteriological and histological outcomes.
This research was supported by Institutional fundings.

Characterization of a novel potential virulence factor in Vibrio anguillarum

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Introduction: Vibrio anguillarum is a causative agent of vibriosis which causes significant economic losses in aquaculture. V. anguillarum harbours several virulence factors like haemolysins, metalloproteases, lipopolysaccharides and toxins. However, our understanding of its virulence mechanisms remains incomplete. A previous study of a transposon mutagenesis library identified a mutant with reduced virulence in a Galleria mellonella insect infection model, suggesting a possible involvement of the disrupted gene in virulence. The aim of the present study was to characterize phenotypic differences between wild-type (WT) and the transposon mutant to determine the potential role of this novel gene in virulence.

Methodology: Localization of the protein was predicted by PSORTb. Phenotypic differences between WT and mutant strains were assessed by comparing growth in TSA at 22oC for 24 h using measurements of absorbance and colony counts, and the effect of reduced osmolarity on bacterial viability using distilled water, and 0.3%, 0.6% and 0.9% saline solutions. The effect of the membrane-active antimicrobial peptide polymyxin B on growth was monitored for 24 h with 3, 6, and 12 ug/ml concentrations.

Results: The protein encoded by the mutated gene was predicted to be secreted or localised in the outer membrane, periplasm or inner membrane, suggestive of a transmembrane protein. There was no notable difference in growth trend in terms of absorbance, although the viability of mutant cells reduced over time indicating that the mutant cells lose viability during culture. Moreover, WT bacterial cells showed higher survival in hypotonic solutions, perhaps suggesting

the mutant has a compromised outer membrane. The mutant failed to grow in polymyxin B, whereas the WT strain had a slightly reduced growth, again supporting a suggestion that outer membrane integrity was compromised.

Conclusion: The mutated protein may have a role in maintaining membrane integrity and protection against insults like membrane-active peptides and hypo-osmolarity. Future studies will focus on identifying the function and localization of this protein and discovering the triggering conditions for its expression. Characterization of the protein will enhance our understanding of V. anguillarum virulence.

Tenacibaculum maritimum evading strategies against Senegalese sole (Solea senegalensis): an in vitro study

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Introduction: To date, little is known about Tenacibaculum maritimum evading strategies and the data regarding host-pathogen interactions are still not fully elucidated. The present study aimed to contribute to this endeavour and was designed to assess in vitro Senegalese sole immune responses following stimulation with live or UV killed T. maritimum.

Material and methods: Three T. maritimum strains (ACC20.1; ACC13.1; ACC6.1) isolated from Senegalese sole were used during experimental assays. These isolates belong to the serotype O3 described for T. maritimum and were kindly provided by Prof Alicia E. Toranzo (University of Santiago de Compostela, Spain). When required, bacteria were killed by ultraviolet (UV) exposure for 4 h. Loss of bacterial viability was confirmed by plating on marine agar plates and MTT assay. Senegalese sole head-kidney leucocytes (HKL) were isolated and adherent cells were exposed to live or UV killed T. maritimum. Cellular responses including ROS, NO and a killing assay based on the reduction of MTT were assessed. HKL were also collected for gene expression studies following stimulation live or UV killed T. maritimum (i.e. 4 h, 12 h, 24 h, and 48 h)

Results: Preliminary data did not show any significant variation in NO and ROS among different T. maritimum strains for a given stimulus. However, UV killed bacteria increased NO in sole HKL compared to live strains. Moreover, evidence for the induction of necrotic cell death using lactate dehydrogenase as marker was recorded HKL exposed to live strains. Interestingly, interleukin-1β, hepcidin, cyclooxygenase-2 and g-lysozyme mRNA levels decreased at 24h and 48h following stimulation with live strains, while interleukin-10 transcripts were upregulated at 48h post-stimulation. In contrast, the expression levels of all analysed genes increased following stimulation with UV killed strains.

Conclusions: The present study showed that UV killed T. maritimum were more effective in activating sole HKL innate immune responses, and further suggests the existence of T. maritimum evading strategies according to downregulation of genes related to inflammatory responses and iron homeostasis.

RNA-seq as an useful tool to study the impact of nanoplastics ingestion in fish

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Introduction: Nanoplastics (NPs) are one of the main concerns regarding plastic pollution. Several studies have revealed a negative impact by NPs in aquatic organisms such as behaviour, reproduction or locomotor activity. Although several studies have been conducted to examine the toxicity of NPs on marine organisms, the effects of NP ingestion remain largely unclear and need to be further investigated. Thus, the aim of this study consisted on the evaluation of the impact of a diet containing NPs in one of the most cultivated species in the Mediterranean sea, the European seabass (Dicentrarchus labrax).

Methodology: Polystyrene NPs (0.25 g NPs / kg feed) were added to the commercial diet. Fish were fed for 21 days and the transcriptomic changes were measured in the intestine through RNA-seq. Liver was also sampled and enzymatic activities were measured to evaluate the organism stress.

Results: We failed to observe relevant changes in the liver enzymatic activities upon dietary NPs. However, we were able to observed changes in DEGs between treatments. After NPs ingestion, we observed an up-regulation of genes involved in biological processes related to complement activation and the immune response, cellular maintenance and membrane transport as well as morphogenesis. On the other hand, we observed a down-regulation of genes participating on the SMAD and BMP protein signalling, critically important for regulating cell development, replication and DNA repair.

Conclusions: Our results suggest that NPs ingestion affect biological pathways that are extremely important on fish development and growth. This work can serve as a basis for future investigations on the prevention and treatment of such pollutants.

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Nanoplastics might influence biological pathways that are essential for fish survival during NNV infection

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Introduction: Nowadays, nanoplastics (NPs) are one of the main concerns regarding plastic pollution. Several studies have revealed a negative impact by NPs in aquatic organisms such as behaviour, reproduction or locomotor activity. However, the impact of NPs on disease resistance is almost unknown. The objective of this study was to assess whether the ingestion of NPs affects fish susceptibility to viral diseases in vivo. In particular, we focused on the nervous necrosis virus (NNV), which affects many fish species causing great economic losses in marine aquaculture. For this purpose, one of the most cultivated species in the Mediterranean Sea, the European seabass (Dicentrarchus labrax) was used.

Methodology: We fed fish for 21 days with a diet containing NPs (0.25 g NPs / kg feed) and then fish were infected with NNV creating 4 treatments (control, NP, NNV and NP-NNV). Changes at transcriptomic profile were studied in the brain after 3 days post-infection (dpi) through RNA-seq.

Results: A significant increase in the viral replication was observed in fish from the NP-NNV group while similar percentage of mortality was registered in both NNV and NP-NNV groups, ranging the 40%. Enrichment analysis showed interesting results highlighting that NPs strongly affected fish brain altering biological routes involved in the immune response, leucocyte activation and cytokine catabolic production assembly, whereas the main routes impacted by NNV were those associated with the complement activation, regulation of peptidase proteolysis activity and DNA metabolic recombinant process. The analysis of NP-NNV revealed that, in addition to the routes activated by NNV, NPs were able to alter other biological pathways extremely important on the viral infection processes such as antigen binding.

Conclusions: Our results suggest that ingestion of NPs negatively influence biological pathways that are essential for fish survival during infection, which can have a strong impact on fish aquaculture. This work can serve as a basis for future investigations on the prevention and treatment of NPs´ pollution.

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Virological application of two novel brain cell lines from European eel and Senegalese sole

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Introduction: Cell lines are crucial models in animal physiology or immunopathology in terms of reducing the use of animals in scientific studies contributing to the 3Rs principle of reduction, refinement and replacement. Senegalese sole (Solea senegalensis) is a particularly promising flatfish species for Spanish aquaculture while European eel (Anguilla anguilla) counts with populations of traditional farming and fishing in Spain, being both of them of special interest for the national sector. Generation of cell lines from these fish species will be helpful for the research community and reduce the use of laboratory animals.

Methodology: In this study, we have established two new brain cell lines from Senegalese sole and European eel, which are target tissues for various neurotropic viruses. These cell lines have been characterized and exposed to several viruses of relevance to the aquaculture sector. Additionally, we have analysed markers of antiviral response.

Results: Our results showed the differential susceptibility to nervous necrosis virus (NNV), spring viremia carp virus (SVCV), infectious pancreatic necrosis virus (IPNV), viral haemorrhagic septicaemia virus (VHSV), eel virus European X (EVEX) or anguillid herpexvirus (AngHV1) together with their antiviral response activation.

Conclusions: The establishment of these two new cell lines from sole and ell will be valuable tools for the study of immunovirology will allow us to better understand the susceptibility to viruses and the response to them while minimizing the use of laboratory animals.

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How presence of substrate impacts the immune response of the Atlantic blue crab challenged with a pathogenic bacterium

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Introduction: This research focuses on how the presence or absence of substrate at the bottom of the tanks impacts on the immune system of the Atlantic blue crab (Callinectes sapidus) during their culture when they are exposed to a pathogenic bacterium.

Methodology: For this purpose, two experimental groups were established with a total of 20 specimens divided into two compartmentalised tank systems. After 30 days of acclimatisation, the specimens were challenged by aqueous medium with Vibrio alginolyticus (2 x 106 cfu mL-1) and haemolymph samples were collected at 24, 48, 72 and 96 hours after challenge. As initial time, haemolymph samples were collected 48 hours prior to challenge. For each of the times, the percentages of cells present in the haemolymph (hyalinocytes, semigranulocytes and granulocytes), the phenoloxidase enzyme activity and the total amount of proteins in the haemocyte lysate supernatant, as well as the lysozyme activity in the supernatant of the haemolymph were measured.

Results: Regarding cell populations, the percentage of hyalinocytes decreased in crabs maintained with substrate at all post-challenge times while this decrease was only observed after 72 hours in animals without substrate. In the case of semigranulocytes, the number increased in animals maintained with substrate at 24, 48 and 72 hours post-challenge, while granulocytes increased, independently of the group, at 48 and 72 hours post-challenge. Phenoloxidase activity was only detected in specimens from both experimental groups at 48, 72 and 96 hours post challenge. The amount of intracellular protein increased in the challenged specimens at 24, 48 and 72 hours compared to the values observed at baseline (0 hours) and end trial (96 hours) independently of the experimental group. Lysozyme activity did not show variations between the challenged experimental groups, except at the initial time, where the activity decreased in animals maintained with substrate.

Conclusions: Therefore, this work could represent an advance in the knowledge of the immune response of the Atlantic blue crab challenged with a pathogenic bacterium under different conditions.

Selective antibacterial role of Piscidin and Hepcidin peptides from gilthead seabream (Sparus aurata) against distinctive species of Vibrio

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Introduction: Vibriosis is an infectious disease that causes significant economic losses in aquaculture. The most important marine bacterium responsible for this disease is V. harveyi. In contrast, V. renipiscarius is a non-pathogenic bacterium that is part of the natural microbiota identified in the head kidney of gilthead seabreams. Other Vibrio species are more closely related to human diseases, such as V. parahaemolyticus, which poses a risk to human health if contaminated seafood is consumed. This study evaluated the antibacterial activity of seven synthetic peptides encoded by identified genes from the piscidin and hepcidin families against V. harveyi, V. parahaemolyticus, and V. renipiscarius.

Methodology: The peptides were serially diluted in phosphate buffered saline and tested against live bacteria. Bacterial growth was measured by optical density at 600 nm to determine minimum inhibitory concentrations (MICs).

Results: The results showed differences in the inhibitory capacity of antimicrobial peptides depending on the type of bacteria. Specifically, no activity was observed against the non-pathogenic V. renipiscarius, except for piscidin-1 and hepcidin-2C, while MICs of V. harveyi and V. parahaemolyticus were lower than 12.5 µM.

Conclusions: These results with synthetic peptides suggest that natural fish antimicrobial peptides (AMPs) may play a role in the selection of antibacterial activity between pathogens and natural endogenous bacteria, and also suggest potential applications of fish AMPs in the treatment of human diseases.

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Transcriptomic characterization of rtp3 and herc4 genes and their potential role upon nodavirus infection

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Introduction: Nervous necrosis virus (NNV) is the causative agent of the viral encephalopathy and retinopathy, which is considered one of the most threatening viruses affecting marine and freshwater fish worldwide. Characterizing the immune responses triggered by this virus in affected fish species has been a priority in developing effective tools to prevent its dissemination and devastating effects, which can result in up to 100% mortalities in species such as European sea bass. Although most studies have focused on typical antiviral responses, like the type-I interferon pathway, as well as antimicrobial or cytotoxic activities, transcriptomic approaches had previously revealed the presence of two different genes potentially involved in the response to NNV infection, the receptor transporter protein 3 (rtp3) and the E3 ubiquitin-protein ligase (herc4). The aim of this work is to characterize the gene expression of both genes in healthy and NNV-infected fish to elucidate their potential role in response to this virus.

Methodology: We used healthy European sea bass to study the pattern of expression of rpt3 and herc4 coding genes in different tissues and during their ontogenetic development. We also tested their expression in vitro in head-kidney leukocytes (HKLs) subjected to different stimuli. Finally, we infected healthy sea bass with NNV and characterized their gene expression in the brain (target tissue of the virus) and head-kidney in vivo.

Results: Our results revealed clear differences in the pattern of expression of rtp3 and herc4 at both tissue and ontogenetic level. When HKLs were treated, only rtp3 expression was up-regulated upon NNV in vitro infection. In contrast, in vivo, both genes showed peaked expression in the brain after 15 days of infection. Strikingly, NNV triggered the complete blockage of herc4 gene expression in head-kidney and the brain at an early time-point.
Conclusions:
The tight modulation of rtp3 and herc4 after infection suggests their potential involvement in the response to NNV infection. The characterization of both genes opens the door to new approaches to preventive tools in the fight against this virus.

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Reassortant and parental nodavirus strains exhibit differential colonization of the ovary and testis of European sea bass in vitro

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Introduction: Fish gonads, including ovaries and testes, are crucial organs for reproduction. Any damage or impairment to these organs can lead to reduced fecundity or sterility, resulting in significant economic losses in aquaculture. Additionally, due to their immunoprivileged condition, gonads are critical targets for pathogens. Viral infections are a significant concern in fish gonads, with several viral pathogens found to infect them, such as the nervous necrosis virus (NNV). The red grouper genotype of NNV has been found to exert a differential pattern of infection and immune responses depending on the species and sex, likely linked to the susceptibility of the species. However, the recent isolation of reassortant genotypes from natural outbreaks opens the door to novel viral adaptation to the species. The primary objective of this study is to characterize the differential pattern of gonadal colonization and immune responses in males and females of European sea bass with diverse NNV genotypes.

Methodology: In this study, we infected fragments of ovary and testis of healthy European sea bass in vitro with the RGNNV strain and the reassortant strains RGNNV/SJNNV and SJNNV/RGNNV. We used real-time PCR to examine the differences in the presence of the virus and immune-related markers depending on sex.

Results: Our results revealed clear differences in the adsorption of the RGNNV strain in the ovary and testis, while the reassortant strains showed similar adsorption between sexes. Surprisingly, the adsorption of both reassortant strains was lower than that of RGNNV in both ovary and testis. We also described the differential pattern of expression.

Conclusions: The marked differences in the NNV absorption and immune responses between strains and sexes in the European sea bass ovary and testis suggest different viral strategies to use the gonad as a reservoir. These findings may aid in the development of new strategies to curb both vertical and horizontal dissemination of NNV.

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Effects on gene expression of head-kidney leukocytes after incubation with different antimicrobial peptides from gilthead sea bream (Sparus aurata)

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Introduction: Antimicrobial peptides (AMPs), also known as host defense peptides, are crucial components of the innate immune system of eukaryotic organisms. AMPs are involved in diverse biological activities. Recently, our team described novel piscidin and hepcidin genes in the skin mucosa of sea bream. The aim of this study is to evaluate the in vitro effects on gene expression in sea bream leukocytes after incubation with various AMPs.

Methodology: Leukocytes were isolated from head-kidney and incubated in Leibovitz L-15 medium supplemented with 0.35% sodium chloride, 1% penicillin and L-glutamine and 3% Fetal Bovine Serum. The cell concentration was adjusted to 107/mL. Cells were incubated for 2 h at 25ºC with one of the following AMPs at 12.5 µM: Piscidin 1, piscidin 2, hepcidin H1, hepcidin C, hepcidin E, hepcidin H2, and hepcidin I. RNA was isolated using the PureLink™ RNA extraction kit, and cDNA was obtained using the second-strand cDNA synthesis kit and SuperScript IV reverse transcriptase. The expression of apoptosis genes [caspase-3 (casp-3), Bcl-2-associated protein X (bax-1), B-cell lymphoma 2 (bcl-2)], proinflammatory cytokines [interleukin-1b (il-1b), interleukin-6 (il-6), tumor necrosis factor (tnf)] and anti-inflammatory cytokines [interleukin-10 (il-10) and transforming growth factor (tgf)] were analysed by rapid real-time PCR, performed with a QuantStudio™ real-time PCR system.

Results: The expression of apoptosis-related genes and proinflammatory cytokines was significantly decreased in most cells incubated with AMPs. In addition, down-regulation of the anti-inflammatory cytokine gene il-10 occurred in most cells incubated with AMP.

Conclusions: The results of this in vitro study corroborate that leukocytes directly exposed to AMP can die by apoptosis and undergo drastic changes in the expression of their inflammatory cytokine repertoire.

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Impact of grapeseed extracts on immune response and disease resistance of European seabass (Dicentrarchus labrax) juveniles

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Introduction: In aquaculture, feed engineering is one of the main ways to prevent disease outbreaks and improve health status, as well as optimising growth capacity. However, few commercial products with proven capacity to improve immunity are available. The aim of this work is to evaluate the capacity of a grapeseed extract supplemented diet to modulate European seabass (Dicentrarchus labrax) immune system.

Methodology: A two-weeks feeding-trial was performed with European seabass juveniles (22.7±1.8 g). Fish were sorted and three diets were distributed into triplicates tanks (Control–commercial diet; positive control diet - supplemented with an immunostimulant; and a test diet supplemented with a grapeseed extract). Following 2-weeks of feeding, the remaining animals (90 per treatment) were i.p. inoculated with Photobacterium damselae piscicida strain MM415 in a concentration of 2.62×105 CFU mL-1 and randomly redistributed into triplicates for each diet. Mortalities were tracked for 14-days, to evaluate disease resistance. Fish were sampled (3 per tank) before and 6 hours after for the evaluation of the inflammatory response. At both sampling points fish were sampled for blood, plasma, mucus and tissue collection (liver, intestine and head-kidney) for immune parameters' assessment.

Results: Preliminary data did not show significant changes regarding the hematological and plasma humoral parameters of seabass fed dietary treatments for two weeks either before or following infection. While the positive control diet was not able to enhance disease resistance compared to the control, the diet supplemented with grape extract induced higher mortalities (72.2%) compared with control (54.4%). Nonetheless, more parameters are being analyzed to better understand the effects of dietary treatments.

Conclusions: Preliminary data suggest that dietary supplementation with a grapeseed extract does not modulate seabass immune status and seems to deteriorate seabass responses against Photobacterium damselae piscicida, at least under the conditions tested in the present study. More data is being gathered to confirm this hypothesis. In such a case, other supplementation levels, as well as, testing other feed additives will be considered in future studies.

Surname: D

Morphological characterization of preural fusions in Senegalese sole (Solea senegalensis)

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Introduction: Senegalese sole (Solea senegalensis) aquaculture is a fast-growing industry in Europe. However, this sector is affected by skeletal deformities, which entail negative impact on animal welfare and production costs. Preural fusions are one of the most prevalent anomalies in this species, although information is still required on if they can be considered as non-pathological as in other fish species. The purpose of this study was to describe the morphological changes that occur in fused preurals in Senegalese sole postlarvae and to provide new insights on the development of these fusions regarding the incubation temperature.

Methodology: Specimens came from a previous experiment where Senegalese sole eggs were incubated at two water temperatures (18°C and 22°C); fish were reared under similar conditions and sampled at 30 days after hatching (dah). Specimens were fixed and stained with alizarin red S staining. For the present work, 76 fish (29 at 18°C and 45 at 22°C) showing fusions in preurals were selected. The caudal complex vertebrae were evaluated using the binocular stereomicroscope, and fusions were categorized into three groups according to the alterations of vertebral bodies and of the intervertebral spaces (IVS).

Results and discussion: Stage 1 category comprised fusions in which the IVS was diminished, but the involved vertebrae still maintained their independence, sometimes coupled with misalignment of the vertebrae involved. Stage 2 grouped fusions in which there was an evident fusion line and Stage 3 included consolidated fusions in which the vertebrae were seen as a single vertebral body with smooth edges. These categories could be perceived as initial, intermediate and late stages of fusions, respectively. Most of the fusions were included in Stage 2 category (80% of the fish). Within Stage 2 fusions, fish from 22°C group showed more advanced re/modelled fusions towards a consolidated stage, comparing with 18°C group.

Conclusions: Depending on the stage of preural fusion, three categories were established. The majority of fusions presented a fusion line indicating an intermediate stage of the process. Most of Stage 3 fusions could be considered as "stable fusions" with scarce repercussion on external appearance and welfare of the fish.

Preliminary investigation on Bluntnose Sixgill shark helminth fauna from the Strait of Messina (Italy, central Mediterranean Sea)

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Introduction: Bluntnose sixgill shark, Hexanchus griseus (Bonnaterre 1788, Hexanchidae), is a little-known deep-water elasmobranch, occasionally found dead along the Italian coasts. Due to the few findings, also the study of the parasitic fauna of H. griseus has not been well described so far. Being localized at the top of the trophic chain, sharks are considered definitive hosts of numerous parasite taxa, which include high commercial value teleost as intermediate hosts in their life cycle.
Given the lack of information on H. griseus, the aim of our study is to describe the helminth fauna of this large predator.

Methodology: One H. griseus female specimen was retrieved by an Italian Coast Guard Unit off the coast of Messina (Italy) (38°15'52.8"N 15°39'04.3"E) and referred to our laboratory for necropsy and parasitological evaluation.

After necropsy, gills, stomach and spiral valve were macroscopically and microscopically investigated for parasite presence, and mucosa scraping was performed to collect all gastrointestinal contents. The collected material was observed under stereo microscope. All retrieved parasites were stored in 70% ethanol for further analysis. Some specimens were clarified in glycerine for 24 h, selected parasites were stained by Semichon's carmine red technique, mounted and observed by optic microscope. Other specimens were dried according to critical point method and sputtered with palladium gold layer before scanning electron microscopy observation.

Results: H. griseus body weight and total length were 65kg and 237cm. No macroscopic and microscopic parasites were retrieved in the gills. Five Trypanorhyncha plerocercoid larvae were retrieved attached to the pyloric area of the stomach mucosa, morphologically identified by the keys as Nybelinia sp. In the spiral valve, six adult cestode and one adult digenean trematode were retrieved. Morphological characteristics of scolex and the distal part of the strobila

allowed to identify all cestodes as Phyllobothrium sinuosiceps; adult digenean trematodes was morphologically identified as Otodistomum veliporum. No other parasite taxa were retrieved in the celomic organs.

Conclusions: The data presented in our study add new information on the parasitic fauna of H. griseus from the central Mediterranean Sea, above all considering that this species has been included in the IUCN red list.

Effectiveness of a novel pathogen inactivation platform (PITTM) to prepare inactivated whole-cell vaccines for aquaculture

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Introduction: The rapid growth and increasing intensification of aquaculture has led to challenges with infectious diseases. Bacterial disease outbreaks often require antibiotic treatment but this can lead to the development of resistance. Vaccines are an attractive prophylactic, but commercial products are not available everywhere and may lack specific strains needed. Autogenous vaccines, made with strains isolated from a specific site for use at that site, can address these shortcomings. Typically, autogenous vaccines are made using heat and chemicals (e.g., formalin), but this approach may impact vaccine effectiveness by denaturing antigens and small batches are often too expensive to produce. In response, Iraka Biotech has developed an inactivation platform (PIT™) that does not require chemicals and is cost-effective at small scale. The present study aimed to: i) determine the inactivation conditions for the fish pathogen Aeromonas hydrophila; ii) prepare an inactivated whole-cell vaccine to assess its ability to induce a specific immune response in vivo, compared to a vaccine made conventionally.

Methodology: A. hydrophila was cultured (28°C, 150 rpm, 16 h) and resuspended for PIT™ inactivation. Samples were taken regularly and plated to assess bacterial viability. Once inactivation conditions were determined, bacteria were inactivated by PIT™ or formalin and then used to prepare two vaccines for comparison. The vaccines were injected into duplicate tanks of 20 Nile tilapia (Time 0), with booster doses administered at 3 weeks. Serum was collected at 2 and 5 weeks for determination of specific immune response by ELISA.

Results: With PIT™ inactivation, there was a near 9-log reduction in viable bacteria achieved in just 8 hours. Complete inactivation was confirmed by inoculating liquid broth medium and culturing for 5 days. When the vaccines were administered by intraperitoneal injection, no adverse reactions were observed for both vaccines; comparisons by ELISA are ongoing.

Conclusion: PITTM is a novel inactivation platform that negates the need for hazardous chemicals when making autogenous vaccines and the technology can be used cost-effectively at small scale. This platform can support the wider uptake of autogenous vaccines, thus serving to reduce disease outbreaks and the need for antibiotics.

Disease-free European Flat Oyster (Ostrea edulis) in the Belgian part of the North Sea: opportunities for restoration and sustainable aquaculture

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Introduction: The European flat oyster Ostrea edulis is one of the most appreciated mollusks due to its gastronomic, cultural and environmental value, and plays an important role in the marine ecosystem through, e.g., its reef-forming capacity. Despite its historical and ecological importance, flat oyster reefs have completely disappeared from the Belgian part of the North Sea (BPNS). Next to overexploitation and habitat destruction by bottom fisheries, diseases caused by Bonamia and Marteilia parasites contributed to the demise of oyster populations. To evaluate the feasibility of restoring and cultivating flat oysters, within the H2020 UNITED project we started a demonstration project inside a Belgian offshore wind farm. The goal of this study was to determine the status of flat oysters introduced in the BPNS and their offspring regarding bonamiosis and marteiliosis.

Methodology: Flat oysters implemented were initially certified Bonamia and Marteilia free and originated from Norway (adult oysters) or from England (oyster spat). After six months to two years, these oysters, and their offspring, were sampled (N = 356) from March to May and July to October from both nearshore and offshore sites within the BPNS.
Each oyster sample was split in two, one part for histology analysis, and the other part for Real-Time PCR analysis. Only in case oysters tested positive for the detection of 149onami asp. Or Marteilia refringens parasites through qPCR, the histologic slides were further analyzed.

Results & Discussion: In none of the tested flat oyster samples, Bonamia or Marteilia parasites were detected, while the control samples were positive. This is the first time these oyster diseases have been monitored in the BPNS, allowing to demonstrate disease-free status, which is a promising result for oyster restoration and cultivation projects envisaged in the BPNS. Nonetheless, other diseases might be present or emerge in this new oyster population. It is thus crucial to continue monitoring by checking health status via histology and qPCR, especially if mortality occurs.

Oral delivery of Bacillus subtilis spores modulates mucus responses in rainbow trout

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Introduction: Bacillus subtilis are spore-forming microorganisms, recognized as safe and reliable probiotic strains. Their endospores are easily produced at a large scale, can be easily dehydrated and maintain their characteristics after long-term storage, providing great advantages for their application in aquafeeds. Nonetheless, the effect of B. subtilis on fish mucosal immunity is still scarce. In this context, the aim of this work was to further explore the immune effects of the long-term oral administration of B. subtilis spores to rainbow trout (Oncorhynchus mykiss), studying their effects on a range of immune and enzymatic parameters of the intestinal and skin mucus.

Methodology: Rainbow trout were fed either a control diet (Skretting) or the same diet supplemented with lyophilized B. subtilis spores (1x10¹º spores/kg) for one month at a rate of 1% body weight per day. After 30 days, fish were sacrificed and skin and intestinal mucus collected, as well as peripheral blood for serum obtention. Different enzymatic activities related to immune function and oxidative status were analyzed in mucus samples, as well as nitric oxide (NO) content, total immunoglobulin (Ig) content and bactericidal activity. Additionally, total IgM levels were quantified in mucus and serum.

Results: Fish supplemented with B. subtilis spores showed increased levels of total Ig in both intestinal and skin mucus, as well as IgM levels in both mucus and serum. Regarding the enzymatic activities analyzed, the B. subtilis spores induced a significant increase of alkaline phosphatase and peroxidase activities in mucus from both sources. Moreover, the protease activity of skin mucus was significantly higher in fish fed the probiotic spores. Although NO production was not affected by the spores, the bactericidal activity of the intestinal mucus was significantly higher in fish fed B. subtilis spores.

Conclusions: The results obtained demonstrate that the dietary supplementation with B. subtilis spores enhances mucosal defense mechanisms by increasing immunological parameters of the skin and intestinal mucus, thus providing novel evidence of the suitability of supplementing aquacultured fish with B. subtilis to increase their immunological status.

L-methionine modulates the functionality of IgM+ B cells in rainbow trout

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Introduction: The interest in dietary amino acids (AAs) as potential immunomodulators has been growing the recent years, since specific AAs are known to regulate key metabolic pathways of the immune response or increase the synthesis of some immune-related proteins. Methionine is among the ten essential AAs for fish, meaning that it cannot be produced endogenously and must be provided through the diet. Although dietary methionine supplementation in fish has been shown to have positive effects on innate immune parameters and disease resistance, to date, the effects that this AA provokes on cells of the adaptive immune system remain unexplored in teleosts. Hence, in the current work, we have investigated the effects L-methionine on rainbow trout (Oncorhynchus mykiss) splenic IgM+ B cells through in vitro studies.

Methodology: Splenic leukocytes were isolated from untreated adult rainbow trout and incubated in complete culture media additionally supplemented cells with different doses of L-methionine (0, 0.5, 1 and 1.5 mM) in the presence or absence of the model antigen, TNP-LPS (2,4,6-Trinitrophenyl hapten conjugated to lipopolysaccharide, 5 µg/ml). After 72 h of the incubation, the survival and proliferation of IgM+ B cells and their phagocytic capacity were determined through flow cytometry. The effect of methionine on the capacity of B cells to secrete IgM was also established through ELISpot.

Results: Supplementation with L-methionine significantly increased the proliferative effects provoked by TNP-LPS. Consequently, the percentage of IgM+ B cells was also increased in these splenocyte cultures. Additionally, L-methionine supplementation increased the number of cells secreting IgM in leukocyte cultures both in unstimulated and in TNP-LPS-stimulated cultures. In contrast, L-methionine did not have an effect on the phagocytic capacity of splenic IgM+ B cells.

Conclusions: The results demonstrate that L-methionine regulates some immune functions of teleost IgM+ B cells, providing novel evidence that supports the beneficial effects of dietary supplementation with this AA as a way to increase the immunological status of fish.

Molecular characterisation of Ricin-B lectin Effector Proteins in Saprolegnia parasitica

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Introduction: Saprolegnia parasitica is one of the most opportunistic fish pathogens that leads to significant losses in aquaculture farms as well as wild ecosystems. This oomycete causes saprolegniosis, a severe infection that leads to lesions in the skin and gills of the fish.

An investigation in the pathogenesis of Saprolegnia parasitica at the molecular level is essential to prevent and find a solution for this ruthless disease. Bioinformatics studies into the genomic profile of Saprolegnia parasitica has revealed that it possesses an armoury of putative effector proteins that all share a ricin-B lectin domain. The objective of the current study is to investigate whether Ricin-B proteins can translocate into host cells.

Methods: Production of recombinant Ricin-B-red fluorescent fusion proteins was optimised, and the recombinant proteins were used in translocation studies employing flow cytometry and confocal microscopy.

Results: The Ricin-B effector fusion constructs were able to translocate into gonad and gill cells of rainbow trout, in the absence of S. parasitica itself. Furthermore, we found that the ricin-B domain is able to bind to galactose residues that are located on the cell membrane of fish cells.

Conclusions: We have demonstrated that Ricin-B effector proteins from S. parasitica can translocate into fish cells. In our future studies, we aim to functionally characterise some of the Ricin-B effectors and determine their role during the infection process.

Perch rhabdovirus in pikeperch (Sander lucioperca) and European perch (Perca fluviatilis) in Swiss aquaculture facilities

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Introduction: Perch rhabdovirus (PRV) belongs to the Perhabdovirus genus. The perhabdovirus genome is made of a linear, negative-sense, single-stranded RNA, which codes for 5 proteins. Differences in the nucleoprotein (N) and phosphoprotein (P) genes are generally used to differentiate genetically close viruses.

PRV can lead to high mortality in European perch (Perca fluviatilis) and pikeperch (Sander lucioperca). Larvae and juveniles are mostly affected. Typical clinical signs are spiral swimming and, later in the disease course, cachexia due to emaciation. In 2013, the virus was detected for the first time in a Swiss aquaculture facility. Driven by intensive fish trade, different variants of PRV are currently circulating in European percid aquaculture.

Methodology: Twenty-two PRV cases were diagnosed in Switzerland since 2018: 13 cases in pikeperch and seven in European perch from aquaculture facilities and two in wild fish from two different river catchments (both in European perch). In these cases, a conventional RT-PCR targeting a 484bp sequence on the N gene followed by sequencing was performed. Finally, a phylogenetic analysis using the Maximum-Likelihood method with 1000 Bootstrap replicates was conducted.

Results: Three main clusters were observed. The largest cluster consisted of 13 isolates from pikeperch. The second one involved seven isolates: six were diagnosed by cultured European perch and one by pikeperch (however these pikeperch were kept in a facility rearing both species and both tested positive with the same isolate). The last cluster consisted of the two isolates detected by wild European perch and was more closely related to the first cluster.

