3rd UK & Ireland EAFP Branch Meeting

Marine Institute

Ireland

Connacht Hotel, 11 – 12 September 2018

Book of Abstracts

In association with:

Tuesday 11 September 2018	
8.30 – 9.20	Registration
Welcome & Opening Address	
9.20 – 9.30	Welcome (Neil Ruane, Marine Institute)
9.30 – 10.10	**Plenary lecture: Wild Atlantic salmon survival and the role of the salmon louse *Lepeophtheirus salmonis* –** (Dave Jackson, Marine Institute)
Viral Diseases (Chair: Eann Munro, Marine Scotland)	
10.15 – 10.30	**An overview of oyster herpesvirus OSHV1-µvar in Ireland** – (Deborah Cheslett, Marine Institute)
10.30 – 10.45	**Preliminary results on the estimation of the minimum infective dose of viral nervous necrosis virus in European sea bass and its stability in seawater –** (Aranҫa Viaplana, AquaBioTech Group)
Break	
11.15 – 11.30	**Experimental investigations into ranavirus (*Iridoviridae*) infections in lumpfish (*Cyclopterus lumpus*)** – (Felix Scholz, Fish Vet Group Ireland)
11.30 – 11.45	**Practical use of syndromic surveillance on detecting cardiomyopathy syndrome in salmon farming** – (Silvia Soares, Marine Scotland)
11.45 – 12.00	**Cyprinid viral diseases in Ireland** – (Susie Mitchell, Fish Vet Group Ireland/Neil Ruane, Marine Institute)
12.00 – 12.15	**RNA-seq analysis of rainbow trout transcriptome reveals suppression of the unfolded protein response by pathogenic VHSV** – (Ronny can Aerle, CEFAS)
12.15 – 12.30	**Wider potential ecological impacts of diseases in bivalves –** (Sarah Culloty, University College Cork)
Lunch	
Bacterial Diseases (Chair: David Verner-Jeffreys, Cefas)	
13.30 – 13.45	**Infectious diseases of cleaner fish in Norway** - (Snorre Gulla, Norwegian Veterinary Institute)
13.45 – 14.00	**Global population structure, endemism and anthropogenic spread of *Yersinia ruckeri* as revealed by whole genome sequencing** – (Ed Feil, University of Bath)
14.00 – 14.15	**Screening for genes enhancing growth of *Aeromonas salmonicida* in fish mucus** – (Andrew Desbois, University of Stirling)
14.15 – 14.30	**Genomic epidemiology of the commercially important pathogen *Renibacterium salmoninarum* within the Chilean salmon industry** – (Sion Bayliss, University of Bath)

14.30 – 14.45	**Assessment of an atypical *Aeromonas salmonicida* bath challenge model in farmed ballan wrasse using molecular methods and histopathology** – (Sean Monaghan, University of Stirling)
14.45 – 15.00	**The Cefas Aquatic AMR Centre of Excellence** – (Edel Chambers, Cefas)
Break	

Surveillance, Management & Research (Chair: Neil Ruane, Marine Institute)	
15.30 – 15.45	**Crayfish plague in Ireland** – (Teresa Morrissey, Marine Institute)
15.45 – 16.00	**Amoebic gill disease: acute immune response of salmon affected by *Neoparamoeba perurans*** – (Adelaide Lerebours, Galway-Mayo Institute of Technology)
16.00 – 16.15	**An improved method for the visualization of sea louse copepodids (Caligidae) from plankton samples by fluorescence stereomicroscopy** (Mark Fordyce, Marine Scotland)
16.15 – 16.30	**Emerging haplosporidia in bivalves around the Irish Coast –** (Sharon Lynch, University College Cork)
16.30 – 16.45	**Connecting industry, science and government for the effective management of serious disease** – (Jason Mewett, CEFAS)
16.45 – 17.00	**Mind the gap: bridging academia and stakeholders to improve disease management in freshwater fisheries** – (Chris Williams, Environment Agency UK)
17.00 – 17.15	**BLUE SMART: Blue education for sustainable management of aquatic resources** – (Tomislav Šarić, University of Zadar)
19.00	**Meeting Dinner @ Connacht Hotel**

Wednesday 12 September 2018	

Early Stage Research Session I (Chair: Sandra Adams, Stirling University)	
9.10 – 9.20	**A rapid test for detecting the infection load of the parasite, *Anguillicola crassus*, in the European eel, *Anguilla anguilla*** – (Michele De Noia, University of Glasgow)
9.20 – 9.30	**Do cleaner fish pose a risk of pathogen transfer? A health assessment of ballan wrasse from the south west of England** – (Jamie McMurtrie, University of Bath/CEFAS)
9.30 – 9.40	**Comparative proteomic profiling of pathogenic and non-pathogenic *Neoparamoeba perurans* proteins associated with AGD** – (Kerrie Ni Dhufaigh, Galway-Mayo IT)

9.40 – 9.50	**Misidentification of bacteria without the use of molecular confirmation** – (Maria Campbell, Marine Scotland)
9.50 – 10.00	**The potential role of invasive species in the transmission of pathogens in the marine environment** – (Katie Costello, University College Cork)
10.00 – 10.10	**Genetic diversity of piscine myocarditis virus in Ireland** – (Andrew Tighe, University College Dublin/Marine Institute)
10.10 – 10.20	**Aquaculture from the inside out: characterizing the gut microbiome in the cultivation of the European lobster** – (Corey Holt, University of Exeter/CEFAS)
10.20 – 10.30	***Yersinia ruckeri* in Norwegian aquaculture**– (Andreas Riborg, Norwegian Veterinary Institute)
10.30 – 10.40	**Metagenomic characterization of the cleaner fish microbiome** – (Lyndsay Christie, University of Bath/CEFAS)
Break	

Early Stage Research Session II (Chair David Hoole, Keele University)	
11.10 – 11.20	**Characterisation of the Atlantic salmon microbial community on gills during an amoebic gill disease outbreak** – (Victor Birlanga, NUI Galway)
11.20 – 11.30	**Cockle health, disease connectivity and trophic interaction dynamics** – (Sara Albuixech Marti, University College Cork)
11.30 – 11.40	**Investigating the prevalence and transmission of a novel *Endozoicomonas*-like organism (ELO) infecting King scallop (*Pecten maximus* L.) populations in a marine protected area** – (Patrick Hooper, CEFAS)
11.40 – 11.50	**Cathelicidins and β-defensin: antimicrobial peptide expression in Atlantic salmon** – (Leisha McGrath, Galway-Mayo IT)
11.50 – 12.00	**Development of laboratory-based protocols for challenging *Crassostrea gigas* with Oyster herpesvirus 1 (OsHV-1 µVar)** – (Chantelle Hooper, CEFAS)
12.00 – 12.10	**Evaluation of new chemotherapeutic approaches to treat amoebic gill disease** – (Cyril Henard, University of Stirling)
12.10 – 12.20	**Population dynamics, reproduction and health of an ecosystem engineer, the common cockle *Cerastoderma edule*: past, present and future scenarios** – (Kate Mahony, University College Cork)
12.20 – 12.30	**Transcriptome analysis via RNA-Seq of the acute immune response in Atlantic salmon challenged with *Neoparamoeba perurans*** – (Grainne Moran, Galway-Mayo IT)
12.30 – 13.00	Close of meeting & presentation of prizes
Lunch	

POSTERS

From detection to regulation – the emergence of carp edema virus disease (CEVD) in freshwater fisheries in England - (Chris Williams, Environment Agency)

Loop mediated isothermal amplification (LAMP) for the rapid in-field detection of *Neoparamoeba perurans* - (Richard Paley, Cefas)

Attempts to culture carp edema virus on *in vitro* gill primary cell epithelia in asymmetrical conditions - (Richard Paley, Cefas)

An *in vitro* model to study salmonid innate immune response to *Neoparamoeba perurans* – (Richard Paley, Cefas)

Use of artificial egg-laying structures to monitor and control *Argulus* infections in still water fisheries – reality or pipe-dream? – (Chris Williams, Environment Agency)

CGIAR research program on fish agri-food systems (FISH) – (Jerome Delamare-Deboutteville, WorldFish)

The impact of UV-B radiation on Pacific oyster performance and development of the pathogens herpes virus *Vibrio aestuarianus* – (Gary Kett, University College Cork)

Proteolytic activity in extracellular products of *in vitro* cultured *Paramoeba perurans* – (Jadwiga Sokolowska, University of Stirling)

Genotype-phenotype mapping of the three-spined stickleback ecology in the Burrishoole catchment – (Floriane Leseur, Marine Institute)

Student prizes sponsored by Elsevier.

WILD ATLANTIC SALMON SURVIVAL AND THE ROLE OF THE SALMON LOUSE *LEPEOPHTHEIRUS SALMONIS*

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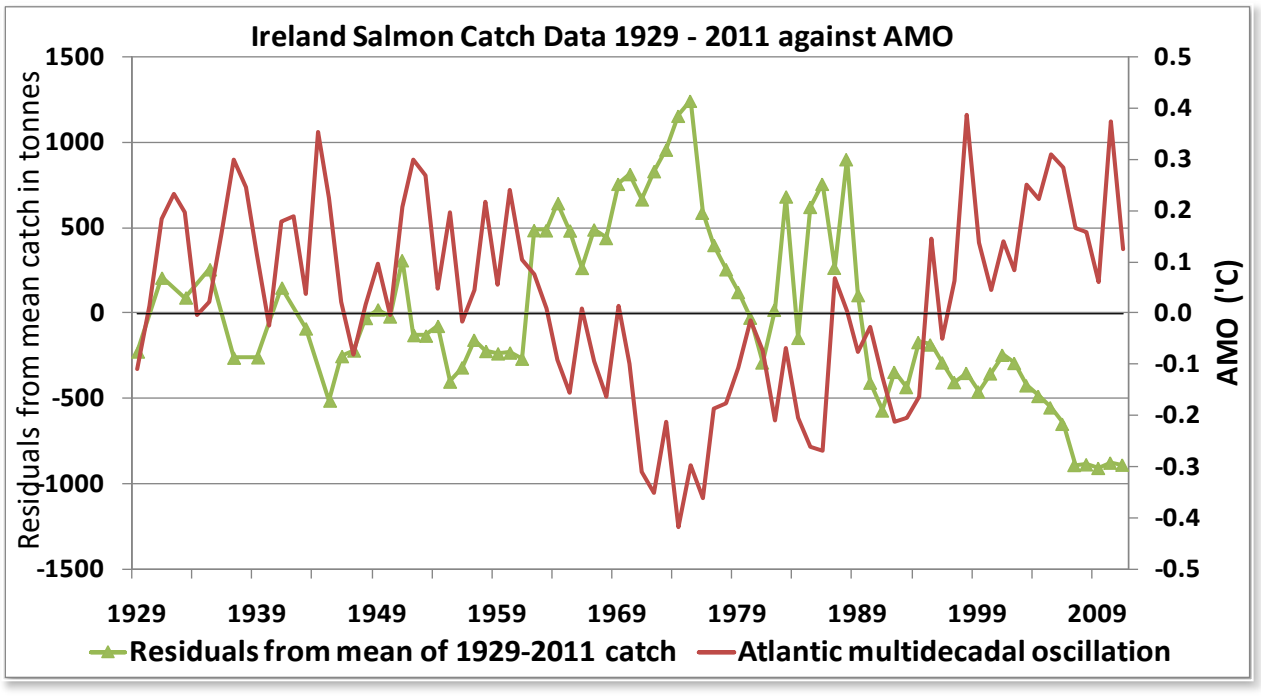
Abstract

A fall in Atlantic salmon marine survival has had profound negative effects on fisheries in the Northeast Atlantic. This has prompted debate as to potential causes for this decline including the potential role of salmon aquaculture. A metadata analysis of available information from 19 groups from 10 locations in Norway and Ireland over a 14 year period was carried out to assess the impact of early infestation with sea lice on marine mortality. The results of the study show that a mortality of approximately 1.0% is attributable to sea lice, over and above mortality from other sources. Over the course of the study period from 1996 to 2009 changes in the climate of the Northeast Atlantic as measured by reference to the Atlantic Multidecadal Oscillation (AMO) have mirrored changes in salmon survival. Over the period 1929 to 2009 salmon catch statistics have co-varied with the AMO. While sea lice infestation does not seem to be linked to declining levels of survival in migrating salmonids over the study period, variations of climatic conditions in the North Atlantic, as measured by the AMO, show a strong relationship with variations in salmon catch and by inference with marine survival over nine decades.