Conclusions: The results confirms that the spread of the virus is strongly supported by the animal trade, which has led to the appearance of two main clusters of isolates, those diagnosed in pikeperch and those in European perch. Control mechanisms to prevent further spread of the disease should be implemented. Data on PRV in wild fish are yet too limited to draw any conclusions.

Vertical transmission of ISAV in Atlantic salmon (Salmo salar) – no strong evidences

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The routes of transmission of the infectious salmon anaemia virus (ISAV) are important to identify when evaluating the risk it represents for Atlantic salmon populations, wild or farmed. Horizontal transmission of ISAV, i.e. between one fish to another through direct contact, contaminated water or equipment, is an important route, but the role of vertical transmission, i.e. from one parent to its progeny through sexual products, is not as clear. Literature do provide as much studies offering evidences of vertical transmission of ISAV than studies offering evidences there is none. Data presented here were obtained by screening matching kidney, ovarian fluids and eggs (fertilized/disinfected) of 26 females broodstock from a population infected by a potentially virulent variant of ISAV (i.e. showing a deletion in the highly polymorphic region of segment 6). ISAV was detected in ovarian fluid of females that showed the highest viral loads of ISAV by RT-qPCR in kidney samples but was not detected in disinfected eggs. Altogether, this data supports that, providing adequate disinfection procedures, vertical transmission in farmed Atlantic salmon is unlikely. However, the existence of vertical transmission in a natural context, when eggs exposed to infected ovarian fluids would not be disinfected, needs further study. Without this crucial information, including testing of progeny, vertical transmission of ISAV is still debatable.

Droplet digital PCR assay for detection and quantification of Philasterides dicentrarchi

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Introduction: Scuticociliatosis is a serious parasitic disease in marine fish, mainly caused by the ciliates Philasterides dicentrarchi in Europe, without effective control measures. Fast and accurate diagnosis and quantification of pathogens in field samples are essential to predict their level of risk, and to stablish appropriate control measures. Real time polymerase chain reaction (qPCR) is the method most frequently used in diagnostic laboratories because it is fast, highly sensitive, reproducible and allow quantification, although qPCR provides relative quantifications.

Droplet digital PCR (ddPCR) is a novel, sensitive, accurate methodology that combines microfluidics technology with TaqMan-based PCR. It is based on the creation of thousands of partitions (droplets), each one with a template, in the reaction mixture. This provides absolute quantification performed directly on the sample, without external calibration or 152ormalization to reference genes. It provides a much lower limit of detection and quantification, and moreover it is less sensitive to inhibition and suboptimal PCR efficiency due to the end-point detection approach.

Methodology: In this study we have developed, optimized, and validated a ddPCR procedure for detection and quantification of Philasterides dicentrarchi using the QX200™ droplet digital PCR system. In addition, a comparative analysis with qPCR has been carried out. P. dicentrarchi extracted genome and plasmid containing the ITS-2 PCR fragment of 78 pb were used as reference sample. The level of detection (LOD) and the level of quantification (LOQ), the dynamic range, specificity and repeatability and reproducibility (R&R) were evaluated.

Results: The LOD was determined as low as 3 copies/reaction with plasmid and genomic DNA. The LOQ was in all cases 1 $\log_{10}$ higher, with a dynamic range of at least 5 Log_{10}, and the procedure demonstrated high R&R. Comparison of the results with TaqMan real-time PCR (qPCR) using the same primers and probes showed an improvement of LOD of at least 2 Log_{10} with respect to qPCR.

Conclusions: The ddPCR developed here is a highly reliable diagnostic method which might substitute qPCR in diagnostic laboratories.

Histopathological changes in case of mass mortality of juvenile farmed Atlantic sturgeon Acipenser oxyrinchus

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Introduction: A case of mass mortality was observed in april 2022 in juvenile (0+) farmed Atlantic sturgeons Acipenser oxyrinchus farmed in recirculating aquaculture system (RAS) in Poland.

Methodology: Clinical examination, gross necropsy, standard bacteriology, were performed. Feed was tested for occurrence of micotoxins. Samples (liver, spleen, kidney, heart, intestine with pyloric caeca) from n=9 sturgeons were fixed in Davidson's fixative for 48 hours for standard histopathology.

Results: The most expressed histopathological changes in all examined fish were observed in heart, spleen and kidney. Main changes observed in heart were: moderate to severe papillary hypertrophy of subepicardial lympho-hemopoietic tissue, pericarditis, myocarditis. Main changes observed in spleen and kidney were xenomas without a thick wall with multifocal necrosis. In liver perivascular hepatitis was observed. Minimal unrelated changes were observed in intestine and pyloric ceaca.

Conclusions: Xenomas, also known as a 'xenoparasitic complexes' are mostly caused by microsporidia. Transmission occurs through contact via the release of infectious spores, predominantly per os. Their migration is possible via infecting red blood cells, T cells, lymphocytes, monocytes, and other migratory cells. That is why we observed more chronic changes in kidney, and less chronic changes in spleen. Although normal subepicardium in the young sturgeons is characterized by the presence of nodular structures that contain lympho-hemopoietic (thymus-like) tissue and a large number of lymphocytes, we observed moderate to severe haemangioma-like hypertrophy of this tissue, macroscopically visible as nodules with cauliflower appearance. This is likely implicated by the great participance of subepicardium in the immune responses.

Exploring variability in the myxozoan mitogenome gene order: Evidence for rapid evolution within Myxozoa

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Mitochondrial (mt) gene order is typically conserved among metazoans, including anthozoan and medusozoan cnidarians. However, myxozoans, which are highly reduced cnidarian parasites, display unique characteristics in their mitogenomes. To date, only five myxozoan species have had their mitogenomes described, with little information on mt gene order. In this study, we present four new myxozoan mitogenomes and report significant variation in gene order, even among closely related species.

We isolated mitochondrial fractions from the parasites and extracted mtDNA, which we then sequenced using Oxford Nanopore technology to generate long reads. These reads were assembled into mtDNA contigs using the Flye assembler. Overlapping PCR products were obtained with specific primers to cover the entire mtDNA molecule, which were then sequenced using Barcode-Tagged Sequencing on an Illumina sequencer. The resulting sequences were mapped to the mtDNA to correct inaccuracies that may have resulted from the nanopore sequencing. Mitogenomes were annotated using MITOS and HHpred.

We constructed mtDNA molecules for Zschokkella nova, Myxidium lieberkuehni, Nephrocystidium pickii, and Zschokkella sp. Due to the high rate of evolution, we identified only a limited number of mitochondrial protein-coding genes (cox1, cox2, nad1, nad3, nad4, and cob), as seen in other myxozoan mitogenomes such as Myxobolus squamalis, which has only two annotated genes. The order of mitochondrial genes in the newly obtained myxozoan mtDNA was different from known mitogenomes, even among closely related species. Furthermore, we found that Zschokkella sp. contains a fragmented cox1 gene consisting of three segments detected on three different contigs, a type of gene fragmentation not previously documented in Myxozoa. Absence of tRNAs and exceptionally fast evolutionary rates were common features of all sequenced mitochondrial genomes of Myxozoa.

The myxozoan mitochondrial genome exhibits significant heterogeneity and is largely unrecognized. We hypothesize that each phylogenetic lineage of Myxozoa has undergone significant changes in mitogenomes throughout its evolution, resulting in variations in mitogenome size, the number of mt molecules, and, as demonstrated here, gene order, even among closely related species.

Internal positive control in duplex assays in qPCR/RT-qPCR diagnostic– what's not to like?

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Introduction: Molecular testing e.g., qPCR and RT-qPCR, is now mainstream in laboratories for the detection of fish, shellfish and mollusc pathogens. Various controls are in place to ensure quality at all steps of the processes, but these controls may not detect issues with individual samples. With many steps in the extraction and qPCR process, involving several liquid transfers in the µL range, undetected errors affecting the sensitivity of detection can be an issue. The use of an internal positive control (IPC), detected concomitantly with the targeted pathogen, can help eliminate this issue.
Methodology: We have selected two IPCs for diagnostic use, the bacteriophage T4 (DNA), and the MS2 virus (RNA). They were grown in Escherichia coli, and stocks were diluted to working concentrations. Duplex qPCR assays were developed for the tandem detection of WOAH listed shrimp pathogens and their respective DNA or RNA IPC. Assays for WSSV, IHHNV, NHP, AHPND, DIV1, YHV, IMNV, MrNV, and TSV were validated for their analytical sensitivity and specificity.

Results and conclusions: The limit of detection (LOD) of each pathogen is comparable to simplex assays. As the IPC is added at the extraction step, and monitored through qPCR and various controls, process issues that would be missed with simplex assays can be detected. We will present the most common and uncommon issues that can be detected with IPC controls. The use of IPCs brings a level of confidence in the testing, which is especially beneficial for surveillance and prevention of accidental pathogen importation, when false negative results can have detrimental consequences.

Exploring plant-based lipids in shrimp feeds: A study into the effects of Aster tripolium inclusion on Penaeus vannamei health

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Some plant-derived oils have been studied for inclusion in shrimp diets both as an alternative to reduce the dependence on fish oil and as a source of health promoting compounds. The aim is to promote sustainability in the aquaculture industry while providing essential nutrients and bioactive compounds for shrimp to grow and thrive. Aster tripolium is a halophyte containing several bioactive compounds that have potential to benefit shrimp health. The primary objective of this study was to evaluate the effect of A. tripolium lipid extract on the immune system and intestinal microbiota of whiteleg shrimp (Penaeus vannamei).

The trial in 40L tanks, with 22 shrimp per tank and 6 tanks per treatment. Experimental diets were prepared including 0.1% and 1% of A. tripolium lipid extracts in soybean oil to the control diet. After one month of feeding trial, three shrimps per tank were sampled, and the remaining animals were subjected to a bacterial challenge with Vibrio parahaemolyticus. At 24 and 48 hours after challenge haemolymph was collected for immune parameters assessment, and gut was collected for microbiome evaluation and proteomic expression.

Regarding growth parameters, no differences were founded among treatments. Protein content and innate immune parameters in haemolymph showed no significant differences between shrimps fed with A. tripolium lipid extracts and those fed a control diet. These findings suggest that the inclusion of A. tripolium does not have harmful effects on shrimp immune status. Proteomics data revealed a higher abundance of 195 and 29 proteins in the 0.1% and 1% A. tripolium supplemented diet, respectively, when compared to control (AVG Log2 Ratio>2, $p<0.05$). Still, other 79 and 114 proteins were less abundant in the these diets, compared to the control (AVG Log2 Ratio<-2, $p<0.05$). Additional data on proteomics and also microbiome analysis are currently being analyzed and may provide further information to complement this study.

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Adaptation of antibiotic disc diffusion method for halophilic Vibrio

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To determine antimicrobial susceptibility, there are various guidelines but the most internationally used are those of the EUCAST and CLSI. These standards give interpretation criteria mainly for bacteria's affecting humans but little information is given for environmental species including marine bacteria. Recently, the EUCAST published some guidelines specifically for Vibrio but mainly for species affecting humans. The recommended protocol to carry out the antimicrobial susceptibility is identical to those for other bacteria and does not take into account the strictly halophilic Vibrio which cannot grow on Mueller-Hinton media. In the context of a research project called REGIS on antimicrobial susceptibility on oyster industry, a protocol for determination of antibiotic susceptibility of Vibrio has been developed based on the EUCAST recommended protocol. The influence of different factors, such as salt in the media, temperature and time of incubation were tested on 30 different isolates of Vibrio belonging to 9 species which were not strictly halophilic. The isolates were cultivated on Mueller-Hinton media with or without 1.5% salt, at 22°C and 35°C and the plate reading was done at 24 and 48H. No difference on antimicrobial susceptibility results was found for the different strains between Mueller-Hinton media with 1.5% salt and Mueller-Hinton media without salt. The plate reading was easier at 24H than at 48H and at the temperature of 35°C. However, some Vibrio strains did not grow at 35°C. To confirm the absence of salt influence on the inhibition diameter, a repeatability test was performed: an antibiogram was made 20 times for a same strain on Mueller-Hinton with or without 1.5%. No difference was noted. Thus, for our study, the antimicrobial susceptibility of Vibrio was tested on Mueller-Hinton media with 1.5% salt at 35°C with a plate reading at 24H.

Dynamics of Vibrio aestuarianus in cockles in wild beds of Hauts-de-France

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Since 2012, significant mortalities of cockles, Cerastoderma edule, have been reported in several French wild beds, including wild beds of Hauts-de-France. During these abnormal mortalities, bacteria belonging to the Vibrio aestuarianus species were isolated from dying animals. These bacteria isolated in cockles were shown to belong to a new subspecies: Vibrio aestuarianus subsp. cardii. In order to better understand the transmission dynamic of this subspecies and its role in cockle mortality, a monthly monitoring of two age class of two different wild beds (one in bay de Somme and another in bay d'Authie) was carried out between 2019 and 2021. Results showed that V. aestuarianus cardii was present in these two beds and affected the different cohorts of cockles (spat/juvenile and adult). This bacterium seemed to develop preferentially during spring and summer but remained present in cockles in winter at low titter and prevalence. V. aestuarianus cardii was also found in the sediment and mussels in the vicinity of the cockles only in spring and summer i.e. during cockles infection pick. Phylogenetic relationships among the different isolates (sediment, cockles and mussels) suggested that, during this period, cockles excreted enough bacterial particles in water to allow their accumulation in sediment and/or other host species. A comparative genomic study showed that several V. aestuarianus cardii genotypes were circulating in the studied area with contrasted virulence profiles. Strains virulent to cockles in experimental pathology were found throughout the year regardless of location and isolation matrix. These strains coexisted in cockle populations with non-virulent strains. Further studies will be necessary to better identify characteristics and specificities of virulent isolates and characterize mechanisms involved in their development in cockles at a given period.

Preliminary study on potential impact of antibiotic use in oyster hatchery*

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In shellfish industry, the use of antibiotic is generally limited to inland facilities such as hatcheries. Antibiotics can be used at all stages mainly during the broodstock conditioning and the larval stage. Our study focused on the use of two antibiotics during the larval phase in Magallana (ex Crassostrea) gigas using unselected and selected oysters, either resistant or susceptible to both OsHV-1 and Vibrio aestuarianus. The objectives were to check if antibiotic resistances appeared during the life history of the oysters, and if germplasm of the oysters could influence the development of antibiotic resistance. Thus, larvae of the three batches were raised with or without antibiotics in an experimental hatchery, and oysters were sampled at the larval, spat and juvenile stages in order (i) to detect specifically Escherichia coli, resistant to third generation cephalosporins, and (ii) to characterize the antibiotic resistance profile of some Vibrio spp isolated from oysters. Resistance profiles were established using the disc diffusion method for Vibrio or using a specific medium for E. coli. E. coli resistant to third generation cephalosporins were not detected in any samples.

Moreover, no resistance to the antibiotics used during the larval stage was noticed. Meanwhile, one of the antibiotics presented a toxic effect on oyster larvae development. Several Vibrio species were isolated and most of them showed resistance to Beta-lactams and some to quinolones such as Vibrio alginolyticus. Resistance profiles of Vibrio were similar among the oyster batches; it was mainly certain bacterial species that showed resistance while others had a wild-type phenotype. Our results need to be confirmed, and it would be interesting to evaluate the impact of antibiotics when used on older stages in particular on the broodstocks.

Effect of water temperature on head kidney macrophages capacity to reduce bacteria

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Introduction: As fish are ectothermic organisms, variations in water temperature have an impact on their physiology, starting at biochemical and metabolic levels, and therefore, affecting also the immune response. The only escape from high temperatures fish have is to seek for cooler waters, but that is quite difficult in aquaculture tanks. However, as far as the temperature range for the species is not exceeded increases do not seem to be alarming.

Methodology: We have studied the in vitro response of macrophages from cultured rainbow trout to a bacterial agent at different natural temperature conditions. Macrophage response was evaluated by the bacteria phagocytosed at 4h post infection (hpi), bacterial survival rate 24hpi, and by the respiratory burst (NBT reduction test). Fish were sampled at slaughter (230-310g), therefore, after several months in the facilities, hence with time to be acclimatized. For that reason, temperature was considered as the mean of daily values recorded by the fish farm in the 7day period ending on the sampling day.

Results: Respiratory burst (RB) was first evaluated between early and late Spring (February to June). A significant drop was observed when temperatures exceeded 15°C, and RB values remained similar once after that temperature was surpassed. RB was also affected when the temperature increase between the sampling week and the week before was 10°C.

According to those results, phagocytosis and bacterial survival were evaluated at temperatures below and over 15°C. As it could be expected from the above results, macrophage capacity to face a bacterial agent was significantly decreased at temperatures over 15°C. The rate of bacteria phagocytosed was lower, while bacterial survival was significantly higher when temperature surpassed that temperature.

Conclusions: Macrophage capacity to face an infection seems to be impaired, even at temperatures well below the higher values of accepted temperature range for the species. That would mean that pathogens would find better conditions that could facilitate their spread and would increase probabilities to cause disease.

Identifying natural bioactive peptides from the common octopus (octopus vulgaris) skin mucus by-products using proteomic analysis

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The common octopus (Octopus vulgaris) is one of the world's economically important species, subject of active fisheries and highly appreciated as food. In addition, they have a great potential as aquaculture species, and serve as model species for biomedical and behavioral studies.

Bioactive compounds from marine organisms' by-products can exert health beneficial properties and are considered as lead compounds for the development of pharmacological, functional foods or nutraceuticals. Particularly, the common octopus skin mucus has been described as anti-microbial and anti-oxidant properties, among others.

We applied the emerging science of Proteomics-based Systems Biology to generate a common octopus reference proteome for the screening of potential bioactive peptides from fishing discards and by-products such as skin mucus. Octopus were captured in NW Atlantic waters (Galicia, Spain), by local fishermen and transported in ice to the laboratory where they were processed for mucus protein extraction/purification and freeze-dried. A shotgun proteomics approach combined with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using an Orbitrap Elite instrument was then used to create a reference dataset from octopus skin mucus. Protein-based bioinformatics analyses were then carried out to predict and characterize potential bioactive peptides.

The first proteomics analysis for Octopus vulgaris mucus skin proteome is presented in this work. This library was created merging a total of 5937 identified spectra (PSMs) from 2038 different peptides. Finally, a total of 510 non-redundant annotated proteins were identified. The results obtained show the potential of bioactive compounds from octopus body parts that are usually discarded and therefore highlight their application in biomedicine and in the pharmaceutical and nutraceutical industry.

Improving farmers' ability to predict anoxia and harmful algal blooms in aquaculture using in-situ and earth observation data

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Introduction: Anoxia and harmful algal blooms (HABs) can affect aquaculture animals, causing potentially massive losses. Anoxic events or periods of low dissolved oxygen (DO) can be caused by several factors including high water temperatures, no/low currents and algal blooms (Pankhurst and King, 2010, Kvamme et al, 2008). Oxygen depletion poses several welfare risks, resulting in reduced appetite, welfare and in some cases increased mortality. Good water quality and DO levels are particularly important during periods of disease outbreaks. (Kvamme et al, 2008). BiOceanOr was part of the Sansithau project, which deployed sensors in the Thau lagoon in France, which is known to be prone to anoxic events, algal blooms and bacterial contamination. The aim was to develop algorithms to predict DO and HABs using real-time, site-specific measurements and satellite imagery to take preventive measures to reduce their impact on aquaculture.

Methods: In situ sensors, local sampling and satellites measured several parameters. The data collected were analysed before, during and after an event of interest, such as anoxia or HAB. Key parameters and their variation over time were identified. Machine learning analysis was then used to develop predictive algorithms.

Results: Using only in situ measurements, BiOceanOr was able to develop an algorithm that predicted DO 48 hours in advance with only 4% error. HAB prediction algorithms using in situ measurements and satellite imagery are under development and is expected in 2024.

Conclusion: The development of the Internet of Things is facilitating the collection of large amounts of data in real-time and at high frequency, allowing us to build larger and more robust data sets. There are several risks to different industries that rely on water quality, and the ability to predict some of these events, such as anoxia and HABs, can benefit aquaculture, allowing farmers to protect their farms and livestock. By being able to predict DO 48 hours in advance, a farmer can know in advance of changes that are about to occur and therefore take preventative action to reduce the impact of high oxygen depletion.

Salmon gill poxvirus can infect salmon yolk sac larvae and cause severe infection of the skin

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A specific gill disease causing acute high mortalities with respiratory distress, characterized by severe gill pathology has been described in salmon (Salmo salar) aquaculture since 1995. The virus causing this disease is now known as salmon gill poxvirus (SGPV) and infection of salmon with SGPV is described in hatcheries, in salmon after sea transfer and in brood fish. Previously successful challenge with SGPV was performed on smolts. Now we wanted to study infection of SGPV in Atlantic salmon yolk sac larvae, as this has not been described. This study is the first report of SGPV infection in Atlantic salmon yolk sac larvae which demonstrates infection not only in gill epithelial cells, but also in epithelial cells of the skin.

Changes in haematopoietic organs in Atlantic salmon suffering from salmon gill poxvirus disease

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The salmon gill poxvirus (SGPV) has emerged in the last couple of decades and in some cases lead to acute respiratory disease and high mortality in Atlantic salmon presmolts. In salmon suffering from SGPV disease (SGPVD), characteristic histopathological changes are seen in the gills and in clinical outbreaks of SGPVD, a large part of the gill respiratory surface is impacted. In some salmon suffering from SGPVD, pathological changes is also seen in the spleen and kidney, in the form of accumulation of apparently intracellular, eosinophilic amorphous material interpreted as erythrophagocytosis, i.e., phagocytic destruction of red blood cells. The pathogenesis leading to the severe blood cell break down is unclear. In this study, we investigated fish from field outbreak of SGPVD and focused on the changes seen in the spleen and kidney. Samples representative for the early mortality phase, the peak of mortality phase, and the late phase of the disease. 158ddition to extensive changes in the gills, moderate to extensive erytrophagocytosis was seen in the spleen and kidney in the fish during the mortality phases. RNA-Seq analysis revealed an increased differential expression of genes relative to control during an early mortality and peak mortality phases, while just a few genes were differentially expressed during the late regenerating phase. Common differentially expressed genes

between the two organs showed a classical interferon response, chemotaxis activity and signatures of coagulation and vasoconstriction, especially during the early mortality phase.

Histopathology of Myxobolus lentisuturalis (Myxosporea: Myxobolidae) in farmed and wild gibel carp (Carassius auratus gibelio) from Croatia

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Myxobolus lentisuturalis is a highly pathogenic myxosporean parasite of gibel carp (Carassius auratus gibelio) and goldfish (Carassius auratus). This typical intramuscular species was originally described from wild gibel carp in China, but subsequently reported from both wild and farmed goldfish in Italy and China. The present study reports the occurrence and histopathology of M. lentisuturalis in farmed and wild gibel carp from Croatia.

While investigating the parasite fauna of gibel carp in Croatia, a few fish were observed to have exophytic multinodular lesions on the back, anterior to the dorsal fin. In extreme cases, these lesions progressed to ulceration. Microscopically, the lesions were completely filled with mature spores of Myxobolus sp. On the basis of the morphological and molecular data, the species was identified as M. lentisuturalis.

Histologically, polysporous plasmodia of M. lentisuturalis were found intracellularly in the skeletal musculature. In more advanced cases, masses of spores were released from disintegrated muscle fibers and histopathological changes were characterized by extensive tissue destruction with liquefactive necrosis of muscle fibers and little inflammatory infiltrate composed of macrophages with lymphocytes and plasma cells, and occasional neutrophils. In areas of extensive myonecrosis, mild hemorrhages were noted at the border of the lesion. Occasionally, individual spores and mild to moderate inflammatory infiltrate were also observed in the dermis and epidermis.

In conclusion, necrotizing myositis caused by M. lentisuturalis is important pathological factor per se.

De novo nucleotide biosynthesis pathways in myxozoan (Cnidaria)

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Myxozoans is a diverse group of microscopic cnidarian parasites that are well known for the disease they cause in wild and aquaculture fish stocks. Myxozoan evolution is characterized by the loss of key pathways that are present in cnidarians and other animals. The purine and pyrimidine nucleotide biosynthesis pathways are essential for cell metabolism and proliferation and are highly conserved in most metazoans. It was previously suggested that the myxozoan Thelohanellus kitauei (Myxobolidea) has lost both pathways for the de novo synthesis of nucleotides. The enzymes involved in the pyrimidine synthesis pathway are denoted by three genes (Carbamoylphosphate synthetase (CAD), Dihydroorotate dehydrogenase, and Uridine 5'-monophosphate synthase) while the purine biosynthesis pathway includes six genes (Adenylosuccinate lyase, Amidophosphoribosyl transferase, Phosphoribosylaminoimidazole carboxylase, Phosphoribosylformaylglysinamide synthetase, Bifunctional purine biosynthesis ATIC, and Trifunctional purine biosynthetic protein adenosine-3). In this work, we mined available genomic and transcriptomic databases from a representative of the myxozoans and cnidarian diversity for these genes. In agreement with the observations on Thelohanellus, our study revealed the absence of all genes involved in the purine pathways in all myxozoans investigated, except for the Adenylosuccinate lyase, a bifunctional protein also involved in adenine biosynthesis. On the opposite, we identified all the genes involved in the pyrimidine pathway in all myxozoans for which their genome is available. The only exception was the absence of CAD in most members of the Myxobolidea. Orthology relationships were confirmed by reconstructing maximum-likelihood-based gene-specific phylogenetic trees. Our results suggest that analyses based on a single genome may lead to erroneous conclusions and demonstrates the strength of comparative phylogenomic studies, which combine data from multiple species. In the future, we propose extending the suggested approach to characterize additional pathways in these important fish parasites.

An outbreak of Aeromonas salmonicida subsp. salmonicida in largemouth bass (Micropterus salmoides) farmed in Italy

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Introduction: Aeromonas salmonicida is a bacterial pathogen distributed almost worldwide causing disease in wild and farmed freshwater, euryhaline and marine fish. Among the five subspecies described so far, A. salmonicida subsp. salmonicida is the only “typical” responsible for furunculosis in salmonids. We report here a mortality outbreak due to Aeromonas salmonicida subsp. salmonicida infection in largemouth bass (Micropterus salmoides) farmed in Italy.

Methodology: After an increase in mortality, in January 2023 11 largemouth bass weighing about 200 grams were sent to the Fish Pathology Unit of DIMEVET, University of Bologna, Italy, for diagnostic purposes. All the fish were subjected to necropsy, parasitological and bacteriological examination. For the latter, Tryptone Soy Agar (TSA) and Blood Agar (BA) plates were inoculated with brain, kidney, and spleen, then incubated at 22±1°C. Identification of the isolates was performed phenotypically by MALDI-TOF and API20E.

Results: Necropsy showed diffuse skin hyperaemia, some focal skin ulcers, in some cases furuncle-like in appearance, and severe hepatic necrosis. Parasitological examination showed the presence of ciliates (Trichodina sp. and Chilodonella sp.) on the skin and dactylogyrid monogeneans in the gills at low infection intensity. After 48 hours of incubation, the growth of a significant number of similar colonies producing brown diffusible pigment on TSA was observed in all the fish examined. MALDI-TOF allowed to identify the isolates as Aeromonas salmonicida, and API20E as A. salmonicida subsp. salmonicida. Disk diffusion susceptibility test showed that all isolates were sensitive to Flumequine and Amoxicillin, and resistant to Florfenicol, Oxytetracycline and Trimethoprim/Sulphametoxazole.

Conclusion: To our knowledge, this is the first report of Aeromonas salmonicida subsp. salmonicida in largemouth bass, expanding the spectrum of susceptible hosts to this subspecies and confirming its potential pathogenic role also for farmed non-salmonid fish species. The outbreak reported here highlights also the importance of implementing strict biosecurity measures, as in this case the introduction of A. salmonicida salmonicida in the farm probably occurred via improperly disinfected trucks from a salmonid farm.

Mortality outbreak in turbot (Psetta maxima) farmed in Italy

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Introduction: Turbot (Psetta maxima) is one of the most important fish species in European aquaculture. The increase of its production should pass through the overcoming of some zootechnical and health issues, among which several transmissible diseases strongly limiting its expansion. During 2021-22 in a farming trial of turbot in inland tanks in Italy, some disease outbreaks were recorded, starting from juveniles and progressively reaching a cumulative mortality of around 95% in a few months.

Methodology: Fish samples were submitted to the lab at three different times coinciding with as many severe disease outbreaks that occurred first in juveniles (33g), then in subadult and adult fish (155g and 489g respectively) from the same batch. All fish were subjected to anatomopathological, bacteriological and parasitological examinations according to standard laboratory methods.

Results: The first outbreak involved juveniles showing erosive and hemorrhagic lesions in the mouth and fins; Aeromonas salmonicida was isolated from brain and kidney. The second outbreak allowed to observe the onset of extensive necrotic-ulcerative skin lesions and enteritis with ascites; massive infections of the skin and gills by scuticociliates referable to Philasterides dicentrarchi and of the gut by the myzozoan Enteromyxum leei were found in the affected fish. In the third outbreak, turbot showed enteritis with ascites and the presence of nodular lesions in spleen, liver and intestine; in this case, in addition to observe a severe intestinal E. leei infection, bacteriological exams allowed to isolate Vibrio harveyi and molecular analyses detected Mycobacterium marinum from the nodular lesions.

Conclusion: All pathogens detected during the outbreaks described here are already well known in farmed turbot, with the exception of M. marinum infection of which very few cases were reported several years ago. The frequency and severity of transmissible disease outbreaks recorded during the production cycle of the turbot batch considered here raise concerns about a possible sustainable production of Psetta maxima in the Mediterranean area.

Mortalities outbreaks by Piscirickettsia salmonis in European sea bass (Dicentrarchus labrax) farmed in Italy

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Piscirickettsiosis is a bacterial disease caused by Piscirickettsia salmonis causing high mortality and huge losses in salmonid and non-salmonid fish such as European sea bass (Dicentrarchus labrax), in which disease outbreaks caused by Rickettsia-like Organisms (RlO) then identified as Piscirickettsia salmonis, were reported during the winter in the Mediterranean, in particular in France, Greece and Croatia (Zrnčić et al., 2021). Typical clinical sign is an abnormal swimming behaviour with internal signs such as swollen kidney and pale liver, among others. In February and April 2023, two outbreaks of P. salmonis infection were diagnosed in cage-reared European sea bass in Southern Italy. The mean weight of fish from the two batches (0+ and 1+), was 35 gr and 170 gr, respectively. Cumulative mortality of 6% was recorded in the two batches during the 3-months observation period. Water temperature range was 13.5-19°C. The fish showed nervous symptoms and appeared thin, with haemorrhagic suffusions in the skin, gill necrosis, marbled liver with focal necrosis, brain congestion, splenomegaly, visceral haemorrhages and catarrhal enteritis. Parasitological, bacteriological, histological analyses and RT-PCR for Betanodavirus were performed according standard lab protocols. The monogenean Diplectanum aequans in the gills and a massive infection by the myxozoan Sphaerospora dicentrarchi in the gut were detected, while cultural exam for bacteria on standard media and PCR for Betanodavirus were negative. At histology diffuse necrosis of brain, liver, spleen and kidney was observed in association with intracellular coccoid structures referable to rickettsiae. The identification of the pathogen was achieved by molecular analysis through 16 S EubB and EubA PCR and Nested PCR with PS2S and PS2AS primers (Manuel et al., 1996). In European sea bass some cases were reported a decade ago in Italy as a disease caused by Rickettsia-Like-Organisms (RLO). The occurrence of further outbreaks of piscirickettsiosis indicates that the infection circulates in Italian sea bass farms, promoting the necessity to improve specific diagnostic capacity, including culture-based tools, and to carry out epidemiological studies aimed to identify main risk factors.

Zrnčić S, Vendramin N, Boutrup TS, et al. (2021). J Fish Dis,44:1033–1042.
Mauel MJ, Giovannoni SJ, Fryer JL (1996). Dis Aquat Org,26:189–195

Digenean trematodes in the digestive system of the great cormorant (Phalacrocorax carbo)

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Introduction: Cormorants (Phalacrocorax carbo) are widespread piscivorous birds, which hunt the local fish fauna in large numbers. As a consequence of their predation, they are the definitive hosts of many digenean trematodes, therefore their digestive systems usually contain several parasite species.

Methodology: Between 2019 and 2022, 131 cormorants were collected from Biharugra after a local reduction of the population. Their digestive systems were subjected to parasitological examination. The intestines were collected from all birds, but the stomach and pharynx were recovered only from a portion of the birds.
The 131 intestines, 44 stomachs and 21 pharynxes were carried to the laboratory in a frozen state, then they were thawed for investigation, cut open, and their contents were decanted in water, filtered, and sorted under microscope. For species identification the ITS region was sequenced, and parallelly sequencing of the cytochrome c-oxidase (coxI) was also performed from a part of the samples.

Results: Out of the 131 birds, 105 were infected with trematodes. Sequences were recovered from 72 samples, the majority belonged to the genus Petasiger (57/131). Other digenean trematodes speces were present in lower numbers: Hysteromorpha triloba (12/131), Plagiophorus sp. (2/131) and Metorchis orientalis (1/131). The Petasiger samples identified at species level included Petasiger phalacrocoracis (3/131), Petasiger radiatus (18/131) and Petasiger exaeretus (36/131).

Conclusions: Our data show that these birds have high infection rates of trematodes, but most of the parasites are not considered as human pathogens. Species of the Petasiger genus were found in the vast majority of cormorants in agreement with the results of previous parasitological studies on cormorants. Only one individual of a zoonotic species was recovered, while most of the detected species do not pose any risk to humans. Metorchis orientalis is a known to cause infections in humans. Carp is among its secondary intermediate hosts beside other cyprinids, therefore the risk of human infection is not negligible, if the fish meat is consumed undercooked.