AN OVERVIEW OF OYSTER HERPESVIRUS OsHV-1µVar IN IRELAND

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Abstract

Pacific oysters are one of the most important farmed mollusc species, with global production reaching almost almost 574,000t in 2016. Since 2008, the industry has faced mass mortality events due to Ostreid Herpes Virus-1µVariants. Ten years on from the first detection of Ostreid herpes Virus-1µVar in Ireland the virus is now considered endemic in the majority of Irish production areas. What has been the real impact of the disease and how has the industry adapted to the challenges posed?

PRELIMINARY RESULTS ON THE ESTIMATION OF MINIMUM INFECTIVE DOSE OF VNN VIRUS IN EUROPEAN SEABASS AND ITS STABILITY IN SEAWATER

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Abstract

Viral nervous necrosis (VNN), belongs to the family *Betanodaviridae* and is one of the most harmful diseases for marine aquaculture in the Mediterranean basin, European sea bass (*Dicentrarchus labrax*) being the most susceptible farmed species. Hydrographic models can be useful in developing pathogen dispersal models and assessing transmission risks. However, robust pathogens may spread further. This study was carried out to understand the transmission of the disease by investigating the minimum infectious dose and stability of the pathogen in seawater.

On the day of the challenge, groups of 30 fish (in duplicate) were immersed in different concentrations of VNNv, $2*10^{3}$, $2*10^{1}$, $2*10^{-1}$ and $2*10^{-3}$ $TCID_{50}$ mL^{-1}, for 6 hours. An additional group was mock infected by immersion (in duplicate) with PBS. To evaluate the shedding rate of VNN from infected fish a group of 30 fish (in duplicate) was injected intramuscularly (IM), whilst the stability of the virus was tested *in vitro* in sterile seawater at 27ºC.

Mortalities started on the 4th day post-challenge and continued until day 13. The IM group showed a 45% average accumulated mortality, whilst dilutions $2*10^{3}$ $TCID_{50}$ mL^{-1} and $2*10^{1}$ $TCID_{50}$ mL^{-1} had a 10 and 13% average accumulated mortality respectively (no mortalities occurred in the other groups). Samples of central nervious system and eyes of every single mortality that occurred during the trial were analysed by RT-PCR and all found to be positive for VNNv; the results of these analysis and the presence of VNN clinical signs confirmed the cause of death as the experimentally induced VNN infection. In addition, 40% of all survivors were similarly sampled at the end of the experiment with RT-PCR showing that 100% of the IM and $2*10^{3}$ $TCID_{50}$ mL^{-1} immersion groups were positive for VNN, whilst 57% (12/21) of the fish from the $2*10^{1}$ $TCID_{50}$ mL^{-1} group were positive. Fish from other treatments were negative for VNNv.

The virus could be isolated even 3 months after the challenge was performed showing high stability in seawater; the time needed for a 99% inactivation of VNNv is still underway. The shedding rate of the virus could not be determined as water samples of the IM tanks were negative for VNNv.

From the information obtained in this study, the minimum dose of VNNv required to reliably induce infection in European sea bass juveniles by bath immersion is approximately $2*10^{1}$ $TCID_{50}$ mL^{-1}. The study also showed that it is a highly stable virus. The low dose of virus required to induce infection in combination with the high resistance of the pathogen in the environment are key factors that facilitate the onset of disease outbreaks.

This study was carried out within the framework of the EnviGuard project, which has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement 614057.

EXPERIMENTAL INVESTIGATIONS INTO RANAVIRUS (*IRIDOVIRIDAE*) INFECTIONS IN LUMPFISH (*CYCLOPTERUS LUMPUS*)

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A ranavirus (*Iridoviridae*), closely related to the notifiable epizootic haematopoietic necrosis virus (EHNV), has been repeatedly isolated from lumpfish (*Cyclopterus lumpus*). Isolates from Scotland, Iceland and the Faroe Islands were not associated with clinical disease. In Ireland the virus was isolated from lumpfish fry experiencing high mortality, but to date the virus has not been proven to be the aetiological agent of the disease. However, histopathology was indicative of viral aetiology and no other pathogens were identified using histology, bacteriology or parasitology. Several ranavirus species can cause severe systemic disease in fish and show a low host specificity, raising concerns about potential biosecurity risks posed to cohabited Atlantic salmon (*Salmo salar*). Challenge trials were conducted to evaluate the virulence of the virus to lumpfish and Atlantic salmon. Initially, sea transfer size lumpfish and lumpfish fry were challenged by immersion, Atlantic salmon smolts were challenged by immersion and intra peritoneal (IP) injection with the Irish isolate. Infection was demonstrated in fry but results were considered inconclusive and a second trial was set up using a cohabitation model. In this model, lumpfish fry were injected with Irish, Icelandic and Faroese strains of the virus and cohabited with naïve lumpfish. Atlantic salmon juveniles were IP injected with the Irish isolate without cohabitation of naïve fish. This challenge model demonstrated replication of the virus in the lumpfish, horizontal transmission of the virus and reduced survival in lumpfish injected with the Icelandic and the Faroese strain. A ranavirus qPCR assay was used to monitor the viral load in shedders and cohabitants at set time points and in mortalities. Results will be presented.

This research is funded by the Irish Research Council (Employment Based Postgraduate Program), FishVet Group Ireland, in part by Bord Iascaigh Mhara and through Aquaexcel 2020.

PRACTICAL USE OF SYNDROMIC SURVEILLANCE ON DETECTING CARDIOMYOPATHY SYNDROME IN SALMON FARMING

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Abstract

Aquaculture is an important industry in Scotland, helping to sustain economic growth within rural and remote communities. Scotland is the largest producer of farmed Atlantic salmon (*Salmo salar* L.) in the EU and the third largest producer in the world.

As with any other large-scale intensive aquatic farming, diseases in salmon farming are a great constraint to the development of the industry and account for a substantial portion of the losses borne by the industry. Therefore, the control of disease is vital for the future development and profitability of the sector. In an attempt to identify health problems in aquaculture quickly and efficiently, Marine Scotland Science (MSS) has developed an algorithm to generate alerts based on the diagnostic data generated by MSS. This data includes pathological codes that have been created over 30 years based on histopathological examination of tissues sampled from farm sites by the Scottish government's Fish Health Inspectorate. This algorithm has been used as a tool to generate alerts by the MSS Fish Health Inspectorate for the surveillance of diseases since 2017.

The aim of this work is to describe a practical use of the algorithm as an alert-generator to identify increases of occurrences of health issues. We chose cardiomyopathy syndrome (CMS) as a case study due to the economic impact of CMS on salmon production in the last few years. Cardiomyopathy syndrome is responsible for considerable economic losses in large salmon and to a reduction of animal welfare, making the management practices a challenge when associated with other stressing factors or health conditions.

CYPRINID VIRAL DISEASES IN IRELAND

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Abstract

Koi herpesvirus disease (KHVD) is a significant disease of both koi and common carp and is listed under EU Directive 2006/88/EC. To date, Ireland is declared free from KHVD and the Directive allows the implementation of official controls to prevent the introduction of the disease into the country. Despite this, there have been occasional reports of KHVD in ornamental koi carp kept in garden ponds. Carp edema virus disease (CEVD) is another significant viral disease of cyprinids, which has emerged over the last decade. Unlike KHVD, CEVD is not a listed disease and therefore there are no mandatory requirements for screening for this disease which has contributed to the rapid spread seen in Europe. KHVD was first detected in Ireland in 2005 in ornamental koi and again in 2016. Both instances were isolated cases in private garden ponds and all fish were culled. The first outbreaks of CEVD occurred in two carp fisheries in 2018 with significant mortalities observed in both lakes. This presentation will describe the clinical signs of both diseases observed in Ireland and the results from a phylogenetic study of CEV detections will also be discussed.

RNA-SEQ ANALYSIS OF RAINBOW TROUT TRANSCRIPTOME REVEALS SUPPRESSION OF THE UNFOLDED PROTEIN RESPONSE BY PATHOGENIC VHSV

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Abstract

Introduction: VHSV causes a lethal disease affecting freshwater and marine fish species. Different genogroups of VHSV result in differing virulence to hosts, but little is known about the mechanisms behind the varying virulence observed[1]. In the present study, temporal changes in gene expression of rainbow trout cells inoculated with pathogenic and non-pathogenic viral isolates were analysed to investigate VHSV pathogenicity.

Material and Methods: Two trout pathogenic VHSV isolates from genogroup Ia and Ic and two non-pathogenic marine isolates from the genogroups Ib and II were used to compare the transcriptomic responses of RTG-2 cells to the viral infection. Cells were inoculated at 0.01 MOI and harvested at 0, 3, 6, as 12 h post-inoculation (hpi) for RNA extraction and subsequent sequencing on an Illumina HiSeq2500. Changes in host transcriptome responses were analysed using Trinity[2] and edgeR[3]. Differentially-expressed genes (adjusted $P<0.001$) were clustered according to their patterns of expression across the samples and partitioned into gene clusters. Enrichment of Gene Ontologies in each of these gene clusters was determined using Blast2GO PRO[4] and FatiGO[5].

Results: The number of differentially-regulated genes in inoculated cells by non-pathogenic isolates was 47x, 26x and 4x higher at 3, 6 and 12 hpi, respectively, than the number of genes affected by pathogenic isolates. Up-regulation of transcripts representing the interferon pathway, unfolded protein response (UPR) and transcription factors were observed in cells inoculated with non-pathogenic isolates, whereas a strong suppression of the UPR and alterations in the Wnt signalling, nuclear factors and lipid metabolism pathways were observed in cells inoculated with pathogenic virus. The gene expression patterns of key genes were corroborated by Taqman qPCR.

Conclusions: Transcriptome analysis in rainbow trout cells inoculated with pathogenic or non-pathogenic VHSV isolates revealed key differences in host cell responses, including host cell shut off and inhibition of UPR in cells inoculated with non-pathogenic virus. Differentially-regulated proteins and/or pathways will be further investigated for their use as potential therapeutic targets.

Funding from the Department for Environment, Food and Rural Affairs (Defra) contract C7277B is gratefully acknowledged.

References: [1]Cano et al., 2016, PLoS One 11(7):e0158151; [2]Haas et al., 2013, Nature Protocols 8(8):1494–1512; [3]Robinson et al., 2010, Bioinformatics 26(1):139–140; [4]Conesa et al., 2005, Bioinformatics 21(18):3674–3676; [5]Al-Shahrour et al., 2004, Bioinformatics 20(4):578–580.

WIDER POTENTIAL ECOLOGICAL IMPACTS OF DISEASES IN BIVALVES

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Abstract

In shellfish diseases much of the focus remains on understanding host:parasite interactions and associated external drivers of infection and disease. The host:parasite:environment nexus can identify the drivers of infection and disease and some of the factors that can be used to minimize the impact of disease in the intertidal area. In many shellfish culture sites, aquaculture and the species cultured, share the habitat with a range of other organisms. The potential impact of aquaculture diseases on these other organisms is poorly understood. In herpes virus infections and specifically OSHV1-µVar, infections have been known to re-occur over extended periods of time, in areas where the virus has been detected and where mortalities have been recorded. Understanding how the virus is maintained in an area and how infections can reoccur, requires an understanding of the whole ecosystem and the potential role of other organisms in maintaining the virus. In this study the role of other organisms in the intertidal area as potential carriers, reservoirs or alternate hosts for OSHV1-µvar was investigated. The results indicate that other species can act as carriers or reservoirs and can transmit the virus back to oysters – indicating that control of the virus may be much more problematic due to its wider ecosystem spread.