Keywords: Phalacrocorax carbo,cormorant,Digenean trematodes, Hungary
Funding: This study was funded by OTKA FK 140350.

Generation of a strain deficient in T6SS-effector EvpP from Edwardsiella piscicida and its susceptibility to host fish species

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Introduction: The effector molecule EvpP, secreted by the type VI secretion apparatus (T6SS) from Edwardsiella piscicida, the causative agent of edwardsiosis, is thought to prevent the induction of pyroptosis and facilitate bacterial growth within cells. However, the details of the molecular mechanisms are still unknown. Therefore, in this study, to elucidate the involvement of EvpP in pathogenicity, an E. piscicida evpP-deficient strain was generated, and its susceptibility to host fish species was examined. In addition, changes in the copy number of E. piscicida present were determined in the flounder tissue after infection.

Methods: The evpP-deficient strain NUF806ΔevpP was generated using a suicide vector pRE112 containing the evpP of E. piscicida NUF806 (pRE112-evpP). The deletion of evpP in the E. piscicida chromosome was confirmed by PCR genotyping and its sequencing. Next, the wild strain NUF806 and the defective strain NUF806ΔevpP were examined for infection of Japanese flounder (Paralichthys olivaceus) and medaka (Oryzias latipes) using the immersion method, and the survival rate after infection was observed for 40 days. In addition, qPCR was performed using genomic DNA extracted from medaka tissues (i.e., kidney, spleen, and liver) 1-8 days post-infection (dpi) as a template to calculate the copy number of E. piscicida in the tissues.

Results: Deletion of the evpP gene was confirmed by PCR and its sequencing. Challenge tests showed that death was observed in the wild-type NUF806 group from 7 dpi, with a final survival rate of 13.3%. In contrast, no deaths were observed in the NUF806ΔevpP group, and the copy number in each tissue of NUF806ΔevpP decreased more quickly than that of the wild-type strain.

Conclusions: These results suggest that the EvpP of E. piscicida is important in the mechanism of infection within host cells.

Ornamental freshwater shrimp (Neocaridina davidi, Bouvier, 1904) as helminth host

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Different species of freshwater shrimps are becoming more and more popular among aquarists, which is why research about their diseases has been emphasized in recent years. These shrimps originate from Southeast Asia and can be immensely valuable depending on their color. In our study, we assessed the health status of captive populations of N. davidi in Hungary. During the examination, many worm species were found crawling on the shrimps. The aim of the present study was to identify these worms at the species level, using morphological methods. The helminths were placed to slides covered by cover glass and examined in a live, or intact state, using stereomicroscope and light microscope. Species identification was carried out using morphological methods. The worms found on N. davidi shrimp were identified as Holtodrilus truncatus (Liang, 1963), Scutariella japonica (Matjašič, 1990) and Monodiscus sp (Plate, 1914). Although it was already suspected that these worm species has been introduced into Hungary, this is the first time that S. japonica, H. truncatus and Monodiscus sp. have actually have been identified there. The worms can be observed on the outer body surface, gill cavity and between the pleopods of different animals. Although the literature is currently divided whether these organisms are epibionts, symbionts or parasites, their presence in large numbers on shrimp can cause host stress, oxygen deficiency symptoms, moulting problems and even death. Therefore, in addition to the many pathogens that can cause disease in shrimps, it is important to consider the risk represented by these worm species as well.

GenomeFLTR: Filtering Reads Made Easy

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In the last decade, sequencing technology advances have led to an exponential increase in genomic data. These new data have dramatically changed our understanding of the evolution and function of genes and genomes. Despite improvements in sequencing technologies, identifying contaminated reads remains a complex task for many research groups, in particular for researchers studying fish parasites. Here we introduce GenomeFLTR, a new webserver to filter contaminated reads. Reads are compared against existing sequence databases from various representative organisms

to detect potential contaminants. The main features implemented in GenomeFLTR are: (1) automated updating of the relevant databases; (2) fast comparison of each read against the database; (3) the ability to create user-specified databases; (4) a user-friendly interactive dashboard to investigate the origin and frequency of the contaminations; (5) download the contaminate free file. Availability: https://genomefltr.tau.ac.il/.

Phylogenetic analysis of non-pathogenic salmonid-associated aliivibrios and description of eight novel species and three novel subspecies of genus Aliivibrio

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Introduction: Genus Aliivibrio currently consists of six validly defined species and no subspecies. The interest in and research related to the existing species of genus Aliivibrio is primarily due to inherent curiosity pertaining to the bioluminescence phenomenon in marine species. Examples are the symbiosis of Aliivibrio fischeri in the bob-tail squid and Aliivibrio logei in other hosts combined with an acute need to deal with emerging infections in salmon farming as related to the identification of Aliivibrio salmonicida as the causative agent of cold-water vibriosis in aquaculture.
Methods: In this study, we have taken advantage of two decades of screening of the salmonid tissues for the presence of cultivable bacteria that are non-pathogenic to fish. In this study, we have compared novel candidate species with known aliivibrios by using DNA whole-genome sequencing, carbohydrate degradation repertoire, and electron microscopy analyses. We applied phylogenetic, phylogenomic analyses and average nucleotide identity metrics to establish the species boundaries of the novel isolates.
Results: We propose to establish eight novel non-pathogenic species and three novel subspecies to update the genus Aliivibrio. The results also provide valuable insights into the role of aliivibrio bacteria in the healthy microbiome of salmonid fish.
Conclusions: The findings contribute to the knowledge base of non-pathogenic, fish-associated aliivibrios. These data open for developing strategies for restoring microbial diversity in high-technology intensified fish farming

Unusual chronic granulomatous ovaritis in Epinephelus marginatus due to Philometrid infection

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Introduction: Philometrid species have been reported in different organs of several fish species, in the Mediterranean basin. Regarding the gonadic philometrid infections, the species more frequently reported in the dusky grouper (Epinephelus marginatus) are Philometra jordanoi and P. lateolabracis.
Ovarian lesion of infected fish frequently shows follicular degeneration with consequent gonadal dysfunction. The aim of this study is to describe an unusual chronic ovaritis due to Philometra sp. infection, describing the histological patterns.

Methodology: A female gonad of an E. marginatus specimen was referred to the laboratory of Comparative Animal Pathology of the University of Messina for histopathological investigation. Tissue samples were fixed in 10% neutral buffered formalin and routinely embedded in paraffin wax. Histological examination was performed on 3-µm-thick sections stained with haematoxylin-eosin (HE).

Results: On histology, the gonadal stroma was diffusely expanded by an extensive granulomatous inflammation and abundant connective tissue proliferation, forming well-defined granulomas, surrounding myriads of adults and larval nematodes, separating numerous degenerated and previtellogenic follicles. The inflammatory cell infiltrate was composed of abundant epithelioid and foamy macrophages and lymphocytes, with fewer plasma cells and granulocytes. Diffuse oedema and multifocal haemorrhages, with abundant macrophages hemosiderin-laden were also observed. Inflammatory foci were centred around myriads of free larvae, approximately 30-40 µm in width and 100 µm in length, which display a smooth, 1-µm wide amphophilic cuticle and basophilic nuclei. Adult parasites were approximately 5 mm in length and 500 µm in width, with rounded cephalic and posterior ends, with a smooth eosinophilic cuticle, coelomyarian-polymyarian musculature and a pseudocoelom with distinct dark-brown intestine and a large uterus occupying most of space in body and filled with myriads of larvae. Based on histological findings a diagnosis of severe, chronic, diffuse granulomatous ovaritis, with intralesional philometrid nematodes was performed.

Conclusions: Based on histological finding we can assume a severe gonadal dysfunction. Differently from other reports of philometrid in Mediterranean teleosts, the unusual chronic ovarian lesion here described supports the reproductive failure, sometimes named parasitic castration, that can severely affect the reproduction of E. marginatus, a species already included in the IUCN red list.

Histomorphometry and immune status in atlantic salmon (salmo salar) fed different astaxanthin sources

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Norway has a long coastline and has long traditions harvesting marine resources. Farming of Atlantic salmon started in the 1970's and is currently most valuable fish species exported from Norway (FAO, 2020). Traditionally, salmon has been considered an important source of nutrients. The characteristic pink colour of wild salmonid muscle is the result of deposition of naturally occurring carotenoid pigments mainly astaxanthin (Baker, 2002; Shahidi and Brown, 1998). Astaxanthin is a red/pink carotenoid, produced by a variety of plants, microalgae, or microorganisms (Ho et al. 2018), with various beneficial and biological functions as a feed supplement, such as, skin and flesh pigmentation enhancer, antioxidant, growth and survivability promoter (Li et al. 2020; Wang et al 2020; Lim et al. 2021).

In this study, a feeding experiment was conducted at Nord University research station, Bodø, Norway, with the aim to evaluate the synthetic (AS), whole cell (AW) or extracted (AE) algal astaxanthin. After the acclimation period, Atlantic salmon were randomly distributed to 18 fiberglass tanks, 20 fish per tank. After 75 days, fish weights were recorded and various tissue samples, such as intestine (distal and mid), skin and liver were collected and preserved for further analysis including histomorphometry, expression of immune genes and assessment of liver fatty infiltration.

The analysis is still incomplete, however fish fed the AS feed appeared to increase the height of mucosal folds both in distal and mid part of intestine compared to the other two diets. Skin and liver histomorphometry, and gene expression are underway.

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Rearing density in a specific period determined Francisella halioticida infection of juvenile Yesso scallops Mizuhopecten yessoensis

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Introduction: The Yesso scallop is one of the most important aquaculture bivalve species in Japan, but the scallop industry in southern Hokkaido has suffered mortality of juvenile and adult scallops partly caused by infection of a bacterium Francisella halioticida. Empirically, it has been known that stocking density during intermediate culture is associated with juvenile scallop mortality, and our previous study revealed that juvenile scallops reared at high density from September to October showed high mortality and F. halioticida infection in the following March. The present study examined temporal changes of mortality and F. halioticida infection in juvenile scallops maintained in different densities from September to October.

Methods: In August 2021, juvenile Yesso scallops derived from wild population were reared in cages at 600 individuals/cage. In order to prepare experimental groups maintained in different rearing densities from September to October, half of the juveniles were transferred to other nets at 50 individuals/net in September (S-group), while the other remained half were transferred in the same way in October (O-group). Then juvenile scallops were sampled monthly to examine mortality and infection of F. halioticida by qPCR until March 2022.

Results: Until November, there was no apparent difference in survival rate, prevalence and burden of F. halioticida in both S- and O-groups. However, in O-group, prevalence and burden of F. halioticida sharply increased in December, and survival rate declined, simultaneously. On the other hand, in S-group, prevalence and burden of F. halioticida decreased in December and survival rate retained high until the end of the experiment.

Conclusion: The present study showed that high rearing density from September to October resulted in high F. halioticida infection and the consequent mortality of juvenile scallops in December, suggesting that susceptibility of juvenile scallops to this bacterial infection may be determined by rearing density in a specific period.

Feed formulation and testing in supporting fish homeostasis and performance in common aquaculture stressing events

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Farm management related stressors are common in Aquaculture operations : transportations, transfers, gradings, but also vaccination procedures include fish exposure to hypoxia, injuries, various water qualities, infectious environments, and populations. Moreover, many of these events happen at juvenile stages, when the fish are immature, whether on digestive or immunological aspects. These lead to mortalities and/or loss of performance, which consequences may impact the entire production cycle.

In response to this rising concern in Brazil, a special feed was formulated including specifically selected feed additives to help juvenile tilapia better cope with such stress, then tested in a dedicated R&D trial run in real field conditions with 8 cages per treatment. Results showed significant +23% biomass gain and -13% FCR compared to control, as well as 11% and 22% increased erythrocytes and albumin blood concentrations respectively within one month post-vaccination and/or handling stress. These led to a 5% significant higher body weight at harvest 6 months later, after only four weeks of distributing the special feed at juvenile stage.

This feed was then proposed to a main tilapia producer in Brazil, whose major priority was to reduce the use of antibiotics and particularly as a preventive practice. Their orders continuously progressed until it covered 100% of their juvenile feeding program, totaling 900 MT within nine months. Especially, they have completely stopped using antibiotics in feeds as a preventive practice, allowing them to earn the BAP (Best Aquaculture Practice) certification, opening their sales to new markets and countries. In parallel, antibiotic resistance observations have dramatically decreased. These facts encouraged this producer to re-think its farming and health management strategy, including restricting the use of antibiotics as a curative treatment only when absolutely necessary.

These results highlight contributions possibly brought by specific feed formulations and selected feed additives in supporting fish physiology and homeostasis in various stressing conditions. Doing so, such tools may help fish producers improve their farm management practices and performance, reduce their reliance on antibiotics and chemicals, thus decrease their impact to the environment as well as reducing antimicrobial resistance, a rising top global health concern.

Chitosan microsphere-based oral vaccine against the scuticociliate parasite Miamiensis avidus

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Miamiensis avidus is a ciliate pathogen affects marine fishes worldwide, including olive flounder (Paralichthys olivaceus) in Korea. This parasite infects all size groups of flounder year-round, causing recurring mortalities and huge economic losses to the Korean flounder industry each year. However, few efforts have been made to implement effective remedial measures to control this parasite. Therefore, our study sought to develop a chitosan microsphere (MS)-encapsulated inactivated vaccine (IMa+chitosan) for oral delivery (adsorbed in feed) to flounder fingerlings and assess its protective efficacy. Immunisation trial-1 was conducted to determine the effective concentration of chitosan. Our findings indicated that an IMa+chitosan 0.05% vaccine formulation was safe and effective in providing moderate protection [46.67%–53.3% relative percent survival (RPS)] against M. avidus intraperitoneal (IP) injection challenge at two weeks post-vaccination (wpv) compared to the IMa+chitosan 0.01% and IMa+chitosan 0.005% vaccines (0%–13.3% RPS) irrespective of the antigen doses. In trial-2, the IMa+chitosan 0.05% vaccine elicited similar protective immunity (30.8%–57.1% RPS) in olive flounder against M. avidus at varying antigen doses (high: 2.38×106 cells/fish; low: 1.5×105 cells/fish), immunisation periods (2 and 5 wpv), and challenge modes (IP injection and immersion). Furthermore, experimental trial-3 validated the use of chitosan MS as an IMa antigen carrier to improve survivability (41.7% RPS) in the host by significantly ($p < 0.05$) upregulating specific anti-M. avidus antibody titres in the fish sera and mucus of the group immunised with IMa-containing chitosan MS. In contrast, non-specific immunomodulatory effects (16.7% RPS and enhanced mucosal antibody titres) were observed in the group treated with chitosan MS without IMa. Therefore, our findings suggested that oral administration of chitosan MS (0.05%)-encapsulated IMa vaccine is a promising immunisation strategy against M. avidus that can protect the IMa antigen from digestive degradation, facilitates its targeted delivery to the host immune organs, and helps in orchestrating protective immune induction in olive flounder, thus controlling parasite infection.

Massive production of recombinant hagfish antibody for passive immunization treatment for shrimp disease

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It has well known that the causative agent of acute hepatopancreatic necrosis disease (AHPND) is the bacterium, Vibrio parahaemolyticus, which secretes toxin A and B into the gastrointestinal tract of its host. These toxins have been implicated in the pathogenesis of this disease and are, therefore, the focus of studies developing treatments for AHPND. For the purpose to develop these toxin A/B-specific hagfish Abs, we first expressed and purified the recombinant toxin A and B proteins from bacterial expression system. The purified toxin A and B showed strong and rapid shrimp mortality especially when injected together. Using our established hagfish immunization/screening platform system, the toxin A-specific hagfish Abs (A4D10, A7C12, A1C8) and toxin B-specific hagfish Abs (BN13, BNB1, B5C5) were found. The screened hagfish Abs expressed/secreted from HEK293 cells showed all the selective Ag-binding activity although A4D10 and A1C8 had weak binding with toxin B too. For large-production, these hagfish Abs were also expressed from bacterial system. However, the Abs (A4D10, A7C12, A1C8 and B5C5) revealed non-specific binding activity except two hagfish Abs (BN13, BNB1) that showed selective and strong binding with toxin B. We guess that three-dimensional shapes of the hagfish Abs could not be correctly formed due to inefficient alpha-helical folding by repeated Leu amino acids and/or unsuccessful formation of disulfide bonds in the bacterial expression system. This result suggests that modification of the bacterial system and other expression systems such as algae or plant are required for mass production of the effective hagfish Abs for commercialization.

Is it possible to anaesthetize sturgeons with a bolt gun in a manner that is appropriate for animal welfare?

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Introduction: The application of a penetrating as well as a non-penetrating bolt gun for stunning large fish, such as sturgeon, is to be evaluated. The aim is to develop scientifically robust data on the effectiveness of the captive bolt method and the animal welfare impacts associated with the method in comparison to the previously approved stunning method by percussion as a basis for a possible approval of the captive bolt method.

Methodology: First, the skull morphology and the position of the brain were analysed in detail to define the correct approach for the bolt shot. To assess whether a loss of perception was achieved after the application of the bolt shot, brain functions were first derived using visual evoked potentials (VERs) in anaesthetised sturgeons. The aim was to show a correlation between the absence of certain behavioural parameters and the loss of VERs. Electrodes were implanted in sturgeon under anaesthesia. The awake fish were anaesthetised to record VERs to test whether the loss of body reflexes and body tone also resulted in a loss of perceptual ability. Furthermore, the resulting stress on fish during anaesthesia was estimated by blood parameters. Blood samples were taken from sturgeons before and after stunning and stress parameters were determined.

Results and Conclusions: The location of the brain varied between different sturgeon species, highlighting the importance of accurate anatomical knowledge in the context of stunning. Recording of VERs showed that brain functions were still measurable despite the absence of externally detectable behavioural parameters, suggesting a perceptual ability of the sturgeons and making it difficult to assess the success of stunning in practice. The stress-associated blood parameters sodium, potassium, lactate, glucose, and total protein showed that no additional stress occurs for the sturgeons due to stunning by bolt gun, as no significant differences were found between the different stunning methods and the removals before and after stunning.

Spinal curvature in forkbeards - and an anatomical peculiarity

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Introduction: A forkbeard (Osteoglossum sp.) from a zoo had been showing progressive curvature of the spine for several months. As the animal had also been eating significantly worse for a few weeks, an extensive investigation was initiated.

Methodology: The forkbeard was examined on site under anaesthesia using MS222. Swabs were taken from the skin and gills and radiographs were taken in two planes. As the general condition of the fish deteriorated over the following weeks, it was 167ineraliza and a necropsy was performed.

Results and Conclusion: No parasites were detected in the smears. Radiographically, fractures of vertebral bodies and ventral processes were found in the curvature of the spine. The 168ineralization of these processes was found to be markedly reduced and the changes in the spine were classified as chronic. A radiographic abnormality was seen in the area of the swim bladder. This had a portion within the abdominal cavity that appeared to be elongated oval and tapering caudally. Other air-filled, segmented areas extended to the tip of the tail. In section, these extra-abdominal areas presented as limited compartments, some contiguous and some separate. A swim bladder compartment outside the abdominal cavity is very unusual and not found in most fish species. The curvature of the vertebral column may have occurred due to trauma that occurred some time ago and may have become more clinically apparent due to deterioration in the general condition of the animal. Differentially, changes in the spine of the forkbeard may also be due to alimentary causes.

Molecular cloning and characterization of galectin-9 gene from sevenband grouper (Hyporthodus septemfasciatus)

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Grouper is one of the most valuable cultured fish in the world. However, many groupers are reported to have been susceptible to several types of pathogens and made huge economic losses.

In this study, transcriptome of kidney from sevenband grouper was generated using next generation sequencing (NGS) technology, and the galectin-9 gene of sevenband grouper Hyporthodus septemfasciatus (SGGal-9) was identified from the transcriptome data and conducted gene cloning and recombinant protein overexpression for analyzing its molecular characteristics.

The full-length cDNA consists of 2004 base pairs (bp), including 162 bp of 5'-untranslated region (UTR), 829 bp of 3'-UTR and 1014 bp of open reading frame (ORF) encoding 338 amino acids. The SGGal-9 has two conserved carbohydrate-recognition domains (CRDs), including N-terminal CRD domain of 135 amino acids and C-terminal CRD domain of 129 amino acids. Each CRD has two conserved β-galactoside binding motifs (H-NPR and WG-EER). The putative protein does not include transmembrane domains or signal peptides. The SGGal-9 was sub-cloned into the pCold I vector expressing 6X His-tag in N-terminal. Overexpressed recombinant protein of SGGal-9 (rSGGal-9) was about 34 kDa molecular weight and expressed as a soluble form in E. coli BL21 (DE3), induced with 0.1mM isopropyl-β-D-1-thiogalactopyranoside (IPTG). To validate the activity of the rSGGal-9, erythrocytes of human Homo sapiens, olive flounder Paralichthys olivaceus, and sevenband grouper were subjected to hemagglutination and aggregated with the rSGGal-9 at minimum concentrations of 6.25, 3.125 and 12.5 (μg/ml) respectively. In addition, the aggregation effect of the rSGGal-9 on various bacteria (Lactococcus garvieae, Photobacterium damselae, Streptococcus iniae, and Streptococcus parauberis) was confirmed. Our results point out that galectin-9 is possible to use as preventive agent against bacterial infection in sevenband grouper aquaculture.

Keywords: Molecular cloning; Galectin-9; Sevenband grouper; Recombinant protein; Bioinformatics

Fish invasion with Eustrongylides excisus (Nematoda: Dioctophymatidae) in the Don river delta and the eastern part of the Taganrog Bay

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Eustrongylides spp. are cosmopolitan parasitic nematodes with several freshwater fish species as intermediate or paratenic hosts, rarely reported as zoonotic agents. This work aims to report about the occurrence of Eustrongylides excisus in specimens of round goby, Neogobius melanostomus (Pallas, 1814), monkey goby, N. fluviatilis (Pallas, 1814), syrman goby, Ponticola syrman (Nordmann, 1840) and 122 specimens of pike-perch, Sander lucioperca, (Linnaeus, 1758) caught in the Don river delta and the eastern part of the Taganrog Bay (southern Russia). Totally 353 specimens of the Azov fish were collected between 2019-2022 years in spring. Fish were visually examined, visible parasites were collected and counted after both procedures, and then they were identified to genus level by microscopic examination. According to the data obtained, the body cavity and serous integument of the intestine are the main places of the localization of parasites for gobies, and muscles and the abdominal wall - for pike-perch. In 2019-2022 the prevalence of the nematode invasion in the Don river delta varied between 53.3-93.3% among sirman goby, 10-46.7% - round goby, 26.7-64% - monkey goby. The highest level of average intensity of invasion was noted for sirman goby (3.5-9.0 spec.), while for round goby it varied between 5-7 spec., and for monkey goby – between 1-9 spec. Pike-perch infected with Eustrongylides was observed in the Don river delta and the eastern part of the Taganrog Bay since 2020. For prevalence of invasion from 26,7 till 73,3%, for average intensity from 1,2±0,25 spec. till 7,9±1,7 spec. E. excisus raised a natural interest as a factor in the dynamics of natural animal populations. Moreover, as Azov gobies and pike-perch are highly valued species in southern Russia, the presence of Eustrongylides might imply an impact on human health and the quality of fish products. Until now, the species E. excisus was not proven to be the causative agent of human disease. In any case, its zoonotic potential cannot be ruled out until more molecular genetic studies are performed, the species causing human cases are studied, and the geographic distribution of the various species is clarified.

Determining transcriptomic response of kidneys of olive flounder to viral hemorrhagic septicemia virus infection using next-generation sequencing

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Viral hemorrhagic septicemia (VHS) in olive flounder (Paralichthys olivaceus) causes considerable economic losses in the flounder aquaculture industry in Korea. This study was aimed to identify the immune-associated genes contributing to VHSV infection progression in the kidneys of the olive flounder. Ten fish were divided into two groups via an intraperitoneal injection with either diethylpyrocarbonate-treated water or 10 to the power of 6 50% tissue culture infective dose VHSV 100 µL-1 fish-1. At 24 h post-infection, kidney tissues were used for RNA sequencing to identify the differentially expressed genes (DEGs). The upregulated DEGs in the VHSV group included many genes linked to interleukins, chemokines, interferons and immune cell types. Functional enrichment analysis indicated that VHSV infection broadly affects the host metabolism and innate immune responses in the olive flounder. The present study provides a theoretical basis for the molecular mechanisms of VHSV that can be further explored in future studies.

DNA vaccine dual-expressing VHS virus glycoprotein and C-C motif chemokine ligand 19 induces the expression of immune-related genes in zebrafish

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Glycoprotein (G protein)-based DNA vaccines are effective in protecting aquaculture fish from rhabdoviruses but the degree of immune response they elicit depends on plasmid concentration and antigen cassette. Here, we developed a DNA vaccine using the viral hemorrhagic septicemia virus G (VG) gene and chemokine (C-C motif) ligand 19 (CCL19)a.2 regulated by the CMV promoter as the molecular adjuvant. After transfection of the prepared plasmid (pVG + CCL19) into epithelioma papulosum cyprini cells, mRNA expression was confirmed through quantitative real-time polymerase chain reaction.

The vaccine was intramuscularly injected into zebrafish (Danio rerio), and 28 days after immunization, viral hemorrhagic septicemia virus (10 to 5 TCID50/10 µl/fish) was intraperitoneally injected. A survival rate of 68% was observed in the pVG + CCL19 group but this was not significantly different from the survival rate of fish treated with pVG alone, that is, without the adjuvant. However, the expression of interferonand cytokine-related genes in the spleen and kidney tissues of zebrafish was significantly increased ($p < 0.05$) on days 1, 3, 7, and 14 after immunization. Thus, CCL19a.2 induced an initial immune response as a molecular adjuvant, which may provide initial protection against virus infection before vaccination-induced antibody formation. This study provides insights on the functions of CCL19a.2 adjuvant in DNA vaccines.

Establishment of Epidemiological Cut-Off Values for Vibrio harveyi and Lactococcus garvieae Isolated from Aquatic Animals

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Introduction: In 2022, the production amount of cultured marine fish was recorded at about 89 thousand tons, and olive flounder, rock fish, gray mullet and red seabream are important varieties in Korea aquaculture industry. Major bacterial fish diseases are edwardsiellosis, streptococcosis and vibriosis in Korea. Usage of antimicrobial agents has been increased to treat several bacterial diseases. The present of antimicrobial resistant bacteria has become significant issue. No epidemiological cutoff values (ECVs) have been reported for Vibrio harveyi and Lactococcus garvieae. In this study, we investigated the distribution of minimum inhibitory concentrations (MICs) for V. harveyi and L. garvieae and determined the ECVs.

Methodology: Antimicrobial susceptibility tests were performed according to the broth microdilution methods described by the Clinical and Laboratory Standards Institute (CLSI, 2021). The ECVs were calculated using NRI method. The NRI method is a fully automatic and freely available Excel spreadsheet calculator (last updated in 2019; http://www.bioscand.se/nri). The ECVs determination was based on the distribution of antimicrobial MICs for each antibiotic against 169 V. harveyi isolates and 75 L. garvieae isolates.

Results and Conclusions: Distribution of the MICs for 14 antimicrobial agents (oxytetracyline et al.) against V. harveyi and 13 antimicrobial agents against L. garvieae were evaluated. The MICs obtained for V. harveyi isolates and L. garvieae ranged from 0.25–256 µg mL−1 for oxytetracycline. Based on the MIC distributions, the ECV of V. harveyi for oxytetracycline was 2 µg mL−1. This categorized 38 (21.2%) isolates as non-wild type (NWT). The ECV of L. garvieae for oxytetracycline was 2 µg mL−1. This categorized 11 (14.7%) isolates as NWT.

Open reading frame 150 – mode of action during cyprinivirus infection

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Introduction: Ubiquitination is a wide-spread mechanism in eukaryotic cells. Most know for its function as degradation signal leading to protein degradation in proteasome. However, it may have multiple functions depending on its linkage type. A recently discovered ubiquitin E3 ligase of Cyprinid Herpesvirus 3 causes complete attenuation and protective immunity if deleted in CyHV3. Even though the mode of action is unclear, the importance is underlined by this result and its presence in CyHV1 and CyHV2.

Methodology: The expression of cellular signalling pathways was investigated by comparison of wildtype virus and deletion mutant. Additionally, in vitro protein expression was used to find interacting proteins.

Results: Expression analysis were employed to get a deeper look into the mechanism behind ORF150. The influence on cellular signalling processes were tried to uncover. Moreover, cellular interaction partners shall be identified.

Conclusions: ORF150 is a suitable target for attenuation of CyHV3. However, a deeper insight into its mechanism is important to understand its role during infection.

Impairing replication of CyHV3 for vaccine design

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Introduction: Since the late 1990th, global carp production is endangered by the Cyprinid Herpesvirus 3, also known as KHV. Until now, no approved vaccine is available. The only produced vaccine, by KoVax (now Phibro), is no longer available. Multiple attempts were undertaken to develop a safe and protective vaccine, without applicable products for aquaculture. One drawback of all vaccines seems to be the remaining persistence of live attenuated vaccines. Resulting in a possible spill over from aquaculture to adjacent natural aquatic systems.

Methodology: Terminases are essential for the packaging of the viral genome and for cutting into genome length. If this process is hindered, the capsid remains empty and virus replication stops. Deletion of one or both of the known terminases of CyHV3 may result in a replication defective variant suitable as live attenuated vaccine. Deletion mutants will be tested for their immunogenic potential in vivo. Furthermore, the potential risk of complementation by wild type virus and related viruses (e.g. CyHV2) will be examined.

Results: Recent data will be presented on the effect on viral replication. Additionally, possible complementation by either CyHV1 or CyHV2 will give first insights into the suitability this strategy.

Conclusions: Interfering with the replication cycle of Alloherpesvirus offers a good opportunity for future vaccines. Live attenuated vaccines without the potential risk of a wide spread in nature may be a prospective direction for this virus group.

Safety of levamisole, fenbendazole and ivermectin anthelmintic baths for juvenile carp (Cyprinus carpio)

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Antiparasitic drugs levamisole, fenbendazole, and ivermectin are not approved for use in fish for human consumption, but MRLs have been established for the meat of sheep, goats, pigs, cattle, and poultry. Therefore, it is possible to use them based on the “off-label” principle as a veterinary medicinal product intended for other food animals. The safe use of levamisole, fenbendazole and ivermectin applicated as a therapeutic bath, was tested in juvenile common carp (C. carpio). The effect on haematological and biochemical blood parameters, oxidative stress and antioxidant biomarkers in gills, liver and muscle, and histological changes on the gills were monitored and compared with the control group.

Levamisole bath (50 mg/l, 2 h) did not affect the haematological profile. Some biochemical parameters in plasma were significantly increased: glucose, lactate, ammonia concentrations, alkaline phosphatase, and alanine aminotransferase activities; on the other hand, aspartate aminotransferase activity was significantly decreased. TBARS in muscle and liver, total superoxide dismutase activity in muscle, and catalase activity in the liver are significantly increased. In contrast, glutathione reductase activity in gills, liver and muscle was significantly decreased. There were found no histological changes in the gills.

Fenbendazole bath (25 mg/l, 12 h) did not affect haematological and biochemical indices, oxidative stress parameters and antioxidant indices. There were found no histological changes in the gills.

Ivermectin bath (0.031 mg/l, 12 h) led to a significantly greater muscle total superoxide dismutase activity, whereas the haematological and biochemical indices remained unchanged. In gills, slight histopathological changes (++) were found, including focal epithelial lifting and hyperaemia of the lamellar vessels.
The bath in fenbendazole can be recommended for safe antiparasitic treatment in carp. When such “off-label” use is considered, a tolerance test must be conducted before administration, and the specified withdrawal period of at least 500 degree-days must adhere.

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Effect of RTGE gastroenteritis on the microbiota of rainbow trout intestine

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Introduction: Rainbow trout gastroenteritis (RTGE) has been reported in Finland since 2010. RTGE is summer enteric syndrome and has been repeatedly isolated especially in recirculating aquaculture systems (RAS) from 0–1-year-old rainbow trout causing serious effects on production. In direct microscopy and in histology segmented filamentous bacteria (SFB) are observed in the intestine of fish in association with RTGE. The SFB cannot be cultivated or isolated by traditional methods, which has hindered studies of its association as the agent of RTGE. With molecular methods SFB has been included in “Candidatus arthromitus” bacterial group. The aim of this study was to confirm the presence of C. arthromitus in the microbiota of rainbow trout with RTGE symptoms and to compare the results with ones obtained from healthy fish. Furthermore, studies of the effect of fish diseases on fish intestinal microbiota are scarce thus the microbiota changes in the intestine affected by RTGE was studied.

Methodology: All together 39 rainbow trout with and without RTGE symptoms were collected from several fish farms. Pathological changes were noted as well as the presence of SFB in the intestine by direct microscopy. Samples of the intestine were collected for DNA extraction and sequenced with NGS method using Illumina MiSeq with 16S V3-V4 primers.

Results: From rainbow trout with clinical symptoms of RTGE, and SFB suspected in the intestine by microscopic observation, C. arthromitus bacteria were confirmed abundantly in the intestine. From healthy fish C. arthromitus was not detected. Microbiota of the healthy fish intestine contained abundantly bacteria from phyla Proteobacteria, while they were less abundant in the intestine of RTGE fish. On the contrary, Clostridia bacterial group was more abundant in RTGE fish than healthy fish.

Conclusions: This study shows that RTGE infection changes the microbiota of rainbow trout by abundant presence of C. arthromitus but also by reducing Proteobacteria, of which many are considered beneficial for healthy intestinal microbiota of fish.