INFECTIOUS DISEASES OF CLEANER FISH IN NORWAY

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Abstract

The use of cleaner fish has expanded dramatically in Norwegian salmon farming in recent years due to increasing chemoterapeutant resistance in salmon lice. While wild-caught wrasse species dominated initially, cleaner fish used in Norway today predominantly consist of farmed lumpsucker, which tolerate lower water temperatures. In 2017, just over 50 million cleaner fish were used (official statistics) in Norway, and estimates for 2018 range around 60 million. These high numbers reflect the short life expectancy of cleaner fish after stocking in salmon farms.

A large proportion of cleaner fish losses are related to infectious diseases. While bacterial diseases are known to play a leading role, the significance of viral infections (such as Lumpfish Flavivirus) and the various parasites infecting these fish species remains to be established. Bacterial pathogens of cleaner fish regularly detected in Norway include 'atypical' *Aeromonas salmonicida*, *Pasteurella* sp., *Pseudomonas anguilliseptica*, *Moritella viscosa*, *Tenacibaculum* spp. and *Vibrio anguillarum*/*ordalii*, in addition to a range of other *Vibrio* species. *A. salmonicida* subsp. *salmonicida* and *Paramoeba perurans*, both serious pathogens of Atlantic salmon, have also been sporadically detected in cleaner fish. While vaccination of farmed Norwegian cleaner fish against some bacterial pathogens may have contributed towards some mitigation of losses, much optimisation work remains in terms of cleaner fish vaccinology.

The high and relatively rapid mortalities experienced amongst cleaner fish today undoubtedly represent a major animal welfare concern, raising a legitimate question mark over the ethicality of current cleaner fish practices. Current and historic trends from cleaner fish diagnostics and research performed at the Norwegian Veterinary Institute will be presented, with a focus on relevant bacterial pathogens.

GLOBAL POPULATION STRUCTURE, ENDEMISM AND ANTHROPOGENIC SPREAD OF *YERSINIA RUCKERI* AS REVEALED BY WHOLE GENOME SEQUENCING

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Abstract

Yersinia ruckeri is the etiologic agent of enteric red mouth (ERM) disease, or yersiniosis, in farmed salmonids world-wide. Transmission occurs mostly in freshwater, and historically the disease has been most commonly associated with rainbow trout. However in recent years the salmon industries of Scotland, Chile and Norway have also been impacted. Population genetic studies using a variety of methods (eg MLST, MLVA and WGS) have revealed that isolates recovered from cases of infection in farms fall in to a limited number of evolutionary lineages. These clusters tend to show significant phylogeographic clustering and are also strongly associated with host species. In particular, the majority of infection in rainbow trout Is caused by a single lineage designated ST1 by MLST, and CC2 by MLVA. This lineage is thought to have emerged in North America and spread to the UK and continental Europe. This contrasts with the picture for infection in Atlantic salmon where different lineages, possibly each endemic to the local environment, cause the majority of disease burden. Here we explore global population structure by analysing 170 novel *Y. ruckeri* genome sequences from global sources, supplemented with 85 previously published genomes. Our analysis confirms the overall clonal structure of this population, but sheds additional light on the emergence of the major lineages with respect to transmission patterns and host adaptation. We also consider the distribution of major plasmids within the context of the phylogenetic tree.

SCREENING OF GENES FOR ENHANCING GROWTH OF *AEROMONAS SALMONICIDA* IN FISH MUCUS

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Abstract

Aeromonas salmonicida causes furunculosis in farmed fish and it can be transmitted to susceptible hosts through the water or by fish-to-fish contact. Outbreaks compromise fish welfare and reduce farm productivity, and vaccination is used effectively and widely to control this disease. However, vaccines are not available for all species and do not protect at early life stages where the adaptive immune system is insufficiently developed. Hence, deciphering the molecular mechanisms underlying bacterial pathogenicity and virulence may lead to improved disease prevention and treatment solutions. To initiate an infection *A. salmonicida* must survive and grow in the mucus that covers the skin, gills and gastrointestinal tract, and this is the first line of the host immune defences. Therefore, the objective of this present study was to identify genes facilitating survival and growth of *A. salmonicida* in *S. salar* skin mucus. A *mariner*-based transposon mutant library was generated for the pathogenic *A. salmonicida* Hooke isolate. This library contained mutants where the transposon had inserted at random at a single site in the bacterial genome to knock out the function of just a single gene. Each transposon mutant was screened for growth in the presence of mucus that had been collected from the surface of *S. salar*. As the *A. salmonicida* Hooke parent isolate has enhanced growth in this mucus, transposon mutants lacking this phenotype would be expected to contain a transposon insertion in a gene with a role in growth enhancement in this milieu. Transposon mutants were screened for a lack of enhanced growth in mucus and the transposon insertion site (and thus the gene disrupted) determined by arbitrary polymerase chain reaction (PCR), sequencing of the PCR products and mapping back to the reference genome. In one mutant, the transposon had inserted into the gene encoding the ABC-type transporter protein abcA, which is involved in regulating the A-layer protein virulence factor. This present study furthers our understanding of *A. salmonicida* virulence and may assist with the development of new approaches to reduce the burden of furunculosis outbreaks in aquaculture.

GENOMIC EPIDEMIOLOGY OF THE COMMERCIALLY IMPORTANT PATHOGEN *RENIBACTERIUM SALMONINARUM* WITHIN THE CHILEAN SALMON INDUSTRY

Sion C. Bayliss[1]*, David W Verner-Jeffreys[2], David Ryder[2], Rudy Suarez[3,4], Roxana Ramirez[3], Jaime Romero[5], Ben Pascoe[1], Sam K. Sheppard[1], Marcos Godoy[3,4,6,7§], Edward J. Feil[1§]

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Abstract

Renibacterium salmoninarum is the causative agent of Bacterial Kidney Disease (BKD), which is a commercially important disease of salmonids. Typing by conventional methods provides little information on the evolution and spread of this pathogen as *R. salmoninarum* has a low mutation rate and there is limited standing variation within the population. Here we apply whole genome sequencing to 42 *R. salmoninarum* isolates from Chile, primarily from salmon farms, in order to understand the epidemiology of BKD in this country. The patterns of genomic variation are consistent with multiple introductions to Chile, followed by widespread geographical spread of the disease over a 30 year period. The estimated dates of introduction broadly coincide with major events in the development of the Chilean aquaculture industry. We find evidence for a Coho salmon associated lineage, indicating that host switching may not be as frequent as previously thought or that other significant barriers to transmission exist in the Chilean salmon production chain. Understanding of the genomic epidemiology of BKD can inform disease intervention and improve sustainability of the commercially important aquaculture industry.