The transcriptome of VHSV resistant and susceptible rainbow trout strains provides insight into immune response leading to different disease outcomes

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Introduction: Viral haemorrhagic septicaemia virus (VHSV) is a highly contagious pathogen affecting salmonid fish populations. Recent evidence suggests that host genetics play a key role in susceptibility to the virus, with differences in innate antiviral immune responses underlying VHSV resistance in rainbow trout strains. The aim of the present study was to investigate gene expression signatures in a VHSV-resistant local German rainbow trout strain (R7) under VHSV infection and to compare them with expression signatures in a highly susceptible commercial strain (R9).

Methods: Fin, gill, gut, kidney and spleen tissues were collected from susceptible and resistant adult rainbow trout at 2 and 4 days post infection with VHSV. RNA-seq was performed from kidney samples at 2 dpi. In addition, gene expression of 27 selected genes was determined by RT-qPCR in all samples collected. The results were compared with gene expression in primary cell cultures from fins, scales and kidneys of fish of the same strains infected with native and inactivated virus.

Results: It was found that the genes involved in the interferon response of rainbow trout showed clear differences in gene expression levels between the two origins. Especially on day 2 after infection with VHSV, a large number of genes differed significantly ($p < 0.05$), especially in spleen and kidney. Particularly striking were the gene expressions of pro-inflammatory cytokines and type II interferons. While gene expression in tissues of resistant origin increased steadily over the observation period, there was a rapid increase (day 2) followed by a decrease in gene expression (day 4) in samples of susceptible origin. The in vitro experiments confirmed the in vivo results that VHSV induces a stronger immune response in cells derived from the R9 strain.

Conclusions: Our results could not confirm that an increased antiviral response is the main factor for increased resistance. Furthermore, it can be concluded that trout from the susceptible R9 origin died because of a dysregulated inflammatory response and cytokine storm.

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Evaluation of alternative carp cell lines for in vitro studies on CyHV-3 pathobiology

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Introduction: Cyprinid herpesvirus 3 (CyHV-3, aka Koi herpesvirus), a double-stranded DNA virus from the Alloherpesviridae family, is a WOAH-notifiable fish pathogen causing disease and mortalities outbreaks in Common Carp and Koi. Other cyprinid species can be infected by CyHV-3, although showing mild clinical signs or acting as asymptomatic carriers. The Great Lakes ecosystems are threatened by invasive Asian carp species, with an unknown status of susceptibility towards CyHV-3 and other viruses affecting cyprinid and native fishes. Few cell lines, from common carp brain (CCB) and Koi fin (KF-1), are currently suitable for CyHV-3 isolation and in-vitro propagation for viral pathobiology studies. New continuous cell lines were generated from Asian Carp species to assess their differential susceptibility upon CyHV-3 exposure.

Methodology: New cell lines were developed from the gills and fins of Asian carp species, including from Grass Carp (Ctenopharyngodon 174della), Bighead Carp (Hypophthalmichthys nobilis), and Silver Carp (H. molitrix). Cell lines were exposed to two reference strains of CyHV-3 (one from Taiwan and one from Germany) to assess their susceptibility to the viral replication. After inoculation of virus, cells were incubated at 25℃ and monitored for the occurrence of cytopathic effects (CPE) over 14 days. Quantitative real time PCR (qPCR) and TCDI50 were performed to measure the viral loads upon any CPE occurrence.

Results and Conclusions: The new Grass Carp fin cell line (GRC-fin), along with the well the reference cell lines CCB and KF-1, showed distinct CPE starting from 7 days post CyHV-3 exposure, showing vacuoles formation and cells detachment. The gills and fin cell lines developed from Silver and Bighead carp did not show any appreciable CPE. This study proved that GRC-Fin cell as an alternative cell system for isolation of cyprinid viruses specifically CyHV-3, however, further characterization and study is needed despite initial indications that CyHV-3 can be grown in this cell line. Having new permissive cell lines can be useful to facilitate virus isolation, test its propagation, and allow further studies on the role of viral ORFs in interfering with the host immune responses.

Case study: Identifying Piscirickettsia salmonis in Scottish aquaculture

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Introduction: Piscirickettsia salmonis was identified in 1992 as the cause of the disease known as ‘Coho salmon syndrome’ which had been affecting farms in Chile since 1981 and causing mortality rates in infected farms of up to 90%. Piscirickettsia salmonis is a Gram-negative, coccoid, facultatively intracellular bacterium. A risckettsia like organism was detected in Scottish waters in 1996, but not until 2002 was P. salmonis confirmed in Scotland. Losses in Scottish waters have not reached the levels seen in the southern hemisphere as Atlantic salmon (Salmo salar) seems to have higher resistance to P. salmonis than Coho salmon (Oncorhynchus kisutch), but different strains have been shown to have different virulence.

In November 2022, P. salmonis was detected by qPCR from DNA extracted from kidney samples taken from fish from two salmon farms on the West coast of Scotland. Piscirickettsia salmonis was not isolated on bacteriology plates from either farm due to the difficulty of growing this pathogen on standard media. The genotype of the detected P. salmonis will be determined to compare it to previous strains detected in Scotland.

Methodology: Samples are taken by the Scottish Fish Health Inspectors as part of their routine sampling of salmon farms. Bacterial samples are taken onto agar and colonies which grow are 174uppressing174d using bacteriology techniques. Isolates are further analysed using molecular biological methods. Kidneys from sampled fish are taken into ethanol for DNA extraction and analysis by qPCR.

Results/ Conclusion: Work is ongoing to identify the genotype of the P. salmonis using RFLP and multiplex PCR, however this has not yet been successful as working with DNA extracted from kidney tissue presents more challenges than DNA extracted from pure bacterial cultures due to cross reaction with the salmon DNA. The use of targeted whole genome sequencing of these samples following Piscirickettsia enrichment is also being considered. The challenge of isolating P. salmonis on agar plates is being addressed, trials of different media which will allow P. salmonis to grow while 174uppressing other common bacteria found in Scottish aquaculture are ongoing.

Tryptophan modulatory role in European Seabass (Dicentrarchus labrax) immune response to acute inflammation under stressful conditions

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Tryptophan has recognized roles in the neuroendocrine system working as a player in the neuroendocrine–immune axis. Therefore, it is of utmost importance to unveil its nutraceutical potential in the quest for the definition of new strategies to empower fish in the mitigation of the non-ideal production conditions. Recent works have put into evidence the immune tolerance role of tryptophan dietary supplementation in fish. The present work aimed to study the role of dietary tryptophan supplementation in modulating the European seabass (Dicentrarchus labrax) immune condition during stressful rearing conditions (15 days exposure to high density), as well as the immune response to acute inflammation after intraperitoneal injection with Photobacterium damselae subsp. piscicida.

A clear peripheral and local inflammatory response was observed in response to bacteria. Moreover, exposure to a high stocking density seemed to exacerbate the inflammatory response at early sampling points, compared to fish stocked at a lower density. Stressed fish presented some immune-suppressing effects on the T-cell receptors expressions at a late sampling point following inflammation. Regarding the effects of dietary tryptophan, no changes were observed on seabass immune indicators prior to inflammation, while a small number of immunosuppressive effects were observed in response to inflammation, supporting tryptophan's role in the promotion of tolerance signals during inflammation. Nonetheless, tryptophan improved the inflammatory response against a bacterial pathogen during stressful conditions, supported by a reduction of plasma cortisol levels, an up-regulation of several immune-related genes at 48h, and an inversion of the previously observed, stress-induced T-cell suppression. Finally, the involvement of tryptophan in macrophages was confirmed by the up-regulation of genes involved in the kynurenine pathway.

The present study brings new insights regarding the immune modulatory role of tryptophan during stressful conditions in fish, thus allowing for the development of novel prophylactic protocols during vaccination by intraperitoneal injection in the European seabass.

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Multiple cutaneous soft tissue sarcomas with myxoid differentiation in reared sturgeons (Acipenser gueldenstaedtii)

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Introduction: The Russian sturgeon (Acipenser gueldenstaedtii) is one of the most ancient species, native to the Black and Caspian Seas. It has considerable commercial importance for caviar production. So far, the occurrence of neoplastic cutaneous masses in sturgeons has never been described. A preliminary investigation has been carried out.

Methodology: The Italian commercial farm rearing sturgeon sourced its water from a spring with a constant temperature of 15°C throughout the year. Six adult fish belonging to the runts' group reared in dedicated tanks were presented with several cutaneous masses on pectoral, anal fins and barbels areas, heavily affecting swimming and feeding ability. After euthanasia, complete necropsy was performed; the masses were analysed by means of microbiological, virological and pathological investigations, including Transmission Electron Microscopy (TEM) focusing on searching for etiological agents.

Results: Cutaneous masses were cauliflower-shaped, at cut section showed a homogeneous, pale white-grey colour and firm consistency; fluid-filled cystic masses with a liquid consistency were also present. Other necropsy findings were unremarkable. Histologically, the cutaneous masses affected the dermal component, were arranged in well-demarcated, expansile, moderately cellular, multilobular areas composed of spindle to stellate neoplastic cells, often arranged in bundles. The neoplastic cells were immersed in an abundant, dense, fibrillar and eosinophilic ground substance;

multifocally, the stellate neoplastic cells were periodic acid-Schiff (PAS)-positive, lying in metachromatic, alcianophilic matrix consistent with mucin. Anisocytosis and anisokaryosis were moderate, mitoses were less than one per HPF. Mild, multifocal lymphoplasmacytic infiltration was present at the periphery of the masses. TEM did not show evidence of biological agents; cell cultures were negative as well. Bacteriological analysis from the masses and cystic cavities revealed the prevalent presence of bacteria Morganella morganii and Aeromonas veronii biovar sobria. As a whole, a diagnosis of soft tissue sarcomas (STS) with myxoid differentiation/myxosarcomas was made.

Conclusions: Cutaneous STS have not been previously reported before in sturgeons. Bacteria within the cystic masses were considered incidental and not associated to STS. Similar cutaneous masses were described in European eels, in absence of an evident biological cause. Further investigation into the biotic and abiotic causes of these tumors is warranted.

Three-dimensional primary cultures and zebrafish model: two advanced preclinical platforms to predict chemotherapy outcome in sarcoma

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Introduction: Soft tissue sarcoma (STS) represent a heterogeneous group of rare malignancies, which exhibit an extraordinary heterogeneity of clinical behavior and histological subtypes. Anthracyclines represents the first line treatment of metastatic or unresectable STS, however the role of chemotherapy (CT) has not been completely elucidated yet. Moreover, sarcoma treatment selection is challenging due to the paucity of preclinical models able to predict chemotherapy outcome. To address this issue, we aimed to combine translational platforms in order to provide support to clinician in their daily practice.

Materials and Methods: This prospective study enrolled three patients affected by: leiomyosarcoma (LMS), undifferentiated pleomorphic sarcoma (UPS), and pleomorphic liposarcoma (PLS). We established patient-derived primary cultures, which were tested both in a 3D scaffold platform and in vivo through xenostransplantation into zebrafish embryos. Standard or tailored CT was administered to these models: ifosfasmide (IFO) and epirubicin (EPI) versus gemcitabine (GEM) and docetaxel (DOCE), or gemcitabine (GEM) and dacarbazine (DACA). Drug efficacy was assessed by checking cell survival with MTT assay and by monitoring tumor shrinkage in zebrafish. Moreover, in vivo drug toxicology was evaluated.

Results: We successfully achieved the establishment and engraftment of all the sarcoma primary cultures. Interestingly, PLS cultured within 3D scaffold showed cell viability of 18% when treated with GEM+DOCE. Furthermore, tumor growth inhibition rates in xenotransplanted zebrafish embryos was as following: LMS 33.6 with IFO+EPI and 9.5% with GEM+DACA; UPS 38.7% IFO+EPI and 14.8% GEM+DOCE and PLS 46.3% with IFO+EPI and 28.3% with GEM+DOCE. Severe adverse events in embryos were observed in 12% of CTR PLS and 12% of GEM+DACA LMS, mainly pericardial edema, scoliosis, abnormal embryonic development and enlarged yolk sack.

Conclusions: In this study, we provided the rationale for exploiting preclinical translation models such as patient-derived primary cultures, three-dimensional culture platforms and zebrafish embryos as predictive tools for treatment selection in daily clinical practice. This work highlights the strategic role of precision medicine in pushing the research towards new therapeutic horizons in the next years.

Antimicrobial and antibiofilm activity of postbiotics obtained from Bacillus pumilus isolated from healthy seabream fed a microalgae-supplemented diet

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Postbiotics are non-viable microbial products or metabolic by-products that provide health benefits to the host, have an extended shelf life, and avoid the potential risks associated with live bacterial use. There is a growing body of scientific evidence supporting the beneficial health effects of postbiotics. Additionally, factors such as the culture conditions and the culture medium used can influence the bioactivity of postbiotics. This suggests that it could be used to affect the potential postbiotic bioactivity of a microorganism.

The objective of our study was to evaluate the effects of different media containing microalgae on the postbiotic potential of extracellular products (ECPs) obtained from two strains of Bacillus pumilus (UMA-169 and UMA-216) isolated from healthy seabream fed with diet-containing microalgae. The ECPs were obtained using the cellophane sheet technique from TSAs medium, and from solid medium (1.5 % agar) supplemented with 5 % Spirulina, Chlorella, Nannochloropsis, and a mix of all microalgae. The plates were incubated at 23 ºC for 24 hours. In addition, the different media without bacterial inoculation were incubated at the same conditions and used as internal controls to evaluate a possible background from the media. The antibacterial and antibiofilm activity of the ECPs were evaluated against human and fish pathogens using the agar-well diffusion and the crystal violet technique, respectively.

It was demonstrated that the inclusion of different microalgae in the culture media affected the bioactivity of ECPs. The antibacterial activity of the ECPs varied depending on the strain and the microalgae included in the culture media. Methicillin-resistant S. aureus was inhibited by all ECPs, and Spirulina 216 and Nanno 169 inhibited C. coli and C. jejuni at the lowest concentrations, respectively. The internal controls did not show any antibacterial activity. Furthermore, all ECPs reduced the biofilm of A. hydrophila, and most of them decreased the biofilm of P. aeruginosa and V. anguillarum. However, some ECPs increased the biofilm of S. aureus, while others reduced it.

In conclusion, the results suggest that optimizing postbiotic production might lead to their application in various biotechnological fields.

Biofilm regulation of shewanella pathogenic strains by extracellular products (ecps) of probiotic PDP11

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Introduction: Recent research has focused on anti-biofilm methods and their implication for surface attachment inhibition (1). The Shewanella. sp Pdp11 strain was isolated by our research group, and it has been described as a probiotic in culture fish(2) and presents inhibitory characteristics. In contrast, S. algae (3) and S.hafnensis (4), have some pathogenic strains, for which biofilm production has been described. This study proposes the use of extracellular product (ECP) from probiotic Shewanella.sp Pdp11 can modify the biofilm formation, or changes in its structure, in related strains such as S.algae and S.hafnensis.

Materials and Methods: The probiotics were grown in 50 ml of Tryptic Soy Broth (TSBs) and incubated at 23ºC and 15 ºC during 36 h. The ECPs extraction has been described (5) in different culture media and under different conditions (15 or 23 ºC during 24 or 48h). For biofilm assay, ECPs conditions were selected based on DNAse activity. The biofilm inhibition was determined in 96 well plates using 90µl ECPs + 90 µl TSBs + 20 µl of pathogenic bacterial suspension adjusted to OD600nm ~ 0.5, per well. The plates were incubated at 23ºC-24 h. The biofilm formation assay was performed by crystal violet (CV) staining (6) and quantify at OD595nm in a plate reader. The biofilm structure was analyzed by fluorescence microscopy.

Results and Discussion: Each strain biofilm responded differently to the compounds present in the ECPs, which increased in the majority of conditions. However, S. hafnensis strains reduced biofilm production to 24h in all culture mediums used to extract Pdp11 ECPs, but its biofilm increased with ECPs of Pdp11. The biofilm structure where analyzed in S.hafnensis P14. The image evidence a good coverage by biofilm in control, a reduction of biofilm for ECPs culture condition and it increases with Pdp11 ECPs. Biofilm formation can be affected by several factors including nutrient availability, environmental conditions, and surface characteristics. In addition, other organisms may compete for nutrients, and space or to produce molecules related to quorum sensing mechanisms.

Aspidogaster limacoides and A. conchicola (Trematoda: Aspidogastridae) in freshwater fish and mollusks from the Korana and Mrežnica Rivers, Croatia

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Aspidogaster limacoides and A. conchicola are well known representatives from the family Aspidogastridae. Aspidogastreans use mollusks as obligate hosts and vertebrates as facultative or obligate final hosts. However, the life cycle is known for only a few well-described species. In addition, data on their presence in mollusks and vertebrate hosts in Croatia are scarce. The aim of this survey was to investigate the presence of aspidogastreans in different freshwater fish and mollusks from the Korana and Mrežnica Rivers, Sava River Basin, Croatia.

Northern pike (Esox lucius) from the Mrežnica River was collected by electrofishing as part of the Croatian Science Foundation project “Metal-binding biomolecules and health disturbances of freshwater organisms exposed to industrial

wastes" (IP-2019-04-2636). From the Korana River, specimens of Danubian roach (Rutilus virgo), chub (Squalius cephalus), nase (Chondrostoma nasus), northern pike (Esox lucius), tench (Tinca tinca), and mollusks (gastropods Esperiana esperi and Holandriana holandrii; bivalves Anodonta anatina, Sinanodonta woodiana, Unio pictorum and Unio tumidus) were sampled by angling and diving, respectively. Fish and mollusk specimens were dissected and the precise number and location of parasites was noted for each infected species. Parasites were fixed in 4% neutral buffered formalin, stained with alum-carmine, and examined under Olympus BX41 microscope. Identification to the species level was performed by morphometric analysis and was based on the number and arrangement of alveoli, according to published data.

A. limacoides was found in Danubian roach and gastropod E. esperi, while the presence of A. conchicola was noted in bivalves A. anatina and U. tumidus. The intensity of infection ranged from 5 to 27 specimens of A. limacoides per infected Danubian roach, while infected gastropod and bivalves contained only single aspidogastreans.

In conclusion, this is the first record of A. limacoides in gastropod E. esperi which widens its host range. A. limacoides and A. conchicola are reported for the first time in Croatia. These data contribute to the current knowledge about the distribution and hosts of A. limacoides and A. conchicola.

Clomethiazole and Eugenol inhibit the replication of spring viraemia of carp virus (SVCV) and prevent cellular oxidative stress in vitro

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Introduction: Spring viraemia of carp virus (SVCV) is one of the most significant disease threats for cyprinid aquaculture. Currently, there are no commercially available vaccines for SVCV or specific treatments. SVCV replication down-regulates heme-oxygenase 1 which promotes oxidative stress in the host cell and disrupts the mitochondrial electron transport chain, resulting in increased levels of reactive oxygen species (ROS). We aimed to explore the antiviral potency of compounds with known antioxidant properties and generate baseline data for antiviral screening.

Materials: We scanned an antioxidant compound library containing a collection of 800 compounds in epithelioma papulosum cyprini (EPC) cells infected with SVCV. Viral replication in presence of the compounds was assayed by the development of cytopathic effects (CPE). Cell viability was measured using the CellTiter-Glo Luminescent cell viability kit with 1% H2O2 as a positive control of cell death. We selected the nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of cellular defence against oxidative stress, as a proxy to measure in vitro oxidative stress. For that, we used a responsive reporter luciferase plasmid that includes Nrf2 responsive of initiating a signalling pathway and transcription of the luciferase reporter gene. A second plasmid encoding a Renilla luciferase reporter gene was used as a control of transfection efficiency using the Fugene™ transfection reagent. To induce the Nrf2 expression, transfected cells were inoculated with either SVCV or 5% DMSO as a positive control of ROS inducer and the luciferase luminescence was measured on an ELISA reader.

Results: Compounds with antiviral properties were selected from those showing at least a 2-log reduction in cell death at a concentration of ≤ 10 µM. A total of 25 out of 800 compounds (3.1% of the compounds tested) showed some antiviral potency in vitro and were selected for measuring in vitro oxidative stress in SVCV-infected cells.

Discussion: We have tested an in vitro pipeline to screen compound libraries for drug repurposing in aquaculture based on their antioxidant properties. Our data showed that clomethiazole and eugenol can inhibit SVCV replication in vitro at a concentration < 1 µM and protect cells from virally induced oxidative stress.

Role and shifts in Crassostrea gigas microbiome in response to Vibrio aestuarianus infections

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Introduction: Despite recent advances in our understanding of Pacific oyster Crassostrea gigas mortality outbreaks associated with Vibrio aestuarianus infections, there are still considerable gaps in our knowledge. Specifically, the role of the oyster microbiota in response to V. aestuarianus infections remains unclear. The objective of the present study was to investigate the role of the microbiome and its shifts in Pacific oysters C. gigas infected with V. aestuarianus.

Methodology: Adult Pacific oysters C. gigas were kept in individual containers and experimentally infected with V. aestuarianus. Oysters were sampled at predefined dates before clinical signs were exhibited, as well as when exhibiting clinical signs. Water samples were also collected before, after, and during V. aestuarianus shedding. The microbiome composition of both healthy and infected oysters, as well as water samples, was determined by nanopore sequencing of amplicons of the almost complete 16s rRNA gene. Additionally, histopathological analyses were carried out to determine host response.

Results: Analysis of the microbiome of infected oysters revealed that V. aestuarianus was the most abundant taxon. A shift in the microbiome composition was observed in moribund oysters. Although a high V. aestuarianus load was observed in the mantle tissue of infected oysters using qPCR, histopathological analysis did not reveal the presence of canonical bacterial infection using H&E staining.

Conclusions: This study provides new insights into the role of the microbiome and its shifts in C. gigas infected with V. aestuarianus.

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Influence of temperature and cohabitation on the growth of the bryozoan Fredericella sultana in culture for transmission studies of myxozoan parasites

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Myxozoans are extremely morphologically reduced parasites that can represent serious threats to fishes. Their complex life cycles involve invertebrate hosts (annelids, bryozoans) and vertebrate hosts (usually fishes), therefore only few myxozoan life cycles are maintained in laboratories. Culturing the invertebrate hosts is challenging because little is known about their biology. Optimizing myxozoan maintenance in laboratories increases our ability to understand these parasites invasion biology.

At the Fish-Health Division, the life cycle of the myxozoan Tetracapsuloides bryosalmonae, the agent of proliferative kidney disease in salmonids, is maintained. The objectives of this study are 1) to determine the influence of temperature in the growth and development of the bryozoan Fredericella sultana, and 2) identify the effect of fish cohabitation and associated temperature change. Bryozoan colonies were grown at three different temperatures for several weeks.

Firstly, the colonies growth and survival were monitored at the different temperatures over time. Afterwards, the group was exposed for one week to PKD infected fish as well as to a temperature change for two weeks. The water was filtrated and analyzed for T. bryosalmonae stages via qPCR to assess the impact of the parasite on the colony's growth. Colonies held at colder temperatures showed higher viability and smaller size but held at higher temperatures showed less viability and larger size. Cohabitation and temperature change seemed to have a negative effect on the colonies growth.

This thesis provides first insights on the optimization of F. sultana culture in laboratory conditions for transmission studies of a pathogenic salmonid parasite.

Diversity of piscine myocarditis virus (PMCV) lacks a consistent time-place association but displays increasing inter- and intra-host sequence variability

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Introduction: Cardiomyopathy syndrome (CMS) is a severe heart disease in Atlantic salmon. It is considered a major problem for both fish health and welfare and causes economic losses to the industry as it usually affects fish late in the sea phase of production. The disease is caused by piscine myocarditis virus (PMCV). The virus is a relatively simple virus with a genome encoding a capsid protein, an RNA dependent RNA polymerase and in addition a separate third open reading frame (ORF3) following ORFs for capsid and polymerase. Previous studies focusing on smaller parts of the genome or selected proteins, such as ORF3, showed that the overall sequence variability between strains is low. Still, PMCV genome sequences vary both between disease outbreaks as well as between individuals within the same outbreak, although there are some variants that always seem to present. This initiated a need to gather full knowledge on how the virus may vary over the full-length genome, including both protein-encoding and non-coding parts, between individuals in an outbreak and between separate outbreaks. In addition, we have further explored whether there is information related to virulence in the genomic sequences.

Methodology: In a project collaboration with University of Minnesota we have used next generation sequencing (RNAseq, Illumina) to sequence full-length genomes of PMCV from several individuals from each of eight different CMS outbreaks. The samples were collected during 2017 and 2018 and compared to samples from an outbreak in 2011 and the at present only full-length genomic sequence available originating from an outbreak in 2007. In addition, ORF3 have been Sanger sequenced on an extended set of samples from the outbreaks, also including outbreaks from 2019-2020, and analysed through phylogenetic studies involving all available sequences originating from 2007-2020.

Results and conclusion: The results of the full genome sequences confirm that infected fish carry several genomic variants found in the field and that the there is significant diversity both between hosts and within single hosts. There are indications of linkage between genome diversity and disease progression and severity with time.

The effect of Lactiplantibacillus plantarum R2 Biocenol™ on gut microbiota, growth performance and health status of rainbow trout Oncorhynchus mykiss

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Introduction: Intensification of fish production may be connected with outbreaks of diseases as well as with negative effects on the growth parameters of fish. One of the possible ways to cope with these problems seems to be the use of probiotics. Our study aimed to evaluate the effects of diet supplemented with Lactiplantibacillus plantarum R2 Biocenol™ (CCM 8674) isolated from intestine of healthy rainbow trout on specimens of the same species. We also compared the effect of two different feeding strategies.

Methodology: There were 3 groups of fish – the control, the fish fed for 7 weeks by commercial feed with added bacterial cells of L. plantarum R2 Biocenol™ (continuous supplementation) and the fish fed with this enriched feed for 4 weeks, followed by 3 weeks of control feed (initial pulse supplementation). The fish were sampled after 7 weeks and growth performance, gut microbiota, immunological, haematological and plasma biochemical parameters were assessed.

Results: L. plantarum R2 Biocenol™ (CCM 8674) was shown to improve growth performance of rainbow trout when provided by both feeding strategies. Although the effect of the pulse probiotic supplementation on the intestinal microbiome community was not permanent, a statistically significant difference was found in the composition of the bacterial community associated with the mucosa in both probiotic-provided groups of fish. Continuous supplementation significantly increased blood haemoglobin and haematocrit levels and reduced plasmatic chloride concentration. Significantly decreased concentrations of plasmatic magnesium and calcium were revealed in probiotic pulse-fed fish.

Conclusions: With regard to assessed indices, the tested strain can be recommended for further studies concerning its use in rainbow trout aquaculture.

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Microbiological risk assessment of Anisakis spp. in the Atlantic chub mackerel Scomber colias sold at Portuguese markets

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Introduction: Anisakiasis is an emerging fish-borne parasitic disease in humans caused by accidental ingestion of live third stage Anisakis sp. larvae (L3), therefore, the consumption of thermally unprocessed or lightly processed traditional seafood represents a risk of anisakiasis for humans. Being an important item of traditional Mediterranean dishes, Atlantic chub mackerel Scomber colias has been recognised among numerous fish species that harbour anisakids in large numbers. Our objective was to genetically identify Anisakis spp. and determine its occurence in fish collected over three months from the fish market in Porto, Portugal.

Methodology: A total of 101 Atlantic chub mackerels (S. colias) (total mean length 24.8 ± 2.2 SD, range 20.7-31.0 cm) were subjected to UV-press method for visual inspection of fillets and viscera, as it conveniently utilises fluorescence of frozen anisakids. All anisakid L3 isolated from fillets were identified to species level using mitochondrial marker cytochrome oxidase 2 (cox2). The same samples were genotyped using the PCR-based restriction fragment length polymorphism (RFLP). Samples were amplified at the internal transcribed spacer (ITS) locus, digested by HinfI restriction endonuclease and visualised in 2% agarose gel. Species was confirmed according to the RFLP pattern reported for genus Anisakis.

Results: The overal prevalence and mean intensity in the mackerel was 96% and 28.9, respectively, while the prevalence and mean intensity in fillets was 39% and 3.3, respectively. Larvae were identified as A. simplex sensu strictu (55%) and A. pegreffii (37%), and their hybrids (8%). Subsequently, collected epidemiological data used to perform risk assessment of human Anisakis spp. infection, indicated a lower risk of consumption of unprocessed mackerel compared to other pelagic fish frequently consumed, such as sardine and anchovy.

Conclusions: Occurrence of anisakids should be frequently monitored in fish of economic importance sold on local markets, and risk assessments should become a standard tool for interpretation of parasite population parameters, especially under the threat of climate change.

Towards developing an economic model for a health and welfare-oriented fish farm

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Introduction: EU aquaculture has lagged behind global growth since 2007. Cure4Aqua is implementing new research to help the EU catch up and keep pace. Priority innovations include better vaccines, alternative therapies, improved biosecurity, monitoring and welfare, but it is unclear how much each farm operation could benefit from these across multiple dimensions including disease burden, product quality, welfare and financial performance. Alongside operational innovations, we are developing simulation tools to quantify the impact of these innovations and, in due course, guide the prioritisation of innovation adoption.

Methodology: A dynamic simulation model based on seabass fish farm operations and economics will quantify the weight, quality and volume of production and integrate influences from environment, disease outbreaks and management processes. The simulation will be benchmarked and validated against a range of operating farm performances, and then the innovations introduced individually and in combination, to allow us to observe the anticipated performance impact in different disease models, e.g., Aeromonas veronii and Tenacibaculum maritimum. The simulation will enable to test 'what-if' questions specifically about the level of vaccine efficacy and treatment strategies needed to improve operations and translates this to economic value added.

Results: The results so far imply that vaccines alone have the potential to deliver substantial improvements to farm performance, but the impact of this is different for each farm. The simulations show that vaccines can transform a farm from modest or unsustainable profitability to consistently high profitability and significantly improves the well-being of the fish and staff. In one case, a 6x improvement in the value of a farm was demonstrated if disease outbreaks were controlled by the vaccine. Other innovations have widely varying impacts depending on operating conditions, farm sophistication and environment.

Conclusions: Cure4Aqua innovations have great potential, and early simulation results indicate that certain farming operations will benefit disproportionately. The performance difference is sufficient to alter the investment case for fish farming and, if deployed widely, could change the European Aquaculture growth from stagnant to matching the robust growth of global aquaculture. We report this "work in progress" to stimulate interest, attract industry collaboration, and help prioritise our efforts.

Carp sleeping disease, an emerging issue in France

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In December 2021, a mortality event of adult common carp (Cyprinus carpio) was reported for the first time at a water recreation park in eastern France, close to Switzerland. Fishermen reported lethargic behaviour with many carps visible on the surface. The event occurred during a period of strong thaw and dry weather (air temperature 4-5 °C; water 9-10 °C). Approximately one ton of animals (22 – 27 kg) was sent for rendering. No other species present in the lake (pike, catfish, perch, tench, rotengles, salmonids) were affected.

At necropsy, the animals exhibited a good general condition; however, an abnormal excess of mucus and a moderate enophthalmia were noticed. Carp Edema Virus was included in the differential diagnosis: qPCR on gills samples confirmed its presence. The sequencing of the complete P4a gene identified a virus of the genogroup I. This agent responsible for carp sleeping disease is an emerging threat that is still emerging in the country.

A few months earlier, in October 2021, about 200kg of carp (40-50 individuals) purchased from a pond fish retailer had been introduced. This was the first introduction of carp in this lake since 2015. The most likely hypothesis to explain this episode would be an introduction of the disease via the delivery of healthy carrier carp in October 2021 with an outbreak two months later when the environmental conditions became favourable (warmer weather).

The curative measures consisted of collecting dead animals and banning fishing for 6 months, while nautical activities were maintained. However, the surviving fish are potentially a source of contamination for future re-stockings. It is therefore essential to avoid the introduction of naive fish or only in small quantities. The preventive measures concern sustainable introduction of biosecurity measures for fishermen (disinfecting fishing equipment) and the implementation of reinforced monitoring (visual + possible analyses) to detect a possible resumption of mortality.

It is crucial to raise the awareness of all stakeholders of the management of water bodies in order to take appropriate prophylactic measures and reduce the impact of this pathology, which is also harmful to wildlife and tourist activities.

Extracellular products of the probiotic Shewanella putrefaciens Pdp11 affect the regulation of the Photobacterium damselae subsp. Piscicida aip56 gene

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Shewanella putrefaciens Pdp11 (SpPdp11) is a strain described as a probiotic in aquaculture; however, there is no knowledge about its postbiotic potential. Postbiotics are referred to bacterial metabolites, including extracellular products (ECPs), for improving host physiology. Their production can be affected by different factors such as growing conditions. SpPdp11 viable cells have been demonstrated to increase the resistance against challenges with Phtobacterium damselae subsp piscicida (Drp), as well as the capability to interact in vitro by reducing the adhesion of the pathogenic cells to skin and intestinal mucus of farmed fish. Drp is one of the most important pathogens in aquaculture, being an important virulent factor its exotoxin aip56, encoded by a plasmid Drp, responsible for inducing apoptosis of fish macrophages.

The objective of this work was to evaluate the effect of the potential postbiotic of the ECPs of SpPdp11 growth under different cultivation conditions, and their effect on the relative in vitro aip56 gene expression. Then, the best-selected postbiotics will be used to study their effect on the respiratory burst and the apoptogenic activity against sea bass macrophages. The ECPs were obtained using the cellophane sheet technique from TSAs medium (T media), and from solid medium (1.5 % agar) supplemented with aquafeed (F media), partial replacement of aquafeed by 25% of a blend of microalgae (Chlorella fusca, Isochrysis galbana, Nannochloropsis gaditana, and Spirulina platensis) (PM media) and a total blend of microalgae (M media). The plates were incubated at 23 ºC and 15 ºC for 24 and 48 hours. Then, Drp was co-culture by adding the different ECP conditions. The in vitro gene expression was performed by real time-PCR and the in vivo parameters by chemiluminescence and cytometry method for the respiratory burst and the apoptogenic activity, respectively.