ASSESSMENT OF AN ATYPICAL *AEROMONAS SALMONICIDA* BATH CHALLENGE MODEL IN FARMED BALLAN WRASSE (*LABRUS BERGYLTA*) USING MOLECULAR METHODS AND HISTOPATHOLOGY

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Abstract

Ballan wrasse (*Labrus bergylta*) is commercially farmed and deployed as cleaner fish in Atlantic salmon cages as an environmentally friendly approach to delousing. Atypical *Aeromonas salmonicida;* a*As*, representing an important bacterial pathogen of *L. bergylta*, was isolated during outbreaks in Scotland between 2016 and 2017. The pathogenicity and virulence of these isolates were assessed in juvenile (approx. 2 g) farmed Ballan wrasse by bath exposure, which enabled the development of a bath challenge model against a*As*. Duplicate tanks of juvenile Ballan wrasse (n= 50) were exposed to two strains of subtype V a*As* including a challenge dose of 2.46 x 10^7 and 3.22 x 10^7 for sub pulsotype B2, and 1.93 x 10^7 and 2.04 x 10^8 for sub pulsotypes B4 for up to 22 days at 15ºC in 5 l sterile sea water (33 ppt). Fish were sampled for bacteriological, molecular and histological assessment to determine bacterial loads and the associated pathology.

Differential virulence was observed with the a*As* subtype V - sub pulsotype challenges resulting in cumulative mortalities of 52 and 60 % with a*As* sub pulsotypes B2 in contrast to 62% and 20% for B4. VapA qPCR, specific for *A. salmonicida*, was more sensitive than conventional VapA PCR, whereby higher bacterial loads in the liver, kidney and spleen were observed in moribund fish compared to sub clinically infected fish. The a*As*-specific Type V-specific PCR was also more sensitive than VapA PCR and confirmed the presence of the atypical strain in these fish tissues. Both moribund fish and subclinical fish exhibited extensive pathology in the liver and pancreas including granuloma formation and peritonitis, respectively, characterised by infiltrating macrophages and eosinophilic granulocytes. Gram and Gram-Twort staining confirmed the presence of Gram negative intralesional bacteria within granulomas. The spleen of infected fish was congested with increased macrophages replacing normal splenic tissue and the cardiac endothelium was moderately active. The a*As* bath challenge model was therefore successful at inducing a systemic disease and will be essential for future vaccine efficacy testing.

THE CEFAS AQUATIC AMR CENTRE OF EXCELLENCE

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Abstract

Antimicrobial resistance, AMR, is one of the biggest threats to our way of life. In 2014 Lord Jim O'Neil was commissioned by the UK government to conduct a comprehensive review into the global risk posed by AMR. AMR threatens most of the important medical advances made in recent years and, if left unchecked, a conservative estimate of the annual increase in death rate due to AMR is 10 million people, with a reduction in global GDP of between 2.0% to 3.5% costing the world up to 100 trillion USD[1].

The aquatic environment, both freshwater and marine, acts as a sink into which antimicrobials and microorganisms come together from industry, health care facilities, agriculture, aquaculture, and human activities. There is opportunity within this environment for resistance to both emerge and transfer between microorganisms. These resistant microorganisms can spread back into the human population via; food, potable water, fish keeping, bathing, leisure and shellfish[2].

Cefas, with funding from UK government, has set up an Aquatic AMR Centre of Excellence, in collaboration with our colleagues from the UK's APHA (Animal and Plant Health Agency) and VMD (Veterinary Medicines Directorate).

This presentation will outline some of the initial studies underway within the Centre which include activities such as; surveillance of environmental *E. coli* samples for AMR within several of the Gulf States, contribution of information towards the establishment of standard methods and interpretative criteria leading to the establishment of epidemiological cutoff points for the human and aquaculture pathogens, *Vibrio parahaemolyticus* and *Vibrio vulnificus*.

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CRAYFISH PLAGUE IN IRELAND

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Abstract

Crayfish plague is a highly infectious lethal disease caused by spores of *Aphanomyces astaci,* a member of the Oomycetes, with five genotype groups of *Aphanomyces astaci* currently having been described.

This OIE listed disease affects the white-clawed crayfish *Austropotamobius pallipes*, which is the only crayfish species native to Ireland. Ecologically, the white-clawed crayfish is an important keystone species which is protected under the EU Habitats Directive and is on the IUCN Red List of Endangered species. Ireland has been regarded as a stronghold for the white-clawed crayfish in Europe, following its widespread decline due in large part to crayfish plague, but existing Irish populations could potentially be eliminated if crayfish plague becomes established nationally. The spores of *A. astaci* can survive for several weeks and can be transported on damp equipment or boats. Also, any introduction, either accidental or intentional, of non-native North American crayfish species poses a high risk of importing the disease. Known carrier species of the plague are established in the wild across Europe (e.g. signal crayfish *Pacifastacus leniusculus*) and several species are thought to be present in Ireland in private ownership as pets, including a species known to reproduce asexually. Established infections within a catchment are very difficult to control, so the focus must be on reducing spread between catchments.

The first outbreak of crayfish plague in Ireland confirmed by the Marine Institute, and reported to the OIE, occurred in 2015 in the Bruskey River, a tributary of the Erne. In 2017, multiple outbreaks were confirmed, initially in the River Suir, then the River Deel, the Lorrha River in Co. Tipperary, which forms part of the Shannon catchment and finally the River Barrow. Genotyping analysis by OIE reference laboratories in the UK and in Finland have shown there are at least three different genotypes of *Aphanomyces astaci* present in Ireland.

This genetic diversity of *A. astaci* suggests there have been at least three separate introductions of the disease into the country. There is now an urgent need in Ireland for a better understanding of the possible sources of the disease, its routes of transmission, and its current spread.

AMOEBIC GILL DISEASE: ACUTE IMMUNE RESPONSE OF SALMON AFFECTED BY *NEOPARAMOEBA PERURANS*

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Abstract

Amoebic gill disease (AGD) caused by *N. Perurans* is one of the most significant diseases for Atlantic salmon (*Salmo salar*) aquaculture in terms of mortalities and subsequent economical losses. Previous studies on host response in salmon affected by the AGD have focused on the expression of genes involved in general pathway or common immune response (Morrison 2006, 2007, 2012), however, post-transcriptional changes need to be considered for biological significance. The first proteomic study on salmon affected by AGD was performed at a late stage of the disease and showed differentially regulated proteins involved in cell signalling and inflammation pathway (Valdenegro-Vega et al. 2014). More recently, a study performed on gill tissue evidenced changes of proteins involved in cell cycle and cytoskeletal regulation, oxidative metabolism and immunity (Marcos-Lopez et al 2017). Most of the significant differences occurred at the clinical stage of the disease at 14 and 21 days post-infection (p.i.) and only subtle changes were noticed at 2 and 7 days p.i. Although these studies are informative there is still a knowledge gap on the molecular events taking place during the pre-clinical stage of the disease.

The proposed research focuses on the acute local and systemic (humoral) response of salmon exposed to an initial amoeba infection.

Gill tissue and serum from control and exposed fish were collected after 1, 2, 3 and 7 days p.i. before the appearance of the clinical stage of the disease and processed for two dimensional electrophoresis (2DE). Several optimisations steps were performed to enhance the sensitivity of the technique. The analysis of the differentially expressed proteins is in progress.

The results will allow a better understanding of the acute immune response of salmon at the earliest stage of the disease and will contribute to the development of biomonitoring and preventive measures to improve fish welfare.

AN IMPROVED METHOD FOR THE VISUALIZATION OF SEA LOUSE COPEPODIDS (CALIGIDAE) FROM PLANKTON SAMPLES BY FLUORESCENCE STEREOMICROSCOPY

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Abstract

The sea louse (*Lepeophtheirus* and *Caligus* spp.) is an important ectoparasite of wild and farmed marine salmonid fishes, resulting in damage to tissues, mortality and substantial industry losses globally. Monitoring of larval sea lice distribution and abundance is routinely performed by the examination of material from plankton nets with reflected visible light under a stereomicroscope. Caligid copepodids are morphologically discriminated by eye from other abundant, similar sized and coloured copepods and planktonic material which is very time consuming and requires a high level of expertise. In this study, *L. salmonis* and *Caligus elongatus* copepodids and zooplankton from other copepod orders were investigated using fluorescence microscopy. Autofluorescence in blue, green and red channels was imaged and quantified. Fluorescence was found to improve visualization of surface details when compared to visible light. Caligid copepodids were found to have a resultant autofluorescence significantly different from other copepod orders. Lower fluorescence in blue wavelengths and higher fluorescence in green/ orange wavelengths allowed easier detection of caligid copepodids by colour. An optimal excitation/ emission wavelength combination was added to a routine stereomicroscope setup and found to reduce screening time. Fluorescence stereomicroscopy presents an improved method to allow faster identification and enumeration of sea louse larvae from plankton samples and potential for development of *in situ* automated lice detection.

EMERGING HAPLOSPORIDIA IN BIVALVES AROUND THE IRISH COAST

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Abstract

The frequency of emerging infectious diseases in marine ecosystems is increasing due to increasing rates of environmental change, caused by changing climates and anthropogenic activities. Such phenomena may provoke previously non-problematic marine parasites to emerge, which may appear for the first time or existed previously but have extended their geographical and host range. Haplosporidia single cell parasites are a major concern for aquatic animal health, in particular in bivalve molluscs, as they have been responsible for some of the most significant marine epizootics on record. In this study, the common cockle *Cerastoderma edule,* mussels *Mytilus* spp. and the Pacific oyster *Crassostrea gigas* from three Irish locations (*C. gigas* culture and nonculture sites) were screened for Haplosporidia spp. by polymerase chain reaction (PCR) using multiple molecular markers. The DNA of two emerging Haplosporidia spp. was detected, isolated and confirmed by Direct Sanger sequencing in surfaced *C. edule* while a single species was detected in the blue mussel *Mytilus edulis*. Of interest, the Haplosporidia spp. were not detected in the cohabiting *C. gigas* on oyster trestles. This finding may indicate that the Haplosporidia spp. are host specific and 'host partitioning' was occurring. The effects of these Haplosporidia spp. on the *C. edule* and *Mytilus* spp. populations is currently unknown. The findings of this study highlight the diversity of emerging Haplosporidia spp. that may be present in coastal marine environments but remain undetected, even in well-studied commercial shellfish species such as cockles and mussels.

CONNECTING INDUSTRY, SCIENCE AND GOVERNMENT FOR THE EFFECTIVE MANAGEMENT OF SERIOUS DISEASE

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Abstract

"Serious diseases pose a threat to aquatic animal health both in aquaculture and in the wild; many have no effective treatment, the potential to cause high mortalities in aquatic animals, cause high economic loss and threaten biodiversity. Official control, through policy and legislation is necessary to ensure a sustainable aquaculture industry, to accord with international standards and to facilitate trade. To be effective, disease controls must use applied science and research to inform Government policy and direct appropriate legislation, requiring strong links between industry, science and government. The Fish Health Inspectorate (FHI) at Cefas provide these links. The resulting specialist relationship that exists between the FHI, the diagnosticians and research scientists at Cefas, contributes to Cefas peer-reviewed science outputs and aquatic animal health controls for diseases of national significance."