Results have shown that some ECP conditions, T1524, T2348 and PM1548, produced a significant down-regulation of aip56, which was corroborated by the in vivo parameters where the respiratory burst and the apoptogenic activity were significantly reduced when the ECPs were co-cultured with Drp.

In conclusion, the culture media affected the bioactivity of ECPs which could be a potential biocontrol agent.

Setting out zebrafish (Danio rerio) as a model to study nervous necrosis virus-host interaction

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Introduction: Viral nervous necrosis is responsible for important economic losses in aquaculture facilities. The causative agent is the nervous necrosis virus (NNV), with an RNA-bipartite genome. Four NNV species have been described, although only RGNNV and SJNNV have been detected in the Mediterranean area. Moreover, RGNNV-SJNNV reassortants have also been isolated from several fish species. In order to design strategies to improve fish resistance to NNV, in vivo studies in commercial and model species are required to study the mechanisms underlying fish susceptibility to viral isolates or species. Zebrafish is a model to study viral infections, as its small size, optical transparency and genome editing tools constitute important advantages. The aim of this work was to set up zebrafish as model of NNV infection. To fulfil this aim, zebrafish susceptibility to three different NNV isolates was determined, and viral replication and innate immune response were characterized.

Material and methods: Three days post-fertilisation zebrafish larvae were infected by intracerebral injection with 107 TCID50/mL of SJ93Nag (SJNNV), Dl956 (RGNNV from seabass), and RG/SJ (from seabream). Larvae were daily monitored for 4 days to record clinical signs and mortality. At 1 and 4 days post-infection (dpi), 3 pools of 6 larvae were sampled for viral genome quantification. Innate immune response was also assessed, focusing on genes related to IFN-I, apoptosis and inflammation signaling pathways. Transcriptional analyses were completed by in vivo 3D imaging approaches on a zebrafish transgenic line expressing GFP in neutrophils (Tg (mpx:GFP) to monitor neutrophils recruitment in brain at 1, 2, and 4 dpi.

Results: RGNNV was the most virulent isolate compared to SJNNV and RG/SJ. These observations were consistent with viral genome replication, as the highest number of viral genome copies was in RGNNV-infected larvae. The immune response, assessed by the induction of immune-related genes and the recruitment of neutrophils in brain, was also higher in RGNNV-infected larvae. Therefore, further experiments can be designed in this successfully established model to better understand the mechanisms underlying NNV virulence in its hosts.

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Effect of glyphosate on the microbiota of rainbow trout, oncorhynchus mykiss

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Introduction: The herbicide glyphosate has been widely used in the past 40 years, under the assumption that side effects were minimal. Glyphosate and co-formulates have been detected in various water sources, but our understanding of their potential effects on aquatic animals is still in its infancy compared with mammals. In this study, we investigated the effect of chronic exposure to an environmentally relevant concentration of glyphosate (pure active substance (AS) and two glyphosate based herbicides - GBHs) on bacterial communities of rainbow trout (Oncorhynchus mykiss, Om).

Methodology: Fish were chemically exposed to glyphosate (G), Round Up Innovert® (R), Viaglif Jardin® (V) and nothing (control C) (one tank with n=12 per condition). They received during 6 months an integrated mean daily expected concentration of approximately 123 ng.L−1 of AS. At 6 months of exposition, water (250 ml, n=3/condition), biofilm (area of about 5 cm2 located in the middle of the submerged area, n = 3/condition) as well as gills, gut content and intestinal epithelium (n=12/condition) were sampled and analyzed by metabarcoding targeting the V3-V4 variable region of the 16S rRNA gene.

Results: Om have their own bacterial communities that differ from their surrounding habitats and possess microbiomes tissue-specific. At the tissue level, the highest richness observed was for the gill (186 ± 54 Operational Taxonomic Unit) in comparison with gut content (55 ± 33) and intestine (16 ± 6). Mean richness values for gill tissue were higher for the control (233 ± 50) than for the exposed conditions (164 ± 40 to 172 ± 47). Glyphosate treatments disrupted microbial taxonomic composition and some bacteria seemed to be sensitive to this environmental pollutant. Lastly, co-occurrence networks showed that microbial interactions in gills tended to decrease with chemical exposure.

Conclusion: Chronic exposure to environmental concentrations of glyphosate or GBHs widely used for several decades may impact the gill microbiota of a fish species of high economic interest. These results open new perspectives for the emerging microbial ecotoxicology discipline. The consequences of glyphosate-induced changes in the gill microbiota remain unknown and require further studies at the functional level.

Hybrids of Anisakis simplex x Anisakis pegreffii and Pseudoterranova decipiens x Pseudoterranova krabbei in cod, Gadus morhua from Baltic Sea

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In the Baltic Sea Anisakidae nematodes are found more abundant in cod, Gadus morhua during over the last decade. Special attention is paid to the presence of zoonotic nematodes in the muscle tissue of cod, which might have implications to human health. One of the aims of this study was to evaluate the level of infection with Anisakis sp. and Pseudoterranova sp. in the muscle tissue of cod sampled in the Polish waters of the southern Baltic Sea. Cod was caught during commercial cruises 2016 - 2017. In total 780 fish were sampled. Fillets of each fish were examined for the presence of nematodes using a white-light transilluminator. Candling revealed the presence of Anisakidae nematodes. All collected nematodes were preliminarily identified to the genus level based on anatomo-morphological features and molecular tools (ITS-1 rDNA). When the distinguishing between species was ambiguous, real-time PCR has been implemented, and the identification was confirmed by sequencing PCR products. The prevalence of cod muscles infection with Anisakidae nematodes was 4.36 %, intensity of infection was up to 5 parasites. Among found Anisakidae larvae, hybrid genotypes of Anisakis simplex x Anisakis pegreffii and Pseudoterranova decipiens x Pseudoterranova krabbei have been detected for the first time in the Baltic Sea. This research was supported by The National Centre for Research and Development under the Strategic Program Biostrateg (grant number BIOSTRATEG2/296211 /4/NCBR/2016).

Contracaecum osculatum and Pseudoterranova sp. in the liver of salmon (Salmo salar) from Polish marine waters

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Over the last decade in the Baltic Sea the dispersion of Anisakidae nematodes (especially Contracaecum osculatum) have been observed. Despite the fact that salmon, Salmo salar, is a popular choice of consumers and therefore one of the most valuable Baltic fish species, the information about the level of infection of salmon liver with these zoonotic nematodes is sparse. In 2020, a total of 120 salmon livers were inspected for the presence of parasites. Livers were digested in artificial gastric juice. All found nematodes were identified based on anatomo - morphological features. Molecular identification was also conducted (ITS-1 rDNA). In total 13% of salmon livers were infected with C. osculatum. Furthermore, a single Pseudoterranova sp. Larva was detected in one salmon liver, representing a host - parasite system that has never previously been reported in the Baltic Sea. (The research was financed from the statutory funds of the National Marine Fisheries Research Institute provided by the Ministry of Science and Higher Education in Poland: DOT21-22-23/ParaSalmon).

Sprat (Sprattus sprattus) as a transmitter of Anisakid nematodes to piscivorous predators in the Baltic Sea

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In the Baltic Sea ecosystem sprat (Sprattus sprattus) is a key fish speciesin the pelagic food web, both as predator on zooplankton and as prey of piscivorous consumers such as cod, salmon and marine mammals. However, sprat may not only be a source of nutrients, but also a transmitter of parasites. We assessed the presence of Anisakid nematodes in sprat from the western Baltic Sea. A total of 140 sprat individuals from Kiel Bight sampled in the first quarter of 2021 were visually inspected for the presence of nematodes during standard ichthyological analyses. Additionally, muscle tissue and all internal organs were digested in artificial gastric juice to reveal the presence of nematodes not detectable by visual inspection. Nematodes were identified based on the their anatomo-morphological features and using molecular analysis (ITS-1 rDNA). Three sprat individuals were infected with Anisakidae nematodes, each fish with one L3 larva. Contracaecum osculatum was found by visual inspection, while Anisakis simplex and Pseudoterranova decipiens were detected after digestion of muscle tissue. This is the first report of sprat infected with Anisakis simplex and Pseudoterranova decipiens in the western Baltic Sea and the first record of Anisakis simplex in Baltic sprat. Sprat is unable to get rid of these nematodes. Despite of the low level of infection with Anisakids, sprat may therefore play a role as transmitter of these nematode parasites to piscivorous predators in the Baltic Sea, considering the large number of

sprat that these predators may consume over their life time. (Funding This work was supported by the European Maritime and Fisheries Fund and The Danish Fisheries Agency (33113-B-20-161)).

Anisakidae nematodes in the liver of European flounder (Platichthys flesus) from the southern Baltic Sea

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The European flounder (Platichthys flesus) natively occurs along the coast of the northeastern Atlantic Ocean and in the Baltic Sea. This euryhaline fish species leads a demersal lifestyle, feeding on benthic invertebrates and small fish. It is presently the third most commercially fished species in the Baltic Sea. Over the past decade, the presence of zoonotic Anisakids has been increasingly reported in numerous fish species across the Baltic Sea, with particular emphasis on the liver nematode Contracaecum spp. Despite the European flounder's significant economic importance, there is a surprising lack of information regarding its infection levels with Anisakidae nematodes. Therefore, the aim of our study was to evaluate the level of European flounder infection with Anisakidae parasite in fish sampled in the Polish waters, southern Baltic Sea. Liver samples were collected from fish caught between 2019 - 2021. In 2019 only visual inspection of 120 livers was conducted and revealed the presence of nematodes. In turn, livers collected in 2019 (n = 51), 2020 (n = 22) and 2021 (n = 104) were digested in artificial gastric juice to revealed the presence of the nematodes. Identification of collected larvae was conducted based on anathomo-morphological features and molecular methods. Prevalence and intensity of infection were calculated only for digested samples. Prevalence of flounder infection with Anisakidae nematodes was 3,92 % in 2019; 9,09 % in 2020 and 9,62 % in 2021; while intensity of infection 1; 1-4; 1-2 respectively. Our findings are consistent with previous research that has shown a rise in the prevalence of nematode parasites, including C. osculatum, among various fish species in the Baltic Sea over the past few years. Moreover, our results further support the idea that the increasing prevalence of Anisakidae nematodes may be linked to a rise in the abundance of grey seals, which serve as the final host in their life cycle.

Survival of 'Pasteurella atlantica gv. salmonicida' in seawater microcosms

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'Pasteurella atlantica genomovar salmonicida' (not yet officially named), has since 2018 been associated with disease and significant mortality in Atlantic salmon farmed along the western coast of Norway. The disease currently affects a relatively limited geographical area and there are concerns over the possibility of further spread. The aim of the present study was therefore, to examine the survival of 'P. atlantica gv. Salmonicida' in seawater at different temperatures under controlled laboratory conditions. The bacterium was inoculated in unsterilized seawater microcosms incubated at 4, 10 and 15 °C and culture and qPCR based estimation of bacterial survival/persistence was performed over a 19 day period. Changes in the total bacterial community were also monitored using Oxford Nanopore 16S rRNA gene amplicon sequencing. While both culture and qPCR based investigations indicated an inverse relationship between increasing incubation temperature and P. atlantica persistence, a relatively rapid decline in both cultivability and number of genome equivalents was apparent at all incubation temperatures tested. In contrast the number of autochthonous bacteria within all studied microcosms increased throughout the experiment. This suggests that 'P. atlantica gv. Salmonicida', which has otherwise relatively fastidious nutritional culture requirements in vitro, probably has a limited ability to survive outside the host organism, and that horizontal sea-borne spread of infection over long distances may be unlikely. The existence and possible role of viable but non-culturable cells (VBNC) in transmission of infection cannot however, be ruled out.

Lysozyme inhibitory effect of Edwardsiella piscicida Ivy and sensitivity of an ivy-deficient strain to host fish

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Intracellular parasitic bacterium such as Edwardsiella piscicida, the causative agent of edwardsiellosis in flounder, are thought to attenuate the immune response in phagosomes upon cell invasion. The vertebrate lysozyme inhibitor gene (ivy) is known to be one of the molecules responsible for this attenuation. However, the lysozyme inhibitory activity of Ivy derived from E. piscicida (Ivy-Ep) and the potential role of Ivy in host infection are unknown. In this study, to clarify the lysozyme inhibitory activity of Ivy-Ep and its involvement in pathogenicity, we tested lysis activity using purified recombinant Ivy-Ep protein (rIvy-Ep), then tested for susceptibility to Japanese flounder (Paralichthys olivaceus) and Japanese medaka (Oryzias latipes), using with an ivy-deficient strain (NUF806Δivy) generated from a pathogenic E. piscicida wild strain (NUF806).

Methodology: The lysozyme inhibition test was evaluated by measuring the effect of rIvy-Ep on the decrease in absorbance of Micrococcus luteus suspension by hen egg white lysozyme (HEWL) over time. Moreover, the ivy sequence of E. piscicida strain NUF806 was deleted using a mobile suicide vector (pRE112) to generate NUF806Δivy. The lysate of NUF806Δivy was used for the inhibition test of HEWL activity in the same manner as rIvy-Ep. Finally, Japanese flounder and medaka were infected by immersion method with NUF806 and NUF806Δivy, and subsequent mortality was observed, and survival rates were calculated according to the Kaplan-Meier method.

Results: The rIvy-Ep inhibited HEWL activity in a concentration-dependent manner. The results of the lysis test showed that the lysozyme inhibitory activity of NUF806Δivy was significantly reduced to the same level as that of the positive control, indicating that Ivy was not functional in this deficient strain. In a susceptibility test against flounder, the survival rate after 30 days of infection was 35.0% for NUF806 and 65.0% for NUF806Δivy. In the test using medaka, the survival rate after 9 days of infection was 0% for NUF806 and 40.0% for NUF806Δivy, indicating a significant difference in survival rates.

Conclusions: These results suggest that Ivy-Ep has lysozyme inhibitory ability and that Ivy is an important virulence factor during host infection in E. piscicida.

Effect of dietary selenium and zinc on the immune response in rainbow trout (Oncorhynchus mykiss)

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Introduction: Dietary immunostimulation represents a valuable method of disease prevention, especially in intensive aquaculture, where frequent exposure of fish to stress may be the cause of immunosuppression. Selenium and zinc are known to have an immunomodulatory effect; however, these effects have not been thoroughly investigated in fish.

Methodology: Selenium (1.5 and 3.0 mg/kg), zinc (15 and 30 mg/kg), and a combination of their higher concentrations (selenium 3.0 mg/kg and zinc 30 mg/kg) were administered to rainbow trout (Oncorhynchus mykiss) in feed for 11 weeks. At week 6, half of the fish from the five selenium/zinc groups and from the control group were experimentally infected with Aeromonas salmonicida. Samples of the head kidney were taken after euthanasia of the fish at weeks 6 and 11 (before and after infection), and a non-specific mitogen-driven, as well as specific antigen-driven lymphocyte proliferation assay, was performed.

Results: With non-specific stimulation, the highest proliferative activity was observed in the groups fed dietary selenium, while the lowest proliferation levels were detected in the control group. The results were similar before and after the experimental infection with A. salmonicida, with the highest non-specific immune response observed first in the group fed the low concentration of dietary selenium and, after the infection, in the group fed the high concentration of dietary selenium. However, with specific stimulation after infection, a significant increase in proliferation levels compared to the control group was observed in the fish fed dietary zinc.

Conclusions: While dietary selenium seemed to induce an increase in the non-specific immune response, dietary zinc was proven to provide a significant enhancement of the specific immune response in rainbow trout. More research to confirm these effects in response to different stimuli, and a complex examination are necessary to prove the positive impact of dietary selenium and zinc on fish health and their suitability for use in aquaculture.

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Experimental infection with 'Pasteurella atlantica gv. Salmonicida' and Pasteurella skyensis in Atlantic salmon – virulence and pathology

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Introduction: In recent years, pasteurellosis has become an increasing problem in salmon farming on the West Coast of Norway. The Norwegian pasteurellosis situation is dominated by the bacterium currently known as 'Pasteurella atlantica gv. Salmonicida' (not yet officially named), although Pasteurella skyensis, responsible for pasteurellosis in Scottish salmon has also been diagnosed.

Methodology: Infection models with Atlantic salmon (Salmo salar) smolt, 58-59 g, using Norwegian isolates of the two species were established. The experimental set up was based on cohabitants held together in seawater (15°C) with either intraperitoneally (i.p.) or intramuscularly (i.m.) injected shedders. Throughout the experiments the fish were supervised twice daily and fish reaching humane end point (HEP) were removed. Samples of fish were taken for bacteriological cultivation, real time PCR and histopathology.

Results: Mortalities were significant in the 'P. atlantica gv. Salmonicida' injected groups, and lower in the P. skyensis injected groups. Mortalities or humane endpoint fish (HEP) among cohabitant fish were only seen in the P. skyensis i.p. experiment (5/20 fish). Only one survivor in each of the two Pasteurella species experiments were infected (one low and one medium dose group). The histopathological study revealed systemic infections with leucocytosis and bacteria present in different organs, especially in kidney and spleen, but also e.g. in gill, pseudobranch and eye. Bacteria were visualized by an immunohistochemical method developed during the study and confirmed by bacterial cultivation and PCR.

Conclusions: The experiments indicate limited horizontal transmission of the two Pasteurella species between Atlantic salmon, but 'P. atlantica gv. Salmonicida' appeared more pathogenic when injected than Pasteurella skyensis, whereas P. skyensis demonstrated better ability to infect naïve fish. When infected, the fish seem to display differences in the pathology produced by the two bacterial species.

Are antibodies used in mammals validated in common teleosts species as immunohistochemical markers?

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Immunohistochemistry (IHC) has become a routine supplement to the classic morphologic approach of investigational pathology in domestic animals. However, comparative immunophenotyping techniques among different species are limited by the low number of validated and/or cross-reactive antibodies.

Immunohistochemistry validation of commercial primary antibodies against intermediate filaments such as cytokeratins (Pan-CK and CK7), glial fibrillary acidic protein (GFAP), desmin and vimentin has never been tested in fish tissues by Western Blot (WB).

The most used commercial primary antibodies against pan-cytokeratin (clone AE1/AE3), CK7 (clone OV-TL 12-3), Desmin (clone M0760), GFAP (clone Z0334) and vimentin (clone V9) were tested for cross-reactivity in a variety of different tissues of European seabass (Dicentrarchus labrax), Gilthead seabream (Sparus aurata), Rainbow trout (Oncorhynchus mikiss) and Goldfish (Carassius auratus) by WB and IHC. After SDS-PAGE, proteins were transferred to nitrocellulose, incubated with primary and secondary antibodies and revealed with chemiluminescent peroxidase substrate. For IHC, histological sections from formalin-fixed and paraffin-embedded tissues (FFPE) underwent heat-based antigen retrieval technique and were incubated with primary and secondary antibodies. 3,3′-Diaminobenzidine (DAB) was used as chromogen. Dog tissues served as a positive control for WB and IHC.

WB using anti-pan cytokeratin antibody showed a band at the expected MW between 52 and 65 kDa in the dog and in all fishes' skin tissues; IHC revealed marked and diffuse staining in fishes' skin FFPE samples.

WB and IHC using anti-desmin antibodies showed a band at 53 kDA and marked staining on skin tissues. WB using anti-GFAP antibody revealed an intense signal at 50 kDA in the fishes' and dog's brain. Western blot using anti-vimentin and anti-CK7 antibodies showed no reactivity in fishes' tissues while a specific WB and IHC signal was noticed in canine tissue.

Commercial anti-pan cytokeratin, desmin and GFAP antibodies showed specific WB bands and IHC stain in the examined tissue of fishes and therefore they are suitable for use in diagnostic pathology settings, while the tested commercial anti-vimentin and anti-CK7 did not cross-react in all fish species tissues examined. We recommend that antibodies used in mammals should be appropriately tested for cross-reactivity by western blot before running IHC in fish.

Effects of Parasites on Food Web Structure and Dynamics: New Ways to Improve Accuracy and Ecological Realism

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Introduction: Scotland's commercial fishing industry is largest in the UK and one of the most productive marine fisheries in Europe. Annually, over one quarter of the total landings is caught from the west coast of Scotland, in 2020 these landings were valued at £142,476,000, thus the ecosystem has a high socioeconomic value. As Scotland moves towards ecosystem-based management for its fisheries it's important to consider the influence of parasitic interactions.

Parasitism is a significant and fundamental consumer strategy in almost all ecosystem communities. Moreover, parasites circumvent metabolically expensive activities such as food-gathering, mobility, and homeostasis, and as such can exhibit higher levels of productivity then free-living species. Since the 1980s there has been a call to include parasites within food web literature. This study aims to identify new ways to include marine parasites within food web models exemplified using Scotland's west coast (ICES fisheries management area 6a).

Methods: The study is focusing on the metazoan parasites of four of Scotland's most commercially valuable species; mackerel (Scomber scombrus), herring (Clupea harengus), anglerfish/monkfish (Lophius piscatorius) and whiting (Merlangius merlangus). Fish dissections and a literature review are being used to identify the main metazoan parasites that could be influencing Scottish fisheries and be detrimental to stock recovery. Fish samples have been collected via two Marine Scotland research trawl surveys in ICES area 6a during 2022. In total 535 fish were collected consisting of 255 mackerel, 222 whiting, 23 anglerfish and 35 herring. Identified parasite species will be input into topological, energetic, and functional food web analyses.

Results: To date 100 mackerel have been dissected. Two highly prevalent parasite species identified through the mackerel dissections are Anisakis simplex (95%) and Kuhnia scombri (74%). Identified parasite species have been input into a topological food web based on Scotland's west coast. Outputs indicate that parasites may be misrepresented as top predators of an ecosystem in food web analyses when only included as consumers.

Conclusion: In conclusion, the study will continue to incorporate parasites in food web analyses to better understand the influence metazoan parasites have on the structure, function, and dynamics of Scotland's west coast ecosystem.

Bacteria isolated from fish infested with Ichthyophthirius multifiliis

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Introduction: Ichthyophthirius multifiliis is a common external parasite of fish. Due to its excellent adaptation to water environment, it is a constant threat to farmed fish. The infestation may result in disease and be a direct cause of fish deaths or reduce immunity and overall condition, predisposing to bacterial infections.
The aim of this study was to analyse the results of clinical examinations of fish and determine how often and which bacteria were isolated in cases of Ichthyophthirius multifiliis infestation.

Methodology: The degree of infestation was described according to the following scheme: single parasites - 1 to 3 parasites in the whole microscopic slide (+), few parasites - 1 to 3 parasites in the field of view (++), numerous parasites - 4 to 10 parasites in the field of view (+++), very numerous parasites - more than 10 parasites in the field of view (uncountable) (++++). A degree of intensity rated as (+) and (++) was considered as carrier state. An infestation of degree (+++) and (++++) was classified as a disease, regardless of whether clinical signs were present. Bacteriological tests were conducted by isolating bacteria on culture media and then identified using API20E tests.

Results: 524 batches of fish were analysed. Presence of Ichthyophthirius multifiliis (ICH+) was confirmed in 16.6% of samples, of which 85% were classified as carrier and 15% as disease. Bacteria were isolated from 24% of ICH+ cases. Aeromonas hydrophila, Pseudomonas fluorescens, Flavobacterium spp and Chryseobacterium indologenes were most frequently isolated bacteria. In 85% of cases, the bacteria were isolated in cases of ICH+ carriers and in 15% from fish with intense invasion, which was classified as disease.

Conclusions: Ichthyophthirius multifiliis is a vector as well as a reservoir of potentially pathogenic microorganisms. Tissue damage by the parasite is thought to promote bacterial infections, as well as invasions by other parasites. The results presented here demonstrate the need for parallel bacteriological testing in cases of Ichthyophthirius multifiliis infestation, as associated bacteria may increase fish mortality in the course of this infestation.

Ultrastructurally diagnosed Mycoplasma-like organism in erythrocytes of anaemic cyprinid hybrids

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Introduction: The present study is based on a severe haemolytic anaemia, sporadically diagnosed in laboratorybred cyprinid hybrids. Results of an etiological study of this disease condition are included.

Methodology: Eight laboratory-reared cyprinid hybrids were included in the study. The fish were examined using fresh materials and Diff Quick-stained blood smears. Next, complete diagnose examination including histology, transmission electron microscopy, microbiological examinations and molecular analysis was used to verify tentative taxonomic assignment of a suspected Mycoplasma-like agent.

Results: The hybrids displayed signs of suboptimal health status and by clearly visible signs of anaemia. The primary role in the development of anaemia was assigned to the Mycoplasma-like organism due to its regular occurrence in erythrocytes both in moribund and fish in early state of the disease. Examination of blood smears revealed a low number of blood cells surrounded by small coccoid organisms. These organisms dominated in ascitic fluid examined using TEM and their ultrastructure resembled yeast organisms.

Conclusions: Novel data on the Mycoplasma-like organism's cytoskeleton were obtained from ultrathin sections of affected erythrocytes. The yeast organism (suspected Cryptococcus sp.) found in ascitic fluid most likely should acts as a concurrent agent.

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Individual approach to fish patients using imaging techniques and surgery

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Introduction: Diagnosing diseases and health conditions in fish almost always involves euthanasia of the individual so that it can undergo a comprehensive examination including invasive sampling, to determine the cause of the health problem. This is followed by specific therapy of fish in the affected population. In some cases, however, it is possible to take an individual approach and attempt a tailored therapy.

Methodology: Here we describe individual curative treatments of two koi carp adults and examination to determine the cause of skull shape changes in rainbow trout. Diagnostic imaging techniques used were X-ray and ultrasound.

Results: The first koi carp had a tumour on the dorsal region of the head in the shape of a spherical canopy with a diameter of about 4 cm and a height of 3 cm. In a sedated fish (clove oil), the mass was ligated and cut away using a surgical suture. The carp was released back into the tank shortly after the procedure. The tumour was histologically classified as a papilloma.

In the other koi carp, the body cavity was gradually enlarging over a period of 6 months. X-ray and ultrasound examination revealed the presence of a tumorous cystic mass within the body cavity. The fish underwent surgery under general anaesthesia (Alfaxalonum). After removal of two masses weighing approximately 750 g, the body cavity was flushed with sterile physiological saline and closed routinely using absorbable monofilament polydioxanone suture. The carp was injected intramuscularly with oxytetracycline and returned to the tank. One hour later, it assumed physiological position and remained at the bottom of the tank. However, after three days, the fish died of sepsis. Bacterial cultivation from the spleen showed massive presence of oxytetracycline-resistant Aeromonas bestiarum.

Adult rainbow trout reared separately in aquaria gradually developed skull deformities. Several individuals with significant deformities were subjected to X-ray examination, revealing soft tissue tumours. The cause of these deformities remains unclear.

Conclusions: Individual curative treatments based on examination with modern imaging techniques are feasible in the fish. However, this approach is not among routine procedures and similar cases are rather sporadic.
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Effect of water treatment with CaviPlasma on survival of disease agents of fish

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Background: This work was aimed at testing a new patented technology named CaviPlasma which was designed for purifying water from microorganisms and pollutants. Biocidal effect of treatment with CaviPlasma was tested on water contaminated with three bacterial pathogens of fish and with parasitic ciliate Ichthyophthirius multifiliis.

Methodology: In Experiment 1, three species of bacteria (Aeromonas salmonicida, Aeromonas hydrophila and Flavobacterium psychrophillum) were suspended in tap water and treated with CaviPlasma. After the treatment, bacterial suspensions were stored at 17 °C and concentration of bacteria was regularly measured using plate count method. Untreated bacterial suspensions served as controls. In Experiment 2, tap water containing invasive stages of Ichthyophthirius multifiliis was treated with CaviPlasma and added at 10% concentration into fish tanks with juvenile common catfish (Silurus glanis). In positive control, untreated water with parasites was added into tanks. After five days, fish were euthanized, and their skin and gills were examined for the presence of parasites.

Results: In Experiment 1, water treatment with CaviPlasma resulted in devitalization of all tested bacterial species. In Experiment 2, adding of untreated water with I. multifiliis into the tanks resulted in the development of severe ichthyophthiriasis in fish. In fish from tanks with CaviPlasma-treated water, no parasites were found on skin or gills and no mortality was observed.

Conclusions: Water treatment with CaviPlasma proved to be effective against three fish bacterial pathogens and against a parasite Ichthyophthirius multifiliis. Effect of CaviPlasma-treated water on fish health, growth performance and microbial community of biofilters remains to be tested.
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Pilot study of behavioural analysis in European sea bass (Dicentrarchus labrax) infected with VNNv

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Introduction: The study of animal behavior is a sector that is attracting increasing interest from the scientific community, also thanks to the new technologies available to researchers. In recent years, fish have also entered this type of study, despite the difficulties of observation due to the aquatic medium.

Materials and methods: In this pilot study, the critical points in setting up studies of this kind in an experimental aquarium were evaluated and the applicability of the system to observe the behavior of sea bass (Dicentrarchus labrax) infected and not with Betanodavirus was evaluated by daily video recording of 2 tanks and subsequent analysis using dedicated software (EthoVision XT, Noldus Information Technology, Wageningen, The Netherlands).

Results: The results highlighted several critical issues in the experimental set-up, both at the hardware level (lighting, aerosol, humidity) and at the software level (reflections, shadows, individual tracking). Concerning behavioral analysis, the infected fish had a lower movement speed and distance covered and a higher state of inactivity than the control group. In addition, the infected fish showed greater dispersion within the tank than the control group.

Conclusions: These results highlight how the progression of the disease under examination influences the behavior of fish, beyond the clinical signs, providing interesting and useful predictive tools for the improvement of animal welfare and the management of diseases.

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Molecular detection of a novel cyprinid herpesvirus in koi carp closely related to cyprinid herpesvirus 3 (CyHV-3)

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Introduction: During spring of 2021 and 2022, in the lake of Castel Trauttmansdorff (Bolzano, Italy), several koi carps (Cyprinus carpio koi) were found showing ulcerative and proliferative skin lesions on the head and at the base of the dorsal fins, as well as, diffused pale discoloration of the skin. However, no mortality events were recorded.

Methodology: Symptomatic fish were collected and tissue samples of target organs were subjected to histological examinations and to bacterial and virus detection.

Results: Histology: skin lesions highlighted neoplastic dermal alteration, attributable to peripheral nerve sheath tumor (PNST), not infiltrating the hypodermis and characterized by numerous mitotic figures; moderate presence of chronic inflammatory infiltrate and neoangiogenesis. Bacteriology: ubiquitous microorganisms (Aeromonas spp.) were isolated. Virology: samples tested negative for KHV, CEV and SVCV PCRs, while some were positive for CyHV nested PCR. Sequence analysis of partial DNA polymerase (Pol) and major capsid protein (MCP) genes of almost all positive samples showed higher nucleotide similarity with CyHV-3 strains, even though percentages were not particularly high (83-85% and 87-90%, respectively). Maximum likelihood (ML) phylogenetic analysis, based on partial MCP nucleotide sequences, placed one sample within the CyHV-1 genotype, but more interestingly, all other samples grouped together without clustering into any of the genotypes already described. This latter result was further confirmed by the ML phylogenies based on partial Pol nucleotide sequences, as well as, on the concatenated partial nucleotide sequences of both genes. Lastly, the presence of a putative novel CyHV was corroborated through haplotype network analysis, which highlighted a large number of characterizing mutations.

Conclusions: A putatively novel CyHV from koi carps was identified and genetically characterized. The association between virus and skin lesions suggests that it can be the causative agent of the disease as no other evident environmental agents or pathogens were reported. Despite this virus showed a close relation with CyHV-3 genotype, koi did not exhibit any typical clinical sign compatible with KHVD. Further investigations are necessary to understand whether the observed lesions were caused by the virus and to confirm the identification of this new CyHV genotype.

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First record of Pseudoterranova decipiens in sprat (Sprattus sprattus) from the Baltic Sea

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Over the last decade in the Baltic Sea a dispersion of Anisakidae nematodes among marine organisms has been reported. This is in line with an increase in the number of grey seal - final host indispensable for the completion of the life cycles of Contracaecum osculatum and Pseudoterranova decipiens. Most attention has been paid to these zoonotic nematode species, which have been noted in commercially important fish in the area. Many reports describe the presence of C. osculatum in the Baltic Sea organisms. Contrary, little is known about the spread and transmission of Pseudoterranova sp. in the Baltic Sea. The aim of this study was to investigate whether sprat may play a role as a transport host for this Anisakidae. Samples were collected in three areas of the southern Baltic Sea (south and east of Bornholm, Słupsk Farrow and Gulf of Gdańsk) during a research cruise in August 2019. Visual inspection of the viscera of 556 sprats was conducted. Parasites were identified using anatomo-morphological and molecular (ITS-1 rDNA) methods. Nematodes were recorded only in sprat caught southeast of Bornholm (prevalence 2.7%; intensity of infection 1-4; abundance 0.05). Molecular identification revealed the presence of Pseudoterranova decipiens. This is the first report of P. decipiens in sprat from the Baltic Sea. Sprat is likely a transmitter of P. decipiens in the Baltic Sea food web.

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Polish report of European eel infection with Anguillicola crassus 2014 - 2022

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The nematode Anguillicola crassus is a common parasite of European eels swim bladder.The aim of the studies was to evaluate the level of eel infection with A. crassus in the Polish EEZ, southern Baltic Sea. Fish has been examined each year between 2014 and 2022, in total 4726 individuals. Parasitological analysis focused on the presence of nematode A. crassus has been performed. The total number of found parasites was 21 370. The correlations between prevalence of infection and infection intensity vs host length, Fulton condition factor, age of the fish, area and time of sampling have been analyzed. The prevalence and intensity of infection have been calculated. Intensity of infection varied from 1 to 95 parasites per fish. We observed decreasing trend of the mean prevalence of A. crassus infection between 2014 and 2020, however, in years 2021-2022 the trend was opposite. Mean prevalence of infection reported previously from Polish waters in 2000-2002 was 73.6-76.2%.