‘MIND THE GAP’: BRIDGING ACADEMIA AND STAKEHOLDERS TO IMPROVE DISEASE MANAGEMENT IN FRESHWATER FISHERIES

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Multiple factors including climate change, the emergence of new disease conditions and greater scrutiny on susceptible and threatened fish species have placed growing pressure on the management of freshwater fisheries, and has emphasised the importance of collaborative work to improve outcomes for aquatic animal health. The importance of academic partnerships to support industry outcomes is demonstrated here using four diseases of freshwater fish. Firstly, the development of an early case description following detection of Puffy Skin Disease (PSD) in rainbow trout. Secondly, the application of molecular methods to identify the diversity of *Saprolegnia* infections in wild salmonid populations. Thirdly, standardisation of approaches for assessing the health of migrating silver eels entrained in pumping stations. Fourthly, evaluating the use of artificial egg-laying substrates for the management of *Argulus* infections in still-waters. In each case, collaborations between industry and academia have been key to improving our understanding of disease impacts, informing new ways of working and translating scientific advances into better management outcomes for freshwater fisheries.

BLUE EDUCATION FOR SUSTAINABLE MANAGEMENT OF AQUATIC RESOURCES – PRESENTATION OF THE PROJECT BLUE SMART

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Abstract

The development of aquaculture is one of the strategic guidelines of the development of fisheries in the Republic of Croatia. Being a strategic sector of the County's development, aquaculture is very often marked in the relevant local and national strategies as an emerging sector with a very high potential for growth and jobs. However, all the data show that there is a big shortage of a skilled workforce which will increase in the coming years, as the sector will grow.

Therefore, the University of Zadar decided to build a strong local consortium composed of the main actors in educational, public, promotional and fishery sector in order to promote and support the development of career opportunities in the blue economy. Hence, the project "Blue Education for Sustainable Management of Aquatic Resources – BLUE SMART" was applied and positively evaluated trough the call: Blue Careers in Europe, within European Maritime and Fisheries Fund-EMFF (Executive Agency for Small and Medium-sized Enterprises- EASME). BLUE SMART project started on 1st January 2017 and will finish on 31st of December 2018.

The general objective of the BLUE SMART project was to create new skills and competences in blue economy sector and increase the employability of current and future sectors' workers in the County of Zadar. First specific objective was to create conditions for training of a new generation of students and professionals equipped with the appropriate skills to match the needs of the industry. This specific objective has been achieved by creating conditions for the establishment of a new graduate (Master) study "Sustainable Management of Water Ecosystems". Second specific objective was to provide people already working in the field with the new skills required, which will be achieved by design of a Training Course for the Vocational Education and Training in "Introduction to sustainable fisheries practice".

Biography

Tomislav Šarić works at the University of Zadar as a Assistant Professor. He holds lectures from the subject "Anatomy and physiology of domestic animals", "Introduction to scientific work and biometrics" and "Aquaculture". His scientific interest are aquaculture and protection of fish and shellfish health. Currently, he is manager at the Blue education for sustainable management of aquatic resources - BLUE SMART project.

EARLY STAGE RESEARCHERS

A RAPID TEST FOR DETECTING THE INFECTION LOAD OF THE PARASITE, *ANGUILLICOLA CRASSUS*, IN THE EUROPEAN EEL, *ANGUILLA ANGUILLA*

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Abstract

Anguillicola crassus is a nematode parasite of the swim bladder originally endemic to Japanese eels, *Anguilla japonica*. *A. crassus* was introduced into Europe in the 80's since when it has spread widely and is thought to contribute to the rapid decline in the European eel. Currently, the only way to detect the parasite is to dissect the eel. We are developing a non-lethal rapid test based on presence/absence of eggs and L2 larvae in the faecal material. A faecal wash had been performed on c.60 European eels in the Burrishoole catchment in Ireland. qPCR primers were designed based on CO1, 18s specific genes and transcriptome available in the literature. Primers were tested using pure *A. crassus* DNA and related nematodes to establish the specificity. To validate the test 24 eels were faecal sampled, euthanized, dissected, and the number of worms counted in the swim bladder. The rapid test is in an optimisation phase and we hope will be a valuable tool for fisheries managers hoping to interrupt transmission of the parasite.

DO CLEANER FISH POSE A RISK OF PATHOGEN TRANSFER? A HEALTH ASSESSMENT OF BALLAN WRASSE (*LABRUS BERGYLTA*) FROM THE SOUTH WEST OF ENGLAND

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Abstract

Hundreds of thousands of wild caught ballan wrasse (*Labrus bergylta*) are translocated from the South West of England to Scottish Atlantic salmon (*Salmo salar*) farms every year[1]. They are deployed as cleaner fish to control sea lice infestations, which are estimated to cost the global salmon farming industry €300 million annually[2]. Very little is known of the prokaryote and parasite fauna of these wild wrasse populations, and subsequently of the risk they may pose to salmon and local wild fish upon translocation. This is the first health assessment of ballan wrasse from the south west, with the main aim to contribute knowledge on the diversity of their pathobiome.

79 wild caught ballan wrasse from Dorset and Cornwall were subjected to a full microbiological and parasite health screening. No viruses were isolated or amplified by PCR. *Neoparamoeba perurans* and *Aeromonas salmonicida* were not detected by culture or Taqman qPCR. A range of metazoan and protozoan parasitic fauna were identified by histopathology, including copepods, nematodes, cestodes, digenean metacercariae, *Cryptocaryon*-like ciliates, sea lice *Caligus centrodonti* and a possible new species of *Eimeria* (apicomplexan) was observed in 20 fish. The endosymbiont ciliate *Pseudocohnilembus persalinus* was cultured from gill tissue, and the mycoplasma *Acholeplasma laidlawii* was identified, associated with cytopathic like effect in viral cell culture, but was not visualised histologically.

The emerging opportunistic pathogen *Photobacterium damselae* subsp. *damselae* was isolated from a single fish, associated with a cryptic systemic bacterial infection. Further work investigating the virulence of this strain is planned.

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COMPARATIVE PROTEOMIC PROFILING OF PATHOGENIC AND NON-PATHOGENIC *NEOPARAMOEBA PERURANS* PROTEINS ASSOCIATED WITH AGD

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Neoparamoeba perurans is the ectoparasite and the causative agent of Amoebic Gill Disease characterised by hyperplasia on the secondary gill lamellae of infected hosts, leading to serious