Diet composition and presence of zoonotic nematodes in the liver of Atlantic mackerel (Scomber scombrus) from southern Baltic Sea

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The Atlantic mackerel Scomber scombrus is an epipelagic and mesodemersal fish species that is most abundant in cold sea areas. It is an important fish species for world fisheries, however, in Baltic Sea is not numerous, therefore is not significant for fisheries industry. Anisakis larvae are quite common in fish such as salmon, mackerel and cod. Nevertheless, the knowledge about diet and parasite fauna of that fish species from the southern Baltic Sea is scarce, especially in case of Polish waters. Generally, Anisakidae nematode can migrate to muscle tissue or to the liver. The aim of the studies was to evaluate the diet composition and the presence of Anisakidae nematodes in the liver of Atlantic mackerel sampled in Polish waters, southern Baltic Sea. The biological material was collected during research cruises in 2016 and 2017. Standard ichthyologic analyses were performed. The stomach analysis was performed and all found organisms have been identified to the lowest possible taxonomic level. The liver of each fish was digested in artificial stomach juice to reveal the presence of parasites. All found parasites were identified on the basis of anatomo-morphological features and using molecular methods. We present and discuss the results in relation to previous findings. The main diet component of Atlantic mackerel was sprat Sprattus sprattus. This is the first record of Anisakis sp. in Scomber scombrus from the southern Baltic Sea.

Nematode parasite infection of fourbeard rockling Enchelyopus cimbrius from the southern Baltic Sea

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The fourbeard rockling, Enchelyopus cimbrius is gadoid fish, common living and reproducing in the Baltic Sea. Due to the fact, that it is non-commercial fish, little is known about the biology of that species. However, non-commercial fish species are important, because they are inseparable part of the ecosystem. They could play a role as a host or transmitter of parasites to commercial fish species. Especial attention should be paid to monitoring of the zoonotic parasite, like Anisakidae nematodes. The aim of the studies was to evaluate the prevalence of infection with Anisakids in muscle tissue of fourbeard rockling from the southern Baltic Sea.

Research material was collected during research cruise. Standard ichthyologic analysis were performered. Muscles were digested in artificial digestive juice to reveal the presence of parasites. Identification of parasites was conducted based on anatomo-morphological features and using molecular methods. This is the first record of A. simplex in fourbeard rockling from the Baltic Sea. Fourbeard rockling is another example of a transmitter of A. simplex in the Baltic Sea food web.

Diet composition of whiting from the western Baltic Sea – potential links to their parasite fauna

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Whiting (Merlangius merlangus, Gadidae) is a demersal fish species, inhabiting the Northeast Atlantic Ocean and the brackish western Baltic Sea. Dietary analyses allow to identify food items that are potential sources of parasite infection. Information on the diet and parasite fauna of whiting from brackish waters are scarce. We assessed the diet composition and parasite fauna of the whiting sampled in the western Baltic Sea. The diet composition of 343 fish sampled in Q4 2020 and 57 fish sampled in Q1 2021 was determined based on stomach content analysis. Food items were identified to the lowest possible taxonomic level. The parasitological analysis focused on the presence of parasites in muscle tissue (transilluminator), liver (digestion in gastric juice) and intestines (visual inspection). Parasites were identified to the genus level based on anatomo-morphological characteristics and molecular analysis. The diet of whiting was dominated by fish (mainly Gobiidae and Clupeidae, i.e. sprat and herring). The Cumacea Diastylis rathkei was an abundant invertebrate; Crangon crangon, Neomysis integer and Mysis mixta were less important Crustaceans. In one stomach nematodes were found among food items. Transillumination of the fillets revealed larval stages of Anisakid nematodes. Livers were digested to detect these parasites. Three representants of nematodes were observed in livers – Contracaecum sp., Anisakis sp., and Hysterothylacium sp. The prevalence of infection was respectively 5,25%; 0,5%; 0,25%. In the digestive tract, only Acanthocephala was found. Sprat and Crangon crangon are known transmitters of Anisakid nematodes to piscivores in the Baltic Sea and are likely sources of whiting infection.

Elevated levels of biliary PAH metabolites and liver lesions in flounder Platichthys flesus from the southern Baltic Sea

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Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous environmental pollutants that can lead to a wide range of physiological dysfunctions in fish, including neoplasia. Monitoring of PAH is used in several environmental programs to assess the biological effects of contaminants. Livers of flounder Platichthys flesus sampled in the Inner Gulf of Gdansk and Outer Puck Bay were examined for the presence of histopathological alterations. Bile samples were taken from the same individuals and analysed for PAH metabolites by high performance liquid chromatography with fluorescence detection (HPLC-F). The concentrations of the following PAH metabolites were determined in the bile of flounder: 2OH Naphthalene, 1OH Naphthalene, 1OH Phenanthrene, 1OH Pyrene, 1OH Chrysene and 3OH Benzo[a]pyrene. Measured concentrations of two PAH metabolites (1OH Phenanthrene and 1OH Pyrene) were compared to internationally agreed background assessment criteria (BAC) and environmental assessment criteria (EAC). Two categories of liver lesions were most frequently found in examined flounder: non-specific inflammatory changes (95.7%) and early toxicopathic non-neoplastic lesions (85.5%). Pre-neoplastic lesions were detected in 26.5% of fish. Less frequent were malignant neoplasms (4.3%) and benign tumors (3.4%). Obtained results revealed associations between exposure to PAHs and the presence of liver lesions in flounder. The highest concentrations of 1OH Phenanthrene, above the BAC value, were reported in flounder with malignant neoplasms (42.9 ng/mL bile) and pre-neoplastic lesions (21.6 ng/mL bile). Fish with malignant neoplasms exhibited extremely high mean concentration of 1OH Pyrene (2422.4 ng/mL bile), many times exceeding the EAC. Financial support for this study was provided by the Chief Inspectorate for Environmental Protection under The State Environmental Monitoring (SEM).

Effect of 2-bromopalmitate and verteporfin on viral replication and persistence in EPC cells

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Innate immune response is the first line of defense against viral pathogens. In fish it is a fundamental arm of the immune system. To get further insight on this issue the effects of two compounds with potential capacity to imbalance the innate immune response pathway have been analyzed in the cyprinid cell line EPC as well as in EPC cells persistently infected with infectious pancreatic necrosis virus (IPNV). 2-Bromopalmitate (2-BP), an inhibitor of protein acylation, can affect both the palmytoilation of viral glycoproteins as well as the acylation of some proteins in the interferon response system. Verteporfin (VTPF) has been reported as an inhibitor of the Hippo-YAP pathway which is involved in cell proliferation and apoptosis. 2-BP showed a mild inhibitory effect on viral replication, while inducing a noticeable increase in syncytia formation by SVCV-infected cells. Treatment of the cells with VTPF before infection with spring viremia of carp virus (SVCV) or viral hemorrhagic septicemia virus (VHSV) did not have a significant effect on viral replication. In the IPNV-carrier EPC cells VTPF disrupted the balance on IPNV persistence, enhancing IPNV replication that correlated with the downregulation of the interferon/mx response.

Population dynamic of epicaridean bopyrid anuropodiona amphiandra parasitizing iridonia speciosa in the eastern Adriatic sea

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Introduction: This study investigated the seasonal population dynamic of epicaridean bopyrid Anuropodiona amphiandra parasitizing the squat lobster Iridonia speciosa. In the Eastern Central Adriatic Sea, Petrić et al. (2010) reported a 7.85% prevalence of this bopyrid in the squat lobster population with evidenced growth inhibition, modification of secondary sex characteristics, retardation of oogenesis, and failure of oviposition as repercussions of bopyrid infestation. We aimed to investigate whether there has been a change in the rate of bopyrid infestation.

Methodology: Sampling was carried out in the eastern central Adriatic Sea from June 2019 to May 2020. Squat lobsters were collected at depths from 120 to 200 m using a commercial bottom trawl. Specimens were frozen on board and transported to the laboratory for further analysis. Sex was determined by the position of gonopores and the presence of first and second pleopods. Individuals with both male and female secondary sexual characters were classified as morphological intersexes. The presence of the parasite and its position on the left and right side of the cephalothorax was noted.

Results: A total of 1494 individuals of squat lobster were collected. The sex ratio of the hosts differed statistically, in favour of males. Of the total 274 infected individuals, 166 were males, 67 females and 41 intersexes. The overall prevalence of bopyrid was 18.3%. The average intensity was 1.00, and the average abundance was 0.18. A statistically significant difference in prevalence was found between the sexes, with a higher infection rate among males compared to females. For both sexes, a seasonal fluctuation in the prevalence was recorded. The highest prevalence of 20.7% was recorded during autumn and the lowest of 15.9% during spring. However, these differences were not statistically significant.

Conclusions: In the studied area, over a ten-year period, we noted a higher rate of infection of the bopyrid A. amphiandra on squat lobster I. speciosa with an increase of intersex individuals and increasing feminization of males. Considering the increase in prevalence, future research should focus on determining the impact on the host reproductive output.

First report of philometroides sanguineus (nematoda: philometridae) in farmed goldfish (carassius auratus) in Italy

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Introduction: The ornamental fish culture and trade represents a sector of great economic interest on a global level, leading ornamental fish species to be the most translocated animals in the world. A frequent consequence of these movements is the translocation of transmissible pathogens in geographical areas where they are not usually present.

Here we report for the first time in Italy the presence of Philometroides sanguineus (Nematoda, Philometridae) in goldfish (Carassius auratus) farmed in northern Italy.

Methodology: After some oral reports on a new goldfish parasitic disease ("red worms in the tail") from consultants and operators working in the Italian ornamental fish sector, in March 2023 ten goldfish (5 alive and 5 dead) were sent to the Fish Pathology Unit of DIMEVET for parasitological analysis. All live fish showed the presence of 1-3 reddish round worms that moved slowly in the thickness of the skin between the bony rays of the caudal fin. Some of them were straight and others were arranged in a “U” shape with the ends directed toward the caudal margin of the fin. After sedating the fish by anaesthetic (MS-222), the worms were extracted by gentle pressure, and fixed in alcohol 70% or in 10% buffered formalin to be subjected to analyses useful for species identification. The dead fish were subjected to full parasitological examination.

Results: The morphological studies allowed to identify all the parasites isolated from the goldfish examined as gravid females (length 3,6-6 cm, width 0,9-1,1 mm) of Philometroides sanguineus. The complete parasitological examination of 5 goldfish did not allow to find males or developmental stages of the parasite in the internal organs.

Conclusion: Philometroides sanguineus is a non-zoonotic nematode species already described in the literature as specific to the genus Carassius and initially reported in Eastern Europe, then in other European and extra-European countries following fish translocations. In light of the high economic value of goldfish farming in northern Italy, epidemiological investigations should be urgently conducted to define the current distribution of this newly introduced parasite and assess the risks for national fish populations.

Sodium percarbonate and peraceid acid application affects the diagnostics of ichthyophthirius multifiliis in rainbow trout (oncorhynchus mykiss) via real-time PCR

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Background: The parasitic ciliate Ichthyophthirius multifiliis (Ich) has the ability to cause significant economic losses in aquaculture of rainbow trout (Oncorhynchus mykiss), with untreated infections resulting in up to 100% mortalities. Current diagnostics of the parasite is based on pathoanatomic signs recognition followed by microscopic examination, with non-invasive methods being developed, such as parasite DNA identification in water.

The management of Ich relies mostly on water disinfection, with sodium percarbonate and peraceid acid representing the most common chemicals used.

Methodology: Flow-trough farm with concrete raceways was monitored weekly. Water temperature, water quality and application of disinfectants was noted. At each sampling, five specimen of rainbow trout (4-6 cm, 8-12 g) were microscopically examined for the presence of ectoparasites. Additionally, 1 L of water was filtered (5,0 µm MCE Membrane), the filter dissolved in acetone and total DNA was extracted with the QIAamp DNA Mini Kit (Qiagen). The same kit was used to extract DNA from mucus and gill tissue of fish. Extracted DNA was analysed for the presence of Ich via real-time PCR using QuantiTec SYBR Green PCR kit (Qiagen).

Results: All three methods (microscopy, real-time PCR from filtered water and skin mucus) showed the presence of Ich one day after the reported onset of increased mortality. After a 5-day peraceic acid treatment, Ich was only detected on the surface of the fish (via PCR and microscope). After return of clinical symptoms, sodium percarbonate was applied, with diagnostics result identical to the peraceic acid application.

Conclusions: Real-time PCR detection of Ich DNA from water was significantly affected by the application of sodium percarbonate and peraceid acid, yielding negative results one and two day after treatment, respectively. The presence of parasite was confirmed at the same time points via real-time PCR from mucus and gills and by the conventional microscopic examination of fish.

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Prevalence of gill epitheliocystis in Atlantic salmon reared in freshwater flow-through and recirculation hatcheries

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Epitheliocystis, an intracellular bacterial infection in the gills and skin epithelium, has been frequently reported in Atlantic salmon (Salmo salar) during freshwater production in a number of countries. This study describes the prevalence and intensity of a natural epitheliocystis infection present in the gills of two strains of Atlantic salmon reared in either a flow-through (FT) or a recirculation aquaculture system (RAS) in Ireland. Repeated sampling of gills prior to and throughout seawater transfer, histology and quantitative real-time PCR were used to determine infection prevalence and intensity. Despite no clinical gill disease, and minor histopathological changes, epitheliocystis lesions were identified in histology sections at all time points. Specific PCR confirmed the presence of Candidatus Clavichlamydia salmonicola in both fish strains and its number of copies was correlated with intensity of epitheliocystis lesions. A significant interaction between hatchery system and fish strain on the prevalence and intensity of gill epitheliocystis was found both using histological and molecular methods. Specifically, fish from FT had higher prevalence and intensity than RAS reared fish and within FT, the Irish cohort were more affected than Icelandic.

Immunomodulatory effects of dietary tryptophan supplementation in gilthead seabream (Sparus aurata)

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Amino acids (AA), besides being building blocks for protein synthesis, are known to be essential for the synthesis of many key metabolites. As such, their inclusion in modern feed formulations with functional purposes seems to have potential. Tryptophan (Trp) in particular is a precursor of several important metabolites that can regulate immune response, modulate behavior, and stress response in fish (e.g. serotonin, melatonin). The present study aimed to evaluate the effects of dietary Trp supplementation on the gilthead seabream (Sparus aurata) immune status and inflammatory response. Triplicate groups of fish (6.28 ± 0.28 g) were either fed a control diet (CTRL) with a balanced AA profile, or the CTRL diet supplemented with graded levels of Trp (i.e. 0.5% and 1% of feed, TRP1 and TRP2, respectively) for a 4-week feeding period. After 2 and 4 weeks, fish were euthanized and blood was collected for blood smears, plasma for humoral immune parameters, whole gut for oxidative stress biomarkers, as well as anterior gut and hypophyses for the measurement of immune and stress-related transcripts. After the 4-week feeding period, fish were intraperitoneal injected with inactivated bacteria and the inflammatory insult was monitored with samplings at 4-, 24- and 48-hours post-insult. Dietary Trp supplementation did not significantly alter the analyzed responses during the feeding trial. In contrast to the lack of effects observed during the feeding trial, it was observed that gut catalase activity increased in fish fed TRP2 upon the inflammatory response (after 4 hours) and gradually decrease after 24 hours reaching a minimum level 48 hours post-insult. This decrease in catalase enzyme activity at the intestinal level might be related to the inflammatory insult. This antioxidant enzyme seems to be allocated to the inflammatory focus (the peritoneal cavity), concomitantly with a general increase of peripheral leucocytes (particularly neutrophils and leucocytes). Moreover, hypophyseal pomca1 expression significantly decreased after 48h post-insult in fish fed TRP2 suggesting mediation and regulation of the stress response. In summary, this study points to a putative immunotolerance role of tryptophan, particularly before immune stimulation, mostly verified by a modulation of oxidative stress and health-biomarkers in the anterior gut.

Characterization of bacteria in commercial probiotics used for aquaculture in Bangladesh to assess concerns over safety

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Introduction: Infectious disease is the prime factor that reduces aquaculture productivity throughout the world. Administration of antibiotics to prevent such losses is common practice in several countries, including Bangladesh. However, inappropriate use of antibiotics can lead to bacterial antibiotic resistance, which is a major One Health concern. Hence, alternatives to prevent disease are being encouraged, with probiotics offering a potential solution. Probiotics contain live microorganisms that, when administered in adequate amounts, may confer health benefits on the host. However, their safety and effectiveness, including questions regarding contamination by other bacterial strains, are issues of concern. This study aimed to isolate and characterize bacteria from probiotics commercially available for aquaculture purposes in Bangladesh, with a view to assessing the possible hazards.

Methodology: Thirty-six products were obtained from Bangladesh, and label information was collected. Bacteria from 15/36 products were isolated by culturing on two agar media, tryptone soy agar (TSA) and De Man, Rogosa and Sharpe agar (MRS), at 28ºC for 24 to 48 hours. Distinct colonies in each product were sub-cultured to purity. Isolates were identified to genus and species levels by primary identification tests (i.e., Gram staining, catalase, oxidase, and motility assays), and 16S rRNA and gyrB gene sequencing. Antibiotic susceptibility and in vitro phenotypic tests of virulence properties were conducted for a subset of 29 isolates.

Results: In total, 42 isolates were cultured to purity from the products. After identification, there were discrepancies between the species declared on the label and those cultured from each product. Two products claiming to contain four species, including Bacillus spp. and Lactobacillus spp., showed no bacterial growth, indicating a possible issue with viability. Overall, 34% of the isolates investigated (10/29) were resistant to at least one of the six antibiotics tested. Furthermore, 48% of isolates (14/29) showed hemolytic activity, while 66% (19/29), 97% (28/29) and 79% (23/29) showed protease, DNase, and gelatinase activities respectively, suggesting they possess potential virulence factors.

Conclusion: Our findings raise questions over the safety and reliability of commercially available probiotics in Bangladesh. Ongoing work is evaluating the feasibility of identifying safe products based on label information.

Surveillance of gyrodactylus parasites in salmonids in the river tornionjoki

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Introduction: Tornionjoki river in the border of Finland and Sweden is the most important spawning river for wild Baltic salmon (Salmo salar). Among Gyrodactylus parasites Gyrodactylus salaris is a dangerous parasite for the salmon strains of the rivers descending into the Atlantic and Arctic Ocean, but no similar effect has been observed in the salmon strains of the rivers descending to Baltic Sea, like River Tornionjoki.

The occurrence study of the G. salaris parasite in the River Tornionjoki from years 2000-2004 has been published. They concluded that especially three factors affected the prevalence of the parasite in the samples collected: the watercourse section sampled, the age of the sampled parr and water temperature during sampling. Surveillance of the G. salaris has continued in the River Tornionjoki also in 2012-2022.

Methodology: Approximately 60 salmon parr were electrofished from four different watercourse sections of the River Tornionjoki in every 1-2 years between 2012 and 2022. Samples were preserved in ethanol and examined under a dissecting microscope in the laboratory for number of Gyrodactylus parasites. If Gyrodactylus parasites were detected, they were counted and some of them were detached from the fish and preserved in ethanol for the PCR confirmation of the species. At first the whole parasite was lysed with Proteinase K treatment and the lysate was used as template to the amplification of ITS1 and CO1 gene regions.

Results: In the beginning of the surveillance Gyrodactylus parasites were observed in 15-48 % of the fish sampled in four different watercourse sections of the river Tornionjoki, while since year 2020 to 2022 it has been observed only in 0-2 % of fish. Considerable decrease can also be seen in the prevalence of Gyrodactylus parasites compared to 2000-2004 study results. In PCR analysis some of the samples were detected as Gyrodactylus salaris.

Conclusions: The occurrence of the Gyrodactylus parasites in salmon parr was found to be reduced, which can also be attributed to a decrease in the number of infections with Gyrodactylus salaris. The factors affecting the prevalence of Gyrodactylus parasites will be discussed in the poster presentation.

Efficacy of dietary praziquantel against important monogeneans infections in Mediterranean farmed fish

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Introduction: Praziquantel (PZQ) is a synthetic broad-spectrum antiparasitic, widely used in veterinary and human and aquaculture medicine. Numerous studies have shown a high anthelmintic efficacy of dietary PZQ in farmed fish. Some palatability issues raised with the use PZQ-medicated diets are commonly overcome with dietary masking agents and specific feeding management. The aim of the present study was to evaluate the efficacy of PZQ against important monogeneans (Sparicotyle chrysophrii, Zeuxapta seriolae) of commercialized Mediterranean farmed fish (gilthead seabream, greater amberjack), as an attempt to replace the laborious, time consuming and weather-dependent chemical baths (formalin, oxygen peroxide).

Methodology: The field experiments were carried out during warm water temperatures (24-26oC) in small cages (3 m3) located in two different farming sites. Greater amberjack (125 g) and gilthead seabream (62 g) infected with Z. seriolae and S. chrysophrii, respectively, were orally given 150 mg PZQ/kg fish for three days. The parasitic load on the treated fish (triplicate) was measured on the outer gill arches and compared against control groups.

Results: PZQ-medicated diets were accepted by the experimental fish. Measurement of both adults and oncomiracidia in sampled gill arches revealed significant parasitic reduction between the tested groups in both cases (3.8 ± 6.3 vs. 19.3 ± 9.8 in amberjack and 1.5 ± 1.9 vs. 11.3 ± 10.2 in gilthead seabream) and a calculated efficacy >80%.

Conclusions: Dietary-administered PZQ (150 mg/kg fish for three days) appeared very effective against important monogenean infections in Mediterranean farmed fish.

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Life cycle dynamics of a myxosporean parasite (Cnidaria, Myxozoa) infecting the urinary bladder of reared zebra seabream Diplodus cervinus

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Introduction: Myxosporeans are endoparasitic cnidarians with complex life cycles that usually involve fish and annelids as temporary and definitive hosts, respectively. Species of Ortholinea mostly infect the urinary system of marine fish. Thus far, only O. auratae and O. labracis have their life cycles clarified and use marine naidids (Oligochaeta) as definitive hosts. This study aimed to explore the diversity and life cycle dynamics of myxosporeans parasitizing Diplodus cervinus experimentally reared in a polyculture semi-intensive fish farm.

Methodology: Specimens were dissected, and their internal organs microscopically examined. Annelids collected from sediment samples obtained from the earth ponds and estuarine wild locations were also surveyed for myxosporean development. Infected tissues were photographed and prepared for molecular procedures targeting the 18S rDNA. Distance estimation and maximum likelihood analysis were performed using MEGA X.

Results: Spherical to subspherical plasmodia and mature myxospores complying with the definition of Ortholinea were observed in the urinary bladder. Plasmodia were mostly disporic, and less frequently polysporic. Myxospores were subspherical in valvular view and ellipsoidal in sutural view, with ornamented valves, and two pyriform polar capsules opening to opposite sides of the suture line. Distance estimation matched the 18S rDNA sequence of the novel isolate with a triactinomyxon type recorded from the marine oligochaete Limnodriloides agnes Hrabě, 1967 collected from a wild location. Ortholinea labracis and O. auratae were retrieved as closest relatives, with 96.5% similarity. Maximum likelihood tree topology positioned the case isolate within the oligochaete-infecting lineage, specifically among congeners that together form a well-supported subclade of the excretory system clade.

Conclusions: This study describes the first occurrence of a myxosporean parasite in zebra seabream, demonstrating that parasitological studies are required to better understand the sustainability of rearing this fish species. Comprehensive morphological and molecular comparisons suggest the case isolate as a novel species of Ortholinea, closely related to congeners occurring in the same geographic location. Triactinomyxon actinospores are reinforced as counterparts for Ortholinea, with marine oligochaetes belonging to the family Naididae suggested to be the preferred hosts of these myxosporeans in estuarine environments.

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Myxosporean diversity parasitizing thicklip grey mullet Chelon labrosus in a Portuguese estuary

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Introduction: Myxosporeans are widespread cnidarian endoparasites that mostly infect fish as vertebrate hosts. Mullets are a diverse fish group playing crucial ecological roles in estuarine environments, and which ubiquitous nature leaves vulnerable to parasitic infections [1]. This study aimed to acknowledge the myxosporean diversity infecting thicklip grey mullet Chelon labrosus in a Portuguese estuary.

Methodology: Six specimens were obtained from fishermen operating in the Aveiro estuary. Their internal and external organs were macro- and microscopically examined for the detection of myxosporean development. Cysts and infected tissues were individually photographed and processed for sequencing of the 18S rDNA. Distance estimation and maximum likelihood analyses were conducted using MEGA X.

Results: Infections by Myxobolus spp. were detected in the scales, fins, branchial arches, gill filaments, kidney, and intestine. Infection by a Sphaerospora sp. was also observed in the kidney, being co-infective with the Myxobolus isolate found in this organ. Morphological comparisons and distance estimation revealed 4 of the 6 Myxobolus isolates in study, as well as the Sphaerospora isolate, as potential novel species. Phylogenetic analyses of the novel Myxobolus sequences retrieved them positioned within the mugiliform-infecting clade of myxobolids, specifically within the main lineage of Chelon-infecting species. The novel Sphaerospora sequence clustered within the Sphaerospora (sensu stricto) clade, together with Sphaerospora sp. ex Chelon ramada (JX286626) and Sphaerospora sp. ex Chelon labrosus (JX286625).

Conclusions: The rising number of Myxobolus spp. found in C. labrosus reinforces the known hyperdiversification of this myxosporean genus in mullets [1], further evidencing careful molecular-based comparisons as a requirement for exploring Myxobolus diversity in this fish group. Phylogenetic analyses of the novel Myxobolus sequences maintain the monophyly of the mugiliform-infecting lineage, and support clustering according to the host genus. Similarly, the positioning of the novel Sphaerospora 18S rDNA sequence in the tree topology obtained for Sphaerospora (sensu stricto) agrees with the host-driven diversification of this genus [2].

[1] Rocha et al., Parasitol. Res. 2019, 118, 3279-3305.
[2] Patra et al., Parasit. Vectors 2018, 11: 347.

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Artificial infection of Japanese medaka Oryzias latipes with Edwardsiella piscicida by the immersion method

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Introduction: Edwardsiellosis is a general term used to describe infections caused by the members of the genus Edwardsiella. Edwardsiella piscicida is an intracellular bacterium with a broad host range. The Japanese medaka Oryzias latipes is a commonly used model organism useful for understanding the etiology of edwardsiellosis caused by E. piscicida or E. anguillarum (formerly E. tarda).

Method: In this study, four strains (E381, OA-3, HME-1, and NUF806) isolated as E. tarda that were not previously classified in detail were re-classified by phylogenetic analysis using 16S rRNA sequences. We also determined the susceptibility of medaka to E. piscicida and E. anguillarum strains using the immersion infection method. The abundance of E. piscicida in infected medaka kidneys and symptoms of edwardsiellosis in Japanese medaka were evaluated.

Results: The 16S rRNA sequences of E381, OA-3, and NUF806 strains belong to the E. piscicida cluster, and strain HME-1 is also closely related to a type strain of E. anguillarum (ET080813). After immersion infection, the final survival rate of adult medaka challenged with each strain was as follows: 30.8% in HME-1, 10.3% in E381, 5.1% in OA-3, and 0% in NUF806 (at 0.8-3.7 × 107 CFU/mL). In plots challenged with the NUF806 and E381 strains, high mortality was observed in the early stages [up to 4 days post-infection (dpi)]. According to the results of quantitative analysis, the abundance of E. piscicida in the kidneys of surviving fish was significantly higher at 5 and 6 dpi than that at 0 dpi following infection with E. piscicida NUF806 (at 1.0 × 108 CFU/mL). In contrast, the abundance of E. piscicida at 8 dpi was significantly lower than at 6 dpi, with the same range observed at 1 dpi. At 4–6 dpi, the base of the dorsal fin reddened, and the spleen swelled in surviving fish.

Conclusions: Thus, a new Japanese medaka infection model was constructed to study infection by the intracellular parasitic bacterium E. piscicida, which causes edwardsiellosis. This can lead to a better understanding of the etiology of this disease.

Are Chondrichthyes also important hosts for Anisakis spp. Life cycle? The case in the south coast of Portugal

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Many Teleostean fishes and cephalopods act as intermediate and paratenic hosts of Anisakis life cycle. But, what about Chondrichthyes? Are they suitable hosts for this parasite too? Will it be safe to eat them?

Anisakis is a genus that include several species already detected in humans infections, as A. simplex (s.s.), A. pegreffii or A. physeteris, thus its occurrence in fishes is very important in terms of food safety.

Most of the fishes surveys for anisakids were made in Teleostean fishes, and only few data was reported from Chondrichthyes, so this work aims to determine the occurrence of anisakids in a sample of different fish species from this taxa. The fish were collected at the south coast of Portugal, and belonged to the by-catch of crustacean bottom trawl fisheries.

The fish were surveyed using the UV-Press method, in the visceral organs and a piece of muscle. The worms detected were molecularly analysed by ITS and Cox2 genes in order to identify they until species level.

In total 266 Chondrichthyes fishes were analysed, and they belong to 14 different species, of rays, sharks and chimera, but only 8 shark species recorded Anisakis spp.

The host species with the highest prevalence / mean intensity was Scymnodon ringens with 57.9% /3.1, followed by Etmopterus pusillus with 33.3% / 50.0, Etmopterus spinax with 32.7% / 6.5, Deania profundorum with 18.2% / 9.5, Galeus atlanticus with 7.4% / 1.5, Galeus melanostomus with 4.6% / 1.0, and in Deania calcea and Centroselachus crepidater only one fish was analysed, infected with 293 and 5 worms, respectively. The 5 Anisakis species found were: Anisakis simplex, A. pegreffii, A. nascettii, A. physeteris, A. ziphidarum, and Anisakis sp., and A. simplex x A. pegreffii hybrid. All the three zoonotic species were here detected in our sample.

Since sharks are top predator fishes, that eats several teleostean fish species rather infected, higher values will be expected. However, in terms of food safety they can not be considered food 100% safe.

Occurrence of Anisakis spp. In the Atlantic chub mackerel Scomber scombrus, off the Portuguese Atlantic coast, and its anisakiosis risk

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Reports of anisakiosis, a fish-borne disease is caused by the ingestion of third stage (L3) Anisakis sp. Larvae are increasing caused by consumption of many Scombridae fishes, comprising tunas, bonitos and mackerels, fishes that are frequently eaten worldwide including in Portugal. In Portugal, to grill the fish is a very common tradition, and one of the most popular and famous dishes is the grilled Atlantic mackerel (Scomber scombrus). Even if the fish is cooked, it's possible that the larvae within can still be active, as this method of cooking can often not reach sufficient high temperatures to cook well all the fish. Therefore, the Portuguese population can be at some risk of exposure to this disease when eating this fish species. So, it is very important to know the distribution of the anisakids species. In this context, this project aims to determine the prevalence and intensity of anisakids in S.scombrus collected from the fish market in Aveiro, and caught on the Atlantic coast, in Portugal.

105 Atlantic mackerels (S.scombrus) with a total length (mean±SD) of 26.8 ±2.2 ranging from 22.0 to 32.5 cm, and with a total weight of 155.3 ± 36.7 with a range from 73.0 to 238.1 g were analysed by visual inspection and UV-press method, for muscle and viscera.

In total, 497 anisakids L3 were removed from all the mackerels sampled, of which 47 (11%) larvae were found in the muscle and 430 (89%) in the visceral organs. The overall prevalence of anisakids in the sample was 67%, and in the muscle 26%. The intensity (mean±SD(range) was 7.1±8.0 (1-39), and in the muscle was 1.7±1.1 (1-6), what was much less. These results suggest that this fish species has a low risk of causing anisakiosis when compared to other fish species traditionally consumed, as the Atlantic hake.

The prevalence and intensity of anisakids is recommended to be checked periodically in the fish populations, in an attempt to evaluate the anisakiosis risk for consumers.

Climatic changes and interference in host-parasite interaction

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The theme on Climate Change has been addressed more frequently, due to the direct and indirect environmental impacts that affect the planet, causing several ecological changes, which cover all life forms. Aquatic organisms, and fish in particular, are affected by a variety of stressors caused by anthropogenic influences that lead to changes in environmental parameters. The fact that climate change mainly affects fish communities, whether in cold or hot waters with the determining factors: ocean acidification, increased temperature, salinity, disposal of pollutants, has shown significant changes in the disposition of host-parasite interactions. The increase in temperature affects transmission system, with direct increase in the metabolism of the parasite in aquatic and terrestrial environment, increasing the feeding or replication of the parasite in the host, increasing the damage and resulting in the production of a greater number of stages of transmission, as well as a faster spread of the disease in a single outbreak. Therefore, understanding parasitism as a crucial component in biodiversity, with the inclusion of these organisms in conservation strategies is extremely important to ensure the maintenance of ecosystems and the survival of host species. The Digeneans, for example, involve mollusks as first intermediate hosts and a number of other organisms, invertebrates or vertebrates, as second intermediate hosts, and usually vertebrates as definitive hosts; and all are necessary to complete parasite ontogeny. With the loss of first host, caused by environmental change, the parasite will also disappear. Climate change may favor the emergence of infectious and parasitic diseases in fish in nature, can increase parasite metabolism, the adaptation of parasitic species to new hosts, the intensification of the most abundant parasitic species in the search for new hosts, contributing to greater economic loss due to parasitic lesions in these hosts, greater parasitic contamination in fish production leading to a higher dietary risk for consumers. Future studies need to be carried out to be able to understand more deeply how climate changes can interfere with host-parasite adaptations and interactions, and how these parasites can be used as bioindicators of the environmental health of the water and the host community.

European seabass (Dicentrarchus labrax) immune status and response after dietary supplementation with algae-derived compounds

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Introduction: Functional ingredients are currently one of the focus of aquatic nutrition research as a potential strategy to face stress-induced conditions in farmed fish. Among them, macro- and microalgae are having a special emphasis due to inherent anti-inflammatory, immune-stimulant and antibacterial properties. The present study aims to evaluate the health-promoting effects of macro- and microalgae-derived compounds in diets for European seabass (Dicentrarchus labrax) juveniles. For this purpose, immune status and response to bacteria as well as disease resistance were assessed.

Methodology: Triplicate tanks of 45 fish (22.7 ± 1.8 g) were either fed a commercial diet (CTRL) or the CTRL diet supplemented with two different levels of a blend containing Tetraselmis sp. Aqueous extract, sulphated polysaccharides and phycocyanin (Dlow and Dhigh) for 14 days. After that, fish were intraperitoneally injected with 2.62 x 10^5 CFU/mL of Photobacterium damselae piscicida strain MM415 and cumulative mortality was followed for 14 days. Samplings were performed immediately before (immune status) and 6 hours after bacterial challenge (immune response). Fish were euthanized and sampled for blood (haematology), peritoneal leucocytes, plasma, mucus (humoral parameters), liver (oxidative stress), intestine and spleen (molecular markers) (n=9 per experimental condition).

Results: No significant differences were found regarding most haematological and plasma immune parameters analysed among dietary treatments. However, total peritoneal leukocytes increased in seabass fed Dhigh at 6 hours following infection compared to their counterparts fed CTRL. Regarding disease resistance, no significant differences were found among different dietary treatments.

Conclusions: Preliminary results suggest that diets supplemented with the blend of microalgae-derived compounds did not enhance fish survival against a bacterial challenge. Nonetheless, an enhanced recruitment of immune cells to the inflammatory focus provided by the highest supplementation level might increase the efficiency to clear a local inflammation. Samples of liver, intestine and head-kidney are currently being processed for analysis of antioxidant activity and immune-related gene expression.

Influence of sea temperature on biochemical profile of greater amberjack seriola dumerili (risso, 1810)

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Introduction: Greater amberjack Serioloa dumerilii is a new fish in aquaculture. The introduction of new species into Mediterranean aquaculture is difficult due to health problems that may be caused by the physiological limitations of the cultured species. Fish blood analyses are very important methods for determining the physiological and pathological conditions of the organism. Changes can be dependent on biotic and abiotic factors.

Methodology: The investigation was performed on cultured S. dumerilii in December 2022 and March 2023. The average sea temperature 30 days before the first sampling was 18.5°C and before the second sampling 13.1°C. Blood samples from each fish were taken from the caudal vein, serum samples were analysed using a Cobas c311 autoanalyzer (Roche Diagnostics, Risch-Rotkreuz, Switzerland).

Results: Serum samples were analyzed for 18 biomarkers as follows, substrates [Albumins (ALB), Glucose (GLU), Lactate (LAC), Total Cholesterol (TCH)], enzymes [Alanine transaminase (ALT), Aspartate aminotransferase (AST), Lipase (LIP), Creatine Kinase total (CK), Cardiac Creatine Kinase isoenzyme (CK-MB), and Lactate dehydrogenase (LDH)], electrolytes and minerals [Sodium (Na), Potassium (K), Chloride (Cl), Calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn) and Phosphorus (P)]. Albumin, ALT, AST, CK, CKMB and Phosphate were statistically lower in March. Lipase was statistically higher in March. Other biomarkers were not significantly changed.

Conclusions: Biochemical parameters of serum can be helpful in determining changes in metabolism during unfavorable growing conditions.

New filo-, hanta- and rhabdoviruses detected in farmed European perch (Perca fluviatilis Linnaeus, 1758)

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Introduction: European perch (Perca fluviatilis) are increasingly kept in aquaculture. Despite this, viral infections remain largely unexplored, and thereby putting farm populations at incalculable risk for devastating epizootics. Fish viruses may also present potential hazard to consumers.

Methodology: To address these concerns, we applied metatranscriptomics in clinically diseased European perch from a Swiss fish farm to identify disease-associated and possibly secondary important viruses. Histology and in-situ 204ncluding204ion was performed to demonstrate associated organ lesions. Organ suspension, 204ncluding CNS, was cultivated on cell cultures to demonstrate virus replication.

Results: Unexpectedly, in clinically diseased fish we detected novel freshwater fish filoviruses, a novel freshwater fish hantavirus, and a previously unknown rhabdovirus. Hantavirus titers were high, and we demonstrated virus in macrophages and gill endothelial cells by using in-situ hybridization. Rhabdovirus titers in organ samples, including CNS, were low, but virus could be isolated on cell culture.

Conclusion: Our data add to the hypothesis that filoviruses, hantaviruses, and rhabdoviruses are globally distributed common fish commensals, pathogens, or both. Our findings shed new light on negative-sense RNA virus diversity and evolution.

How sustainable is stocking of Atlantic trout, e.g. in presence of Proliferative Kidney Disease?

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Introduction: In many Swiss rivers, Atlantic trout (Salmo trutta) populations have declined dramatically over the last decades. As a main contributing factor, Proliferative Kidney Disease (PKD), caused by Tetracapsuloides bryosalmoane, is discussed. To compensate for the losses, trout are regularly stocked. However, it is still unclear whether these measures are necessary to maintain the population or whether they can be recruited exclusively from natural spawning. With this project, we aimed to investigate: (1) if a population remain stable over several years despite PKD without stocking measures? (2) if PKD progression change over several generations in a native trout strain? (3) if differences in survival probability of stocked fish exist between PKD-free and PKD-positive water stretches?

Methodology: We selected a river system in the Swiss midlands with PKD free and PKD affected river stretches. Following a last stocking event in 2015, stocking was stopped. Quantitative electrofishing has been carried out at three sites along the course of the river over the last eight years and trout young-of-the-year (YOY) have been examined for PKD prevalence and associated pathology. In addition, stocked animals were marked during the last stocking in 2015 and the ratio of stocked animals vs. naturally spawned trout was monitored over several years by genetic analyses.

Results: Over the last years, total fish numbers and numbers of YOY trout were highly fluctuating, but never dropped under a minimal threshold. Interestingly, the number of adult trout and the total biomass have remained stable over the examination period. Depending on the location, few stocked trout could be only re-captured one to three years after stocking. Afterwards, no stocked animals could be detected at any site. Therefore, stocked fish hardly contribute to population stability. PKD distribution, prevalence and pathology remained stable over the last years.

Conclusion: Based on our results, we hypothesise that (i) stocks can be recruited by natural spawning and remain stable over several years even in presence of PKD, (ii) stocking of naïve animals in PKD-positive water stretches does not seem sustainable; and (iii) PKD has no significant influence on wild trout population stability.

Influence of Flavobacterium psychrophilum gill infection on immune parameters of rainbow trout (Oncorhynchus mykiss)

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Introduction: Flavobacteria are considered one of the most important fish pathogens worldwide. Flavobacterium psychrophilum, the etiological agent of bacterial coldwater disease and rainbow trout fry syndrome, causes great economic losses in salmonid aquaculture worldwide but little is still known about the effect of infection on the immune system of the fish. The present study examined the changes in non-specific humoral and cellular immunity parameters upon the identification of F. psychrophilum on gills.

Methodology: Mortality occurred in one of four tanks in a local fish farm in rainbow trout weighing 10-15 g. Mortality of affected fish increased till day 7 to over 30%. After that, it rapidly decreased.

After a microbiological examination dystrophic change appeared in the gills and F. psychrophilum was identified.
For 6 weeks at 7-day intervals after the onset of initial mortality material for testing was collected from 10 randomly selected fish from the affected tank and 10 healthy fish. Blood for the study was collected from the tail vein and after centrifugation, the blood serum was used for the determination of lysozyme and ceruloplasmin activity, total immunoglobulin, and total protein levels. Immunocompetent cells were isolated from the spleen and head kidney. After isolation of the cells, the following parameters were determined: the metabolic activity of the spleen phagocytes (RBA, Respiratory Burst Activity), the potential killing activity of the spleen phagocytes (PKA, Potential Killing Activity) and the proliferative response of the head kidney lymphocytes (MTT, Mitotic Transformic Test).

Results: Almost all the immunological parameters studied were reduced compared to the control group. Only ceruloplasmin activity was increased.

Conclusions: F. psychrophilum bacterium (or at least some strains) may have mechanisms that allow it to evade the fish's immune system and suppresses the basic nonspecific humoral and cellular defense mechanisms.

Comparative Genome and Infectivity Analysis of the Fish Pathogenic Oomycetes from the Genus Saprolegnia

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Introduction: Diseases caused by pathogenic oomycetes lead to enormous losses in agriculture, aquaculture, and forestry. The best-known oomycete species infecting aquatic animals is Saprolegnia parasitica, which causes saprolegniosis. The molecular-level studies of the disease origin and evolution are essential to develop an effective treatment.

Recent findings in our laboratory suggested a possible correlation between phylogeny of Saprolegnia species and saprolegniosis outbreaks in Scottish salmonid farms. Therefore, our study focuses on identifying the different levels of virulence and detecting their distinctive phenotypic features to provide insight into the basics of Saprolegnia infections.

Methodology: The collection used in the project included 41 distinct strains of S. parasitica, S. diclina, S. ferax, S. australis and S. delica isolated from all over the world. Sample identification was based on the analysis of PCR amplification targeting three separate regions. Preliminary studies allowed to compare growth at different temperatures and cyst production among the isolates. Variations in aggressiveness were investigated by infection experiments using larvae of the wax moth Galleria mellonella. Genomic DNA from each Saprolegnia sample was sequenced with Illumina, and all genomes were then assembled and annotated.

Results: A combined ITS-COX1-COX2 phylogenetic tree divided samples into ten different clades. Distinction was also observed in the size of the genomes and the number of predicted protein-coding genes. Comparative analysis of putative effector proteins revealed differences in the number of several effector families possessed by Saprolegnia species, as well as compared to other oomycete genera.

Conclusions: Whole genome sequencing and comparative genome analysis provided an in-depth understanding of Saprolegnia diversity. These results may provide a basis for the future research to characterise pathogenic Saprolegnia species at the molecular level and help to combat saprolegniosis disease.

Interactions of predominantly plant-based feeding and handling stress on the expression of selected immune markers in rainbow trout (oncorhynchus mykiss)

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Nutrition has a decisive influence on the well-being of organisms. As the exchange of fishmeal in aquafeeds is still important in the context of sustainability and cost efficiency in aquaculture, the impact of a predominantly plant-based diet on fish health is yet not fully understood. Therefore, this study examined the effect of a primarily plant-based diet on the expression of selected immune markers in rainbow trout (Oncorhynchus mykiss) under the influence of handling stress.

In a feeding trial, a predominantly plant-based diet, where the amount of fish meal was reduced up to 80% (P), and a mainly fishmeal-based diet (F) were compared. Rainbow trout have been stressed by shooing in the tank twice a day

for 50 days. The mRNA expression from whole blood samples of tumor necrosis factor (TNF) α, interleukin (IL) 1β, immunglobulin (Ig) T and IgD were evaluated using a standard real time qRT-PCR.
Significant differences between the two dietary treatments F and P were found in expression of IL1β (p=0.048) and IgD (p=0.0327) for non-stressed fish and in expression of TNFα (p=0.041), IgT (p=0.0068) and IgD (p=0.0070) for stressed fish, while the stressed P group significantly downregulated the genes. No significant differences in gene expression were seen between non-stressed and stressed fish fed the same experimental diet.

Under repetitive stress mainly fishmeal-based and predominantly plant-based fed trout show contrasting gene expression patterns whereby the P group downregulated IgT, IgD and TNFα significantly when stressed. In addition, corresponding differences in the microbiome of the fish could be detected.

The gene expression of investigated immune parameters is significantly impacted by the feed formulation. The more fishmeal the more pro-inflammatory cytokines, while the performance of fish under stress was better than with a predominantly vegetarian diet. Without stress the plant-based feed performs better. Results show, that fish performance under the influence of stress should not be ignored when looking for alternatives to fishmeal. We need to better understand the impact of plant-based protein sources on, for example, gut health and microbiome as one of the most important immune modulators in the body of carnivorous fish like rainbow trout.

The first diagnosis of Myxobolus lentisuturalis a highly pathogenic muscle-infecting parasite of gibel carp (Carassius auratus gibelio) in Hungary

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Introduction: The gibel carp (Carassius auratus gibelio Berg 1932) is an aggressively invasive fish in Europe. It can infect with several highly pathogenic myxozoan parasites in its original biotope of East Asia. The muscle-infecting Myxobolus lentisuturalis was initially discovered in China and described from gibel carp. In Europe, the infection (caused) by M. lentisuturalis has been detected on the goldfish (Carassius auratus auratus) in Italy, and on the gibel carp in Croatia but not in Hungary so far.

Methodology: During the fall of 2021 and 2022, eighteen gibel carp (15 - 20 cm in total body length) with dorsolateral distortion were collected from the fish ponds in Southern Hungary. All the fish showed external alterations manifested in large humps at the dorsolateral region of body infecting the musculature between the head and the dorsal fin. The fresh spores were collected for morphological and molecular studies from the lesion. For histopathological analysis, two fish were selected. The muscle tissues were excised, fixed in Bouin's solution, dehydrated, embedded in paraffin wax, cut into 4–5 µm sections, and stained with hematoxylin and eosin (H&E). The molecular characterization were based on 18S rDNA and 28S rDNA. Universal primers and myxozoan-specific primers were used and the purified products were carried out by the Sanger sequencing.

Results: According to the size and morphology, spores collected from the degenerated tissue correlated with M. lentisuturalis described previously from gibel carp and goldfish. Histopathological investigation showed that the formation of plasmodium containing myxospores causes the severe destruction of muscle tissues. The obtained 18S rDNA and 28S rDNA sequences matched with M. lentisuturalis sequences previously published in GenBank. The phylogenetic analysis clustered our samples in a monophyletic group supported by a high bootstrap value.

Conclusions: This study highlights the incidence of M. lentisuturalis in Hungary. The aim of this study is to draw the attention of fish farmers to this new pathogenic parasite in Hungary.

Keywords: Myxobolus lentisuturalis, gibel carp, muscle tissue, morphology, 18S rDNA, 28S rDNA, Hungary

Funding: This study was funded by the Stipendium Hungaricum Program.

Infectious salmon anemia outbreak in farmed Atlantic salmon (Salmo salar L.) in Iceland, first detection of an ISAV HPRdel variant

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Infectious salmon anemia (ISA) is a serious viral disease of Atlantic salmon (Salmo salar L.) caused by the ISA virus (ISAV) and is notifiable to the World Organization for Animal Health (OIE). Virulent strains ISAV-HPRdel have deletions in a highly polymorphic region (HPR) of the hemagglutinin-esterase (HE) gene on segment 6, whereas avirulent strains ISAV-HPR0 have none.

Routine targeted samplings for Real-time RT-PCR analyses have been performed since 2009. Icelandic Atlantic salmon broodfish farms are formally declared free of ISA by the fish health authority of the European Union. (https://www.mast.is/static/files/aaetlanir/aquaculture-surveillance-programme-fish-diseases-2020.pdf)

ISAV screening with RT-qPCR started at Keldur in 2011. The few samples that have been ISAV positive from broodfish are HPR0 genotype. A research project carried out at Keldur from 2015-2018 screened both wild and cultured salmon juveniles, cultured salmon in sea pens and wild salmon from rivers (a total of about 800 fish) for ISAV, which was not detected.

In November 2021, an increased mortality was experienced in farmed Atlantic salmon in sea pens in Reyðarfjörður, East Iceland. The fish showed macroscopic clinical signs suggestive of ISAV. Tissue samples and organs were sent to Keldur for diagnosis, using ISAV RT-PCR and histopathological analysis.

The histopathology of the diseased fish was consistent with previous descriptions of ISAV-del infections in Atlantic salmon, i.e., characterized by extensive hemorrhage and congestion in most organs, associated with varying degree, often significant, pathological changes. Erythrophagocytosis was commonly observed, due to extensive immune reaction.

RT-qPCR results for ISAV were positive with Ct. values ranging from 14-27. The samples were also run in a ISAVseg6 RT-PCR and further analysed by capillary electrophoresis. Sequencing of the ISAVseg6 PCR amplicons showed that it was an ISAV HPRdel variant. This is the first time that an HPRdel variant of ISAV has been detected in Iceland. The complete HE-gene was sequenced and when aligned with published sequences it showed greatest similarity to HPR0 and HPRdel sequences from northern Norway and HPR0 sequences from The Faroe Islands.

Heart pathology in Atlantic Salmon (Salmo salar L.) in Scotland: a case study

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Introduction: Cardiac diseases are of economic importance and welfare concern in Scottish commercially farmed Atlantic salmon (Salmo salar L.). Viral diseases affecting the heart such as cardiomyopathy syndrome (CMS), heart and skeletal muscle inflammation (HSMI), and pancreas disease (PD) are a cause of mortality and production loss in farmed Atlantic salmon. In Norway, a novel cardiac abnormality has been recently described in farmed Atlantic salmon and rainbow trout (Oncorhynchus mykiss (Walbaum)), with novel histopathological changes observed in the compact layer of myocardium.

Following reports of increased mortality by a farm operator, in late October 2022, the Fish Health Inspectorate (FHI) visited a salmon farm site located in the west coast of Scotland. The stock (1.3 kg average weight) recorded elevated mortalities related to multiple factors as for instance gill health issues and presence of mild to moderate inflammatory pathology observed in the heart, potentially associated with a viral infection. During the visit the FHI sampled one fish for molecular, bacteriological testing and histopathological standard diagnostic examination.

Results: Histopathology findings revealed identical observations to those recently described and observed in Norway in farmed salmonids. Multifocal areas in the heart stratum compactum were observed with lighter stained cardiomyocytes with granular or vacuolated cytoplasm. The lesions appeared to occur around terminal branches of the coronary arteries. Mild epicarditis was also observed.

Conclusion: This finding is important to the Scottish salmon industry as it may be start of a new cardiac abnormality. The prevalence is currently unknown as this is the first record in Scotland. These observations highlights the importance of routine inspection to fish welfare and handling of farmed salmon and other salmonids. Further work could be an investigation into the prevalence of this novel pathology, the contributing factors and the potential impact to the robustness and welfare of farmed salmon. Additionally, an investigation on rainbow trout sites could be also performed to understand whether this species is also affected in Scotland.

Recombinant salmonid novirhabdovirus as vaccine vectors to protect Senegalese sole against viral encephalopathy and retinopathy

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Introduction: The outbreaks of viral hemorrhagic septicemia (VHS) and viral encephalopathy and retinopathy (VER) caused respectively by the viral hemorrhagic septicemia virus (VHSV) and the nervous necrosis virus (NNV) represent

two of the main viral infectious threats for aquaculture worldwide. Our aim is to develop live attenuated vaccine vectors based on VHSV expressing the main protective antigen of NNV and characterize their safety, immunogenicity and protective efficacy against these two important diseases in sole and trout, major host species of NNV and VHSV, respectively.

Methodology: The genome of a salmonid VHSV has been engineered to modify the gene order as a stable attenuation strategy, and to introduce an expression cassette encoding an NNV antigen. The cassette was designed to include a truncated and duplicated form of the NNV coat protein fused to VHSV regulatory signals for transcription and specific domains derived from the glycoprotein G of novirhabdovirus (signal peptide and transmembrane domain). The suitability of the recombinant VHSV recovered by reverse genetics (rVHSVs) was assessed by immunization and challenge in juvenile trout and sole.

Results: We generated eight attenuated rVHSVs bearing an expression cassette encoding the major protective antigen domain of NNV capsid protein. All recombinant viruses expressed and correctly addressed NNV-derived antigen at the surface of infected EPC cells. The antigen was also detected at the surface of the viral particles. Following bath immersion administration of the various rVHSVs to juvenile trout, some of the rVHSVs were fully attenuated (4/6) and protective against VHSV challenge. Results indicated that rVHSV N2G1C4 is safe and protective against VHSV challenge in trout with a relative percent survival (RPS) of 78%. In parallel, juvenile sole were injected with rVHSVs and challenged with NNV. The rVHSV N2G1C4 is also safe, immunogenic, and protective in sole against NNV (RPS of 70%).

Conclusions: rVHSV N2G1C4 represents a promising starting point for the development of a live attenuated bivalent vaccine candidate for the protection of these two commercially valuable fish species against two major pathogens of aquaculture.

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Ionic liquids as novel compounds with applications in the prevention of viral diseases in fish

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Introduction: Viral diseases are responsible for large fish mortalities every year all over the world. The search for new compounds that can contribute to their control is a priority. Ionic liquids (ILs), labelled “green solvents”, are formed entirely by organic salts composed of ions which have low melting points. Their outstanding characteristics and the fact that they can be customized have led to a growing interest in these compounds. In this work, we analyse the antiviral activity of a selection of ILs against the enveloped viral hemorrhagic septicemia virus (VHSV), and the non-enveloped nervous necrosis virus (NNV).

Methodology: Six compounds with different ionic composition were chosen, including the anions [TFSI]– and nitrate, and the cations pyrrolidinium, imidazolium and choline. Different concentrations of the ILs (from 100 mM to 0.125 mM) were added to EPC and E-11 cell lines derived from carp and, snakehead, respectively. Toxicity was evaluated by observation of the morphology and integrity of the cell monolayers. To assess the potential antiviral activity virucide, adsorption and viral replication inhibition assays were performed.

Results: Non-toxic concentrations varied between 10 mM to 1 mM depending on the studied IL. EPC cell line was more sensitive that E-11 to the IL treatments. The results revealed that the selected ILs did not exert a direct virucidal effect against VHSV or NNV at the non-toxic concentrations. Furthermore, ILs did not prevent adsorption of the viruses to the cell surface either. However, several ILs inhibited the viral production of both strains in a dose-dependent manner. While ILs based on the nitrate anion showed the best NNV inhibition results, VHSV production was mostly reduced after treatment with IL based on imidazole. Interestingly, ILs composed of choline, one of the most biodegradable and water-soluble inorganic salts, inhibited replication of both viruses.

Conclusion: Some of the selected ILs inhibit NNV and VHSV replication ex vivo at concentrations that have no effect on cell viability encouraging further studies to investigate its potential therapeutic use in the prevention of viral diseases in fish.

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Occurrence of lymphocystis disease virus (LCDV) in Platichthys flesus from the South Baltic

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Introduction: Lymphocystis disease (LCD) is a self-limiting viral disease characterised by hypertrophy of fibroblastic cells in connective tissues of fishes. Previously, marine isolates of this virus from Baltic Sea has been genetically classified into two different genogroups: LCDV-1, which is found in flounder (Platichthys flesus) and plaice (Pleuronectes platessa), while LCDV-2 is usually found in dab (Limanda limanda). The aim of our study was to determine the spread of LCDV in the species Platichthys flesus caught in the South Baltic Sea and genetic characterisation of isolates.

Methodology. Samples were collected in 2016 and 2022 during a cruise in various fishing areas of the South Baltic Sea. DNA samples were isolated from external nodules stored in ethanol and frozen at -80°C until analysed. After PCR reaction, products of MCP gene fragment amplification were sequenced and analysed using the Genious software.

Results and Conclusions. Molecular detection confirmed presence of LCDV in the external nodules from infected fish. Sequence analysis of the MCP gene of the detected virus revealed significant identity with another LCDV isolate previously reported from the samples from Baltic Sea and assigned the detected isolates to genogroup 1. The tests of the collected samples showed the presence of the LCDV virus in a total of 30 samples out of 170 fish examined. The highest percentage of infected fish was recorded in the Bornholm fishing area (60%). The actual prevalence of infection is difficult to estimate due to the specificity of the fishing method, which uses the tendency of fish to grouping in specific places at the sea bottom.

The results of our research confirmed the presence of LCDV-1 in the species Platichthys flesus from the South Baltic Sea and genetic diversity depending on the fishing area. Occurrence of lymphocytosis in flounder may be related to the recently observed decrease in the number of this sepecies in the Baltic Sea, which is confirmed by annual reports of the International Council for the Exploration of the Sea.

First detection and phylogenetic analysis of piscine orthoreovirus 3 (PRV-3) in salmonid fish from Poland

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Introduction: Piscine orthoreovirus (PRV) belonging to the family Reoviridae was first discovered in 2010 in farmed Atlantic salmon exhibiting Heart and Skeletal Muscle Inflammation (HSMI). This pathogen is thought to mainly affect aquacultured and maricultured fish stocks, and recent research has been focused around the susceptibility of wild stock. However, public concern has been raised regarding the possibility of transmitting PRV to wild salmonid populations and being a factor in their declining. The aim of our research was to test whether PRV is present in farmed or wild salmonid fish in Poland.

Methodology: The samples included in this study were obtained as part of the surveillance program for IPN virus, conducted in 50 chosen aquaculture facilities, producing different salmonid species in years 2018 to 2022. 30 fish were collected at each farm, internal organs were pooled from 10 fish per sample (heart, spleen, kidney) in RNAlater and frozen. Finally, wild sea trout (Salmo trutta m. fario) that migrate to spawning up rivers from the Baltic Sea in 2019-2022 were obtained as part of the National Science Centre research grant, UMO-2019/33/NZ6/02929. Specimens with visible pathological changes on the skin used as material for research on the ulcerative dermal necrosis (UDN) have also been tested for the presence of PRV. After RNA purification from organs molecular detection of PRV by RT-PCR was performed.

Results and Conclusions: Of the 50 farm samples tested, 12 were positive. The presence of PRV virus has not been confirmed in sea trout spawners from the Baltic Sea. Isolates that tested positive for the presence of PRV were sequenced (S1 segment). Comparative analysis of the phylogenetic tree showed that Polish isolates, which were grouped into PRV-3 type, are very similar to German and Danish ones. Due to the predominance of isolates from the oldest, traditional trout farms in Poland, we suspect that PRV was brought to Europe with the species of rainbow trout from North America. Then, together with the roe's transport, the virus could have gone unnoticed to Polish farms. However, so far it does not cause serious problems in wild or farmed salmonid fish populations.

Virological studies of wild salmon (Salmo salar) from the South Baltic

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Introduction: A number of viral diseases are noted in European salmonid fish farms, that have a negative impact on the condition of fish, food quality and safety. The most dangerous fish pathogens include: infectious salmon anaemia orthomyxovirus (ISAV), viral haemorrhagic septicemia (VHSV) and infectious hematopoietic necrosis (IHNV)

rhabdoviruses, alphaviruses (SAV), piscine orthoreoviruses (PRV-1, PRV-3) or salmon gill poxvirus (SGPV) in salmonids, which cause infectious diseases with severe course, often leading to high mortality. Until now, there was no information on the presence of these pathogens in wild salmon (Salmo salar) sampled in the Polish marine waters of the Baltic Sea.

Methodology: In order to determine the prevalence of the above-mentioned viruses, samples from salmon caught in the Polish marine waters of Baltic Sea were collected for testing. Selected organ fragments were secured in RNAlater and frozen. Then, after defrosting the tissues, DNA and RNA nucleic acids were isolated and analysed for the presence of viruses using molecular methods recommended by the World Organization for Animal Health (WOAH).

Results and Conclusion: The results of our research did not reveal the presence of the above-mentioned viruses in samples taken from salmon from the southern Baltic. This may support the theory that the frequency of most viral pathogens in wild fish in marine waters remains at a very low level, in contrast to infections in fish in inland waters. Our hypothesis is also confirmed by the negative results of our earlier tests for the VHS virus of Baltic flounder, sprat, cod and herring conducted in 2015-2017.

Effects of levonorgestrel on the hypothalamus-pituitary-gonad (HPG) and hypothalamus-pituitary-thyroid (HPT) axes in common carp (Cyprinus carpio)

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Introduction: Levonorgestrel is a synthetic progestin widely used in human contraception. Its presence has been proven in wastewater effluents, rivers, and groundwaters, where it can potentially impact aquatic organisms. The present study focused on the long-term effects of levonorgestrel on the early development and mRNA expression of selected genes involved in the hypothalamus-pituitary-gonad (HPG) and hypothalamus-pituitary-thyroid (HPT) axes in common carp (Cyprinus carpio).

Materials and Methods: Common carp were exposed from the embryonic to juvenile period to three levels of levonorgestrel (3, 31 ng L−1 – environmentally relevant concentrations, and 310 ng L−1 – sublethal concentration) for 47 days. Moreover, solvent control was included. The effects of levonorgestrel on the development rate, morphological parameters, occurrence of malformations, histopathology of thyroid follicles, immunohistochemical examination of thyroxine labelling, and mRNA expression of selected genes involved in the HPG and HPT axis have been assessed.

Results: Levonorgestrel treatment did not impact early development and the occurrence of morphological anomalies or cause pathological alterations in the thyroid follicles. The thyroxine antibody labelling was not significantly different between fish exposed to 310 ng L-1 levonorgestrel and the control group. mRNA expression of iodothyronine deiodinases (dio1, 2, 3) was differentially affected by levonorgestrel treatment during carp development. The expression of dio3 was significantly down-regulated in fish exposed to all three levels of levonorgestrel compared to control at the conclusion of the experiment. At the end of the test, mRNA expression of npr, esr1, and esr2b in the body and npr and esr2b in the head of fish exposed to 310 ng L−1 LNG was significantly upregulated compared to the control.

Conclusions: The expression pattern of deiodinases, namely a decrease in dio1 or dio3 or an increase in dio2 transcription observed at different time points of the study, indicates hypothyroidism of levonorgestrel exposed fish. Upregulation of npr corresponds well to the fact that levonorgestrel is an antagonist of fish npr. Levonorgestrel caused parallel changes in the HPT and HPG axes.

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eDNA screening for "Pasteurella atlantica" in Norwegian aquaculture

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Pasteurellosis in cold-water fish is a systemic disease caused by different strains of Pasteurella skyensis and the as yet invalidly described "Pasteurella atlantica". The disease causes reduced welfare and losses primarily in Atlantic salmon farmed at sea in Norway and Scotland. While only a limited number of cases were diagnosed up until 2018 in Norway,

a specific strain of “P. atlantica” has since then become established along the south-western coast of Norway, with recorded disease outbreaks at 52 sites in 2022.

Environmental DNA (eDNA) monitoring is increasingly being used to detect both micro- and macro-organisms in aquatic environments, and has great potential as a surveillance tool of pathogens in aquaculture. While the expected shedding of pathogenic organisms is high during acute disease, and easily detectable during eDNA monitoring, shedding from latent infections is expected to be lower and harder to detect. However, we have demonstrated that during thermal delousing, which is extensively used in Norwegian salmon farms for ectoparasite removal, even small amounts of shed bacteria may accumulate to detectable levels due to high numbers of fish treated in a relative low water volume. eDNA analysis of treatment water from thermal delousing units can thus reveal even latent infections, and we have used this method as a screening tool to map the distribution of “P. atlantica” in Norwegian aquaculture. In some cases, eDNA has revealed the presence of the bacterium weeks before disease outbreaks manifest.

These findings supports the notion that eDNA monitoring in aquaculture can be used as an early warning system of potential disease outbreaks, which could in turn improve biosecurity and fish welfare.

Multiplex PCR assay for simultaneous detection of three important viruses in tilapia

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The recent emergence of different RNA and DNA viruses that are negatively affecting global tilapia aquaculture has highlighted the urgent need to find effective methods to control and prevent diseases among tilapia. Although molecular assays based on PCR have been developed for single detection, no multiplex PCRs have been applied to detect these emerging viruses. In this study, we tested the accuracy (specificity and sensitivity) of a multiplex PCR assay for simultaneous detection of three important viruses in tilapia, namely, TiPV, ISKNV, and TiLV and sample preparation via the simultaneous extraction of RNA and DNA. The multiplex PCR assay was able to detect the presence of three tilapia viruses in only 1,000 copies per reaction and had high specificity. A comprehensive analysis of the multiplex PCR assay using clinical samples demonstrate the robustness, sensitivity, and specificity of the assay to screen samples infected by single or concurrent virus infections in tilapia. In addition, the technique provides a powerful and cost-effective tool for diagnosing and distinguishing between tilapia viruses.

Bacterial Diseases in Intensively Cultured Giant Snakehead in Thailand: Identification, Characterization, and Antibiotic Susceptibility

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The giant snakehead, Channa micropeltes, is a highly valuable freshwater fish species in Asia, particularly in Thailand, where it is widely cultivated. However, due to the intensive aquaculture practices, the giant snakehead is prone to high levels of stress and is susceptible to various diseases. This study presents an outbreak of disease that occurred in a farmed giant snakehead over a two-month period, resulting in a significant mortality rate of 52.5%. The infected fish exhibited signs of lethargy, anorexia, and hemorrhages in the skin and eyes. Bacterial isolation and analysis revealed two distinct colonies of gram-positive cocci and gram-negative rod-shaped bacteria. Further analysis based on 16S rRNA confirmed the isolates as Streptococcus iniae and Aeromonas veronii, respectively. The MLSA placed S. iniae isolate within a large clade of strains from clinically infected fish worldwide. Gross necropsy findings showed liver congestion, pericarditis, and white nodules in the kidney and liver. Histological analysis revealed focal to multifocal granulomas in the kidney and liver, enlarged blood vessels within the meninges of the brain, and severe pericarditis with myocardial infarction. Antibiotic susceptibility tests showed that S. iniae was sensitive to various antibiotics, while A. veronii was resistant to amoxicillin. Our findings highlight the need for appropriate treatment and control strategies for the natural concurrent bacterial infections in cultured giant snakehead.

New histopathological observations in cases of Moritella viscosa septicemia

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Introduction: “Winter ulcer” is a disease with serious negative impacts on fish health and welfare, as well as a cause of substantial economic loss. The primary causative agent is Moritella viscosa. Genetic analysis divides the bacteria into several closely related subpopulations, known as clonal complexes (CC). Currently, there is a lack of knowledge regarding potential virulence differences between CC. Infections with M. viscosa have been an increasing problem in salmon farming in Norway in recent years, despite routine vaccinations. Skin lesions are a hallmark of the disease, and

systemic infections occur commonly. So far in 2023, the Norwegian Veterinary Institute has diagnosed four cases of systemic infection with M. viscosa in atlantic salmon (Salmo salar) where the pathological findings differ from the typical disease manifestation. The main observation in these cases has been a pronounced fibrinous pericarditis, in some cases with evident bacterial infiltration. M. viscosa infection was confirmed at all of these sea-sites; either diagnosed recently or observed in the submitted material. In two cases, fish health personnel reported wounds at the base of the pectoral fins.

Methodology: Tissue samples from the submitted cases were initially examined in hematoxylin and eosin stained slides. To further investigate possible infection with M. viscosa, immunohistochemical analysis (IHC) aimed at this bacteria was performed. The applied antiserum is polyclonal and will detect M. viscosa, and in theory also other closely related bacteria. IHC aimed at Pasteurella sp. was done in two of the cases, as the pathological findings resemble those observed with pasteurellosis.

Results: IHC aimed at M. viscosa showed strong positive staining in pericardial areas with fibrinous exudate, inflammatory and degenerative changes in adjacent myocardial tissue, and bacterial structures. IHC aimed at Pasteurella sp. was negative.

Conclusion: These recent observations indicate that there may be a shift in the disease manifestation in certain cases of M.viscosa septicemia. It is unknown whether these new pathological findings can be attributed to specific clonal complexes or variants of the bacteria.

Effect of b-glucans on rainbow trout (Oncorynchus mykiss) IgM+ B cells

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Introduction: β-glucans are carbohydrates present in the cell wall of many fungi, often used as immunostimulants in feeds for aquacultured species. Their capacity to activate innate immune responses directly acting on innate cell populations has been widely document in fish. However, whether they can affect the functionality of adaptive immune cells has been scarcely explored. In this context, in the current work, we have determined the effects of different β-glucan concentrations on rainbow trout blood IgM+ B cells in the presence or absence of 2,4,6-trinitrophenyl hapten conjugated to lipopolysaccharide (TNP-LPS), a model antigen.

Methodology: Leukocytes were isolated from rainbow trout peripheral blood and incubated with different doses of β-glucans (6, 30 and 60 µg/ml) or media alone in the presence or absence of TNP-LPS (5 µg/ml) for 72 h at 20ºC. At this point, the survival of IgM+ B cells in the cultures as well as their levels of expression of surface MHC II and antigen processing capacities were determined by flow cytometry. The number of IgM-secreting cells in the cultures was also estimated by ELISpot.

Results: β-glucans did not affect the percentage of IgM+ B cells in leukocyte cultures, but significantly decreased the levels of surface MHC II expression and the antigen processing capacities of these cells, especially in the presence of TNP-LPS. On their own, β-glucans slightly activated the proliferation of IgM+ B cells but reduced that induced by TNP-LPS. Finally, β-glucans significantly increased the number of cells secreting IgM in the cultures.

Conclusions: These findings contribute to increase our knowledge regarding the immunostimulatory effects of β-glucans in fish, identifying fish B cells as targets for their regulatory effects.

Characterization of B cell responses in rainbow trout (Oncorhynchus mykiss) experimentally exposed to red mark syndrome (RMS)

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Introduction: Red mark syndrome (RMS) is a disease that affects farmed rainbow trout throughout Europe. Although the etiological agent of RMS has not yet been isolated, the disease is thought to be caused by a Midichloria-like organism (MLO). RMS causes no or low mortalities in the affected stocks but is characterized by red hyperaemic skin lesions, which lead to economic losses due to downgrading. Previous immunohistochemistry and transcriptomic studies pointed to a B cell dominated local immune response, which potentially could be involved in the pathology.

Methodology: In the current work, we further investigated the B cell response to RMS. For this, skin leukocytes were isolated from experimentally infected fish and non-infected controls and the presence of B cells studied by flow cytometry. The number of B cells secreting IgM in the skin was also determined by ELISPOT. Finally, a transcriptomic analysis of a panel of genes related with B cell function was also carried out in the skin as well as in systemic immune organs (spleen and head kidney).

Results: Flow cytometry confirmed that the presence of IgM+ B cells greatly increased in the skin lesions of rainbow trout affected by RMS. Additionally, we established that the number of cells secreting IgM locally also significantly increased, suggesting a local differentiation of B cells to plasmablasts / plasma cells. The gene expression pattern obtained in the skin further confirmed this local differentiation of B cells in RMS lesions. Finally, some changes in B cell associated genes in spleen and head kidney suggested that B cells were also affected to some degree in systemic immune organs.

Conclusions: Our results confirm the prevalence of B cell responses in RMS-related skin lesions and further demonstrate an associated local differentiation of B cells to plasmablasts / plasma cells in the affected fish.

Development of an immersion mucoadhesive nanovaccine to enhance vaccine efficacy against Aeromonas veronii in red tilapia (Oreochromis sp.)

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Red tilapia (Oreochromis sp.) is a freshwater fish species, important for world food security. However, in intensive industrial culture, they are prone to various bacterial diseases, especially hemorrhagic septicemia caused by Aeromonas veronii. Vaccination is widely used as a control strategy to prevent diseases in aquaculture. Vaccination by immersion has many benefits over injection vaccination, although it generally provides lower levels and shorter duration of protection. Therefore, antigen potency and the efficiency of antigen uptake across the mucosal tissues during immersion vaccine can be improved through the use of nano-delivery systems. Our study aimed to develop a potential mucoadhesive nanovaccine as an efficient delivery vehicle to enhance the bioavailability and antigenicity of the vaccine during immersion vaccination and to enhance the immune response and protective efficacy of the vaccine in red tilapia against A. veronii. Sonicated bacterial cells were encapsulated into the nanoparticles. The surface of the particles was modified with a mucoadhesive polymer. The physicochemical properties of the nanoparticles and encapsulated efficiency were assessed, and the particles were visualized by TEM. The average diameter of mucoadhesive nanovaccine particle was 215 ± 20 nm. With a zeta potential of 25 ± 3 mV. And 0.19 ± 0.1 of polydispersity index. The encapsulated efficiency of antigen into the nanoparticle was 97 ± 1.25%. The mucoadhesiveness of the nanoparticle vaccine was assessed by imaging the incorporation of fluorescent bacteria into the gills of the immersion-vaccinated fish and from SEM images. The results consistently showed that the immersion mucoadhesive nanovaccine had higher levels of attachment and uptake into the gills compared to the non-encapsulated vaccine. The vaccine efficacy was assessed by measuring the serum IgM antibody level, serum bactericidal activity, and level of protection against an experimental infection with a virulent A. veronii isolate administered by immersion. The results revealed a marked increase in IgM serum levels with enhanced bactericidal activity in the mucoadhesive nanovaccine vaccinated fish, which also had high levels of the relative percentage of survival (RPS). Our study highlights the potential of this immersion mucoadhesive nanovaccine to generate a protective immune response against A. veronii infection in immersion-vaccinated red tilapia.

European Virus Archives-Global boosts research in aquaculture

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EVAg (European Virus Archives-Global) is a non-profit organisation that deploys a global network with expertise in virology to collect, characterise, standardise, authenticate and distribute viruses and derived products. A unique biological resource in the field of virology has been made readily available online at https://www.european-virus-archive.com. Non-profit users may benefit from free of charge access to their products of interest. An international group of 36 laboratories, including 29 EU and 7 non-EU institutions, are actively involved in this project and represent an extensive range of virological disciplines. Among them, the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE) has recently added to this unique catalogue part of its extensive viral repository of aquatic viruses including: i) all known Betanodavirus species (RGNNV, SJNNV, TPNNV, BFNNV and reassortant strains); ii) several strains of Viral hemorrhagic septicemia virus - VHSV; iii) Infectious haematopoietic necrosis virus - IHNV; iv) Infectious pancreatic necrosis virus - IPNV; v) two strains of Tilapia Lake virus – TiLV; and finally vi) strains of Lymphocystis disease virus - LCDV and Ranaviruses (European catfish virus - ECV and Rana esculenta virus - REV). Uncultivable viruses such as Carp edema virus - CEV and Sturgeon iridovirus European - AcIV-E have also been made available.

The IZSVE deposited viruses meet the highest scientific standards in terms of quality and can therefore be used to develop and standardise diagnostic methods, besides being employed as certified positive controls. Since most of them are live, titrated and fully characterized, they can also be used during in vitro or in vivo pathogenesis studies or within any other scientific investigation involving aquatic viral diseases.

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Dietary beta-glucans supplementation effects on fish after experimental infection

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Introduction: As fish production in aquaculture increases, so does the risk of diseases outbreak. In connection with current trend of antibiotics reduction, the need for substances enhancing fish resistance to various agents increases. Beta-glucans belong to these immunostimulants.

Methodology: We tested the effect of yeast beta-1,3-1,6-glucans supplements in feed on the health status of common carp (Cyprinus carpio) and rainbow trout (Oncorhynchus mykiss) after exposure to Cyprinid herpesvirus 3 (CyHV-3) and Aeromonas salmonicida, respectively. The experiment on common carp included 3 groups of fish; control group and groups provided supplemented feed (beta-glucan concentration 0.1 and 0.5 %). After 3 weeks the fish were challenged with CyHV-3. The experiment on rainbow trout included 6 groups of fish; control group and groups with supplemented feed (beta-glucan concentration 0.1; 0.2; 0.5; 1 and 2 %). After 5 weeks the fish were challenged with A. salmonicida. In both tests, blood samples were taken prior the challenges and at the end of the experiments (i.e. 4 weeks after the challenge). Haematological, biochemical and immunological analyses were performed. The skin mucus was sampled for lysozyme determination and the gut microbiome was analysed in both fish species.

Results: We are still processing the results of described experiments. According the first assessments it seems that beta-glucans had immunostimulatory effects in common carp. The highest titre of specific antibodies against CyHV-3 were detected in the group fed higher concentration of beta-glucans. In the same group, we also observed enhanced oxidative burst of peripheral blood phagocytes.

Conclusions: The observed the enhanced specific and non-specific immune response in common carp demonstrates the positive impact of beta-glucans on fish health, confirms their suitability for further studies and preliminarily suggests their potential for use in aquaculture.

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Modulation of gilthead seabream gill microbiota by Sparicotyle chrysophrii infection

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In the aquaculture industry, there is a growing interest in manipulating microbiota to improve welfare and nutrition, as it plays critical roles in many host functions, including nutrient metabolism, digestion, immunity, and protection against pathogens. In that sense, fish skin and gills' mucosal surfaces are the primary defense barriers against pathogens. Thus, characterizing their microbial communities is pivotal for detecting potential alterations that lead to fish homeostatic imbalance and disease susceptibility. Sparicotylosis is an enzootic disease caused by the gill parasite Sparicotyle chrysophrii. It is well established across the Mediterranean Sea and affects gilthead seabream (Sparus aurata) with limited management, mitigation, and treatment strategies.

This study aims to assess how the gill microbiota of gilthead seabream is modulated during sparicotylosis. Gilthead seabream were infected by exposure to effluent water from infected donor fish. In parallel, an additional tank containing unexposed fish was used as control (C). Infected fish were divided into two groups according to their infection intensity and days post-exposure (dpe): Low (L, 14 dpe, <100 parasites/fish) and High (H, 42 dpe, >100 parasites/fish). Five gill mucus samples from each group were selected for 16S rRNA MinION sequencing and then processed with an in-house bioinformatics pipeline to analyze gill microbial composition. Gill histopathology was performed in parallel.

Proteobacteria was the most abundant phylum in all groups, constituting more than 90% of the bacteria, followed by Firmicutes and Bacteroidota. A significantly lower alpha-diversity was detected in the H group in comparison to the L and C groups, and beta-diversity and discriminant analyses showed significant differences in the gill microbiome structure among the three groups. At the genus level, LEfSe analysis revealed nine microbial markers. Of interest, the bacterial biomarker 2013Ark19i (Candidatus Ichthyocystis sparus), already present in C, increased dramatically in H. This bacterium, proposed to be the causing agent of epitheliocystis, correlated with the increased number of cysts in H gills.

These results show how sparicotylosis changes the gill microbiota of gilthead seabream, facilitating the growth of pathogenic bacteria and the development of secondary infections that aggravate the outcome of the disease.

Unexpected associated bacteria with Crassostrea gigas juveniles during mortality events

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Crassostrea gigas pacific oysters are recurrently affected by mortalities at the juvenile stage both on the atlantic and mediterraneous coast since 2008. Thau lagoon (south of France) is an important farming area, producing between 7000 and 5000 tonnes of oysters each year. However, the recurrent juvenile oysters mortality, ranging from 35% to 70%, causes important economic losses.

The disease involves an herpes virus (OsHv-1 µvar) that causes immunosupression in juveniles, leading to bacterial dysbiosis, then followed by opportunistic bacterial colonization and final death. The underlying mecanisms that triggers mortality, depending both on host and environment interaction, remains still unknown.

In « Microlag project » conducted in Thau lagoon, we investigated the changes in the microbiome composition in juveniles oysters from several months before mortality starts, during the mortality event, and till 2 months later to determine if the microbiome characteristics could help in understanding the outcome of oysters. We also investigated the impact of moribund oysters on their immediate environment (water and sediment) as we suspected the role of the environnment in these recurrent episodes of mortalities.

The microbiome of juveniles oysters (alive and moribund) was investigated by 16rRNA sequencing, from february to august 2021. At the same time, OsHv-1 herpes virus was quantified by qPCR, aside with a suspected primary pathogen (Vibrio aestuarianus).

The results showed unexpected associated bacteria with juvenile oysters : the Mycoplasmatacea 216amily, that dominated quite constantly juveniles'microbiome, and a more discreet pathogen : Vibrio aestuarianus, mainly present during the mortality event. These bacteria were also detected in the environment. The role of these permanent or transient bacteria in oysters in relation with the farming environment will be discussed in the context of oysters mortality.

Larvicidal activity of water extracts of selected herbs and spices against L3 larvae of Anisakis pegreffii

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Introduction: Anisakiasis is a fish-borne zoonosis caused by the ingestion of live and/or dead third-stage larvae (L3) of the genus Anisakis Dujardin, 1845. Undercooked or thermally unprocessed fish and cephalopods, such as carpaccio, cold marinated or lightly salted products present substantial epidemiological risk. To maintain organoleptic properties of the product, the freezing of flesh prior to processing is ignored, especially in households, providing vector for the transmission of this disease. Extracts and bioactive compounds from plants and herbs can increase food safety since they have proven antimicrobial and antioxidant properties and can be safely incorporated in food processing protocols.

Methodology: the larvicidal effect of aqueous extracts of 15 species of selected herbs and spices against larvae of Anisakis pegreffii was tested at 37°C and 6°C, with and without the addition of 5% NaCl. The experiment was performed in six replicates per extract/temperature/salt regime, with 10 – 15 A. pegreffii larvae per replicate. Pure water served as control. The experiment at 37°C lasted for six days, and the one at 6°C for 12 days. Viability of the larvae was checked under dissecting microscope at specific intervals during the experiment. Kaplan-Meier survival curves were used to visualize the results, and a subset of larvae was identified at the molecular level by sequencing of COX2 gene amplicon, and PCR-RFLP assay of ITS1-5.8S-ITS2 region.

Results: nematocidal/larvicidal activity on L3 of A pegreffii was observed for aqueous extracts of clove, garlic, black and green pepper, turmeric, and laurel at 37°C. Of these, only clove showed a similar effect at 6°C, regardless of salt content. Low temperature completely neutralized larvicidal activity of green pepper and bay leaf within 12 days of the experiment. Other extracts (yellow and purple onion, celery, fennel, parsley, olive, sage, rosemary, lemon) did not show larvicidal effect.

Conclusions: aqueous extracts of clove, garlic, black pepper, and turmeric represent good candidates for identification of active compounds in future research to facilitate the development of applications and dosage guidelines, either for medical/ prophylaxis purposes, or in the fish-processing industry to reduce the zoonotic risk for consumers.

Developing stable transformation for saprolegnia parasitica

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Introduction: Emerging oomycete diseases, such as Aphanomyces invadans, Aphanomyces astaci and Halioticida noduliformans, Saprolegnia diclina and Saprolegnia parasitica are responsible for serious ecological disruptions to

aquatic biodiversity and global food security. The prevalence of saprolegniosis is specifically a serious threat to farmed fishes and is mainly caused by the S. parasitica.

Functional characterisation of genes is possible in S. parasitica with a transient RNAi silencing method, but a stable transformation method would be better because reporter genes can be used to follow gene expression and localisation studies of particular proteins, as well as potentially creating stable gene silenced transformants. Therefore, the aim of this study is to develop a S. parasitica transformation method, which has never been achieved before.

Methodology: The DNA-transformation method we developed, thus far, is based on the uptake (entry) and integration of a selection marker gene with the help of PEG and lipofectin in protoplasts of S. parasitica.

Results: Transformation experiments with a CyP51 selection marker gene have been carried out and resulted in the successful creation of transformants as was verified by PCR. In several of the transformation experiments, a green-fluorescent marker gene (GFP) has been introduced into S. parasitica following co-transformation with the CyP51 selection gene and several transformants showed constitutive GFP production.

Conclusions: We have accomplished, for the first time, a method that can generate transgenic S. parasitica strains. The method is continuously refined and optimised and should experiments continue to show promising results, we also hope to generate transgenic strains of S. parasitica in which we have silenced genes that may be involved in pathogenicity.

Surname: V

Production and characterization of chimeric recombinant nanostructured proteins to be used as antigens in a prototype vaccine against Piscirikettsia salmonis

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Introduction: Piscirickettsia salmonis is the etiological agent of Piscirickettsiosis, one of the most serious and harmful diseases that affect the salmon industry in Chile. Currently in Chile there are 34 vaccines for the control of this pathogen. However, none have shown a decrease in continuous outbreaks during the salmon production cycle. For this reason, the development of a new prophylactic method based on high-efficiency antigenic nanoproteins of P. salmonis is proposed, since it releases functional proteins over time, protecting salmon during their productive stage.

Methodology: In the first instance, two recombinant chimeric proteins were designed by reverse vaccinology, verifying the antigenic potential of amino acid sequences with epitope characteristics, using the following servers: Vaxijen v2.0, Bcepred, Rankpep, ProtScale. Selected amino acid sequences that were included in the pET22b(+) vector; those that were overexpressed, produced and purified on a laboratory scale; proteins were induced with 1 mM IPTG for 3 hours at 37°C in the exponential phase of E.coli BL21(DE3) bacterial growth. To then be purified by mechanical and enzymatic disruption techniques. Subsequently, they were quantified using the western blot technique and characterized by SEM. In summary, it was possible to produce chimeric nanoproteins at high concentrations, obtaining ranges between 12.5 mg/ml to 99 mg/ml of protein/production, which were visualized in the SEM as spheres with an average diameter of 160 nm. These proteins were produced in E.coli which maintained an OD600 of 0.8 to 1.2 during the 3 hour induction with IPTG.

Conclusions: In conclusion, chimeric nanoproteins of amino acid sequences with epitopic characteristics of P.salmonis were produced in E.coli BL21 (DE3) in a mg ratio, so it is planned to evaluate whether they induce activation of genes involved in antigen presentation (cd80/86, cd83, mhcii and il-12 gene) in salmon cell lines and verify if the proposed vaccine prototype can induce an adaptive immune response over time, by means of a bioassay in fish.

Diagnostic methods for identifying Aphanomyces astaci, the crayfish plague agent

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Introduction: Crayfish plague is a lethal disease to most freshwater crayfish species, except for North American crayfish species, which function as natural carriers of the crayfish plague agent, the oomycete Aphanomyces astaci. The detection of A. astaci used to be dependent on the isolation of the agent, followed by pathogenicity test, until molecular diagnostic methods became available. Today, quantitative real-time PCR (qPCR) methods are most used for the diagnosis of crayfish plague. However, new discoveries of Aphanomyces species, such as A. fennicus, have demonstrated that false positive results are possible with the established internal transcribed spacer (ITS) based qPCR. Several PCR methods have been developed for detection of A. astaci, some of which amplify genotype-specific regions. Isolation of the pathogen remains a necessity for further research, although it is best suited for samples from acute disease cases. We compared three PCR methods and developed a MALDI-TOF MS method for the quick identification of isolates from cultures.

Methodology: Selected samples from a crayfish plague positive group of noble crayfish were evaluated by three PCR methods: the established ITS-based qPCR, an improved ITS-based qPCR, and a genotyping PCR method.

For the MALDI TOF MS analysis, an A. astaci reference strain was used to create a mass spectrum library against which A. astaci strains of several genotypes, and other oomycete species in Finnish Food Authority culture collection were compared to assess the specificity of the method.

Results: The results of the three PCR methods from same crayfish samples are presented and compared.
Using MALDI-TOF MS pure cultures of different A. astaci genotypes were reliably identified as A. astaci from other Aphanomyces species and several other oomycete species tested.

Conclusions: Although molecular methods have improved the diagnostics of crayfish plague, the possibility of unknown genetic diversity of oomycetes in aquatic environment can affect the reliability of genetic methods. A method best fit for purpose should be chosen.

MALDI-TOF MS appeared suitable as an additional diagnostic tool for A. astaci identification of isolated cultures, with the caveat that the culture needs to be as pure as possible.

A study on the in-vitro instability and the replication functions of the virulence plasmid pPHDP70 of Photobacterium damselae subsp. piscicida

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Introduction: Several bacterial pathogens require plasmid-encoded virulence factors. As opposite to chromosomal genes, which are relatively stable, plasmid genes are subjected to high instability, a condition that may pass unadverted in vitro. Photobacterium damselae subsp. piscicida (Pdp) (formerly Pasteurella piscicida) is a devastating pathogen in marine aquaculture. One of its pathogenicity mechanisms is the acquisition of host iron by siderophore piscibactin, a system encoded within the mobilizable plasmid pPHDP70. Little is known about pPHDP70 stability, and the genetic elements involved in its replication and maintenance remain unstudied.

Methodology: Evidence of the in vitro curing of pPHDP70 was obtained by subjecting a virulent Pdp strain to passages in liquid medium under iron-replete conditions. Aliquots were plated and colony-PCR used to track pPHDP70. To investigate the genetic elements involved in plasmid replication, cloning combinations were assayed by shuffling three candidate sequences in cis and trans. Plasmid incompatibility between pPHDP70 and the related MDR plasmid pPHDD2-OG2 was assessed by mating assays.

Results: We demonstrated that pPHDP70 undergoes curing upon in vitro cultivation. The percentage of individual colonies PCR-negative for pPHDP70 increased from 12% in the first passage to 54% in the fourth. We found that a small, non-protein coding plasmid region constituted the minimum cis-acting sequence required for self-replication, but this sequence needed the in-trans provision of two additional, distantly-located, plasmid regions, one encoding RepA, and a second region encoding hypothetical proteins. These plasmid regions exhibited high similarity to pPHDD2-OG2 carried by its sister subspecies P. damselae subsp. damselae (Pdd). As expected, acquisition of pPHDD2-OG2 (pAQU1-related) under antibiotic selective pressure, caused the loss of pPHDP70 in 100% of the Pdp transconjugants.

Conclusions: The virulence plasmid pPHDP70 undergoes in vitro instability with a great proneness to undergo curing, a fact that should be taken into consideration in laboratories handling Pdp strains for research purposes and for vaccine development. The minimal elements required for pPHDP70 replication have been mapped to three distantly-located plasmid regions. pPHDP70 proved to be incompatible with the family of pAQU1-related multidrug-resistant plasmids. These results might contribute to the future design of strategies to control MDR plasmid spread.

Description of a novel Saprolegnia (Oomycota) species from Lake Velence in Hungary

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Introduction: Water moulds (Oomycota: Saprolegniales) are found in all freshwater habitats, and some species may cause significant economic and ecological losses. The precise identification of water mould species by morphological features is challenging. Species-level identification is mainly based on sexual structures (antheridium, oogonium), but many species do not form these in vitro and often in vivo neither. Therefore, species identification is nowadays aided by DNA sequence analysis of internal transcribed spacer regions 1 and 2 (ITS 1&2).

Methodology: In the course of a survey, we collected macroscopically apparent water mould samples from Lake Velence, the second largest natural lake in Hungary. Saprolegnia isolates were examined morphologically, moreover using molecular and phylogenetic methods targeting ITS regions and RNA polymerase II B subunit (RPB2) gene. In vitro examinations were carried out in three different culture media at two temperature ranges (+20-21°C and +6-8°C).

Results: The examined two Saprolegnia sp. isolates developed most of the structures in the presence of fish extract, regardless of temperature and the mineral content of media. Elongated, large, and branched gemmae were observed, in many cases double gemmae chains, and undeveloped, round, smooth-surfaced oogonia. However, antheridium and mature oogonium were not observed. The structures observed were somewhat similar to those of Saprolegnia ferax, however the DNA sequence comparison and phylogenetic analysis confirmed that the two isolates (with 100% ITS sequence identity) were not identical to S. ferax. They were closely related to S. ferax (sequence identity 94.2-94.5%) and to Saprolegnia australis (93.9-94.2%), but represented a completely new branch in the maximum likelihood (RAxML) phylogenetic tree. By examining a 612-bp-long fragment of the protein-coding gene, RPB2, the two isolates clustered together in the RAxML tree, next to the group composed of S. australis and S. ferax sequences.

Conclusion: Based on our results, the Saprolegnia sp. From Lake Velence cannot be identified with any of the previously described water mould species, thus its description as a new species seems justified.
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Elucidating the dynamics and transmission potential of the aquatic pathogen Renibacterium salmoninarum in Rainbow trout

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Introduction: Renibacterium salmoninarum (Rs) is a facultative intracellular bacterium and the aetiologic agent of bacterial kidney disease (BKD). This chronic infection is associated with severe mortality in salmonid fish worldwide. Rainbow trout (Oncorhynchus mykiss), the main species farmed in Denmark, is highly susceptible to Rs although the mechanisms of transmission and the chronic state of the infection are not yet fully understood. The disease represents one of the main health challenges in Recirculating Aquaculture systems (RAS).

Methodology: Our study aimed to characterize disease kinetics and host survival through in-vivo cohabitation at 6°C and 12°C in RAS. Rainbow trout shedders were intraperitoneally injected with Rs (1x109, 1x108 cells dose-1) or saline water (control), and subjected to survival analysis during 12 weeks post-infection (wpi). Naïve fish were put in cohabitation with shedders to assess the transmission potential and bacterial kinetics through qPCR of bacterial DNA and environmental DNA (eDNA) extracted from fish kidney and water samples, respectively.

Results: All challenged groups were susceptible to Rs, but only the shedders experienced reduced survival due to BKD. Reduced survival began at an earlier stage at 12°C, and the probability of surviving until the end of the trial was 16.10%, 5%, and 0% for the groups injected at low dose at 12°C, high dose at 12°C, and high dose at 6°C, respectively. Infection by cohabitation was established within 2 weeks, and abundance of bacterial transcripts was significantly higher at 4 wpi in the kidney of sampled fish in all infected groups. Interestingly, water eDNA analyses revealed bacterial shedding at its highest at 3 wpi and detected the bacteria at late infection sampling points. Moreover, Rs was reisolated in SKDM media from fish kidney samples also at the latest stage of infection at 6°C.

Conclusion: Our results provide information on disease progression and transmission potential of BKD and insights into the chronic state of the infection. Here we also confirm the possibility to detect Rs in RAS at different infection stages. Overall, this study defines an infection model of Rs in rainbow trout, essential to further explore the underlying mechanisms of pathogenicity of the bacterium.

Documentation of an outbreak of kudoa lutjanus in commercially farmed chicken grunt (parapristipoma trilineatum)

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Chicken grunt (Parapristipoma trilineatum) is a popular species for commercial-scale aquaculture due to its economic value and growing market demand. However, an outbreak of Kudoa 221utjanus was recently detected in two farms in Pingtung County, Taiwan - containing 5000 and 20000 chicken grunt, respectively. Infected fish showed erratic swimming behavior such as whirling and floating on the water's surface. The infected fish displayed white oval cysts in various locations, including the muscle, internal organs' serosa, sclera of the eyes, and cerebral meninges. These specimens were identical to those of other K. 221utjanus by 18S and 28S ribosomal rRNA sequencing. The outbreak of K. 221utjanus in chicken grunt is the first confirmed case in Taiwan, with barely any documented cases of farmed fish infections since 2003, its discovery. As known world, seafood safety is a critical concern, especially in raw fish cuisine, as myxozoa parasites have made it challenging to produce safe and high-value seafood. Its reappearance with significant mortality should serve as a warning to the aquaculture industry and seafood safety.

Case Study: The resurgence of Aeromonas salmonicida in Scottish Aquaculture

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Introduction: Aeromonas salmonicida is the causative agent of the disease furunculosis in Atlantic Salmon (Salmo salar). The disease had a devastating impact on farmed S. salar population until the late 1980s when a vaccine was developed, reducing cases to small isolated incidents. However, since 2022, cases have started increasing in Scottish aquaculture. In 2022, 17% of cases (5/29) isolated A. salmonicida compared to 0% of cases in both 2021 (0/20) and 2020 (0/8), 7% of cases in 2019 (2/30) and 5% in 2018 (1/20). A case has recently been identified in April 2023 from the Western Isles, Scotland which isolated A. salmonicida from 4 out of 5 fish from material taken from the kidney, lesion and gill. The level and purity of growth in this case suggested that the isolate would be implicated as a source of morbidity and, because A. salmonicida is a primary fish pathogen, it poses a significant risk to fish health. Aeromonas was also confirmed through histopathology.

Methodology: Samples are taken by the Scottish Fish Health Inspectors as part of their routine sampling of salmon farms. Tissue material is taken from kidney, gill and lesions (if present) onto TSA/TSAS and colonies of interest are identified using phenotypic and biochemical bacteriological methods which include antimicrobial sensitivity testing. Isolates of A. salmonicida are further analysed using molecular genetics, including the sequencing of the vapA gene.

Results/ Conclusion: The A. salmonicida isolate from the 2023 case shows no difference to historic isolates for sequencing on the vapA gene. There was evidence of increased resistance to AML, but no evidence of resistance to OT, FFC or SXT. Further investigations are ongoing on how this compares to historic isolates. Whole genome sequencing investigations are ongoing to analyse the possible emergence of new variants by comparing recent isolates to archive samples from 1990s. Other factors, including vaccine failure and the robustness of the fish are also being considered.

Time-course proteomic analysis of the early response induced by a DNA vaccine against viral haemorrhagic septicaemia (VHS) in rainbow trout

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DNA vaccine against viral haemorrhagic septicaemia (VHS-DNA vaccine) induces effective and long-lasting protection in rainbow trout. The protective mechanisms induced by this vaccine start a few days post-vaccination when systemic innate responses are triggered. The specific adaptive immunity replaces the non-specific innate immunity a few weeks post-vaccination, which can protect the immunized fish during the whole farmed cycle.

The strong early responses induced by VHS-DNA vaccine could be the key to paving the way for an effective, specific, and long-lasting protection. Therefore, several studies have focused on dissecting the early response by analyzing the differentially expressed genes in different tissues. However, it is known that there is a low correlation between transcriptomic and proteomic (functional) analysis because of the post-transcriptional/translational regulations. Thus, it is not completely correct to explain biological processes by the results of gene expression analysis.

In this study, we performed a proteomic analysis using quantitative mass spectrometry of different tissues (muscle, liver, spleen, head kidney, blood cells, and thymus) of fish vaccinated with the VHS-DNA vaccine. Additionally, several sampling time points were done to have an overview of the kinetics of the mechanism involved. The differentially expressed proteins in each condition were used to perform pathways and network analysis to discover biological processes that could explain the high efficacy of this vaccine. These results could be used to compare and understand the mechanisms induced by other vaccines.

Transcriptome analysis of Pacific abalone (Haliotis discus hannai) challenged with polyriboinosinic polyribocytidylic acid (poly I:C)

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Introduction: Abalone is an economically important marine mollusk with high nutritional and medicinal value. However, they are susceptible to various diseases, including viral infections. Poly(I:C) is a synthetic analog of double-stranded RNA and has been shown to activate the innate immune response in various organisms. In this study, we aimed to identify differentially expressed genes in abalone hemocytes in response to Poly(I:C) treatment using de novo transcriptome assembly.

Methodology: The control group was injected intramuscularly with 100 µL of DEPC-treated water, and the treated group was injected with 100 µg of double-stranded synthetic RNA, Poly (I:C). Hemocytes were isolated from control and Poly(I:C)-treated abalone, and RNA was extracted for sequencing. We performed transcriptome analysis on hemocytes from abalone using the next generation sequencing technology, Illumina Nextseq system.

Result: De novo transcriptome assembly resulted in a total of 448,240 contigs with 327,400,067 bp and an average length of 730 bp per gene. Differential gene expression analysis identified 2,555 DEGs in Poly(I:C)-treated abalone compared to the control, with 1,270 up-regulated and 1,285 down-regulated. Up-regulated DEGs include hemicentin-1, superoxide dismutase, interferon α-inducible protein and retrotransposon. Down-regulated DEGs include α-L-fucosidase, matrix metalloproteinase, Collagen α-1, tubulinA such as actin-related genes (fold change > 2, $p < 0.05$). Among the up-regulated genes, the hemicentin transcript related to the immunoglobulin superfamily was significantly up-regulated (LogFC 4.871).

Conclusion: These results provide insights into the molecular mechanisms underlying the immune response of abalone to Poly(I:C) treatment. The up-regulation of hemicentin, a protein with known functions in cell adhesion and migration, suggests a potential role in the immune response of abalone to viral infections. Future studies could investigate the functional roles of other DEGs identified in this study and their potential applications in abalone aquaculture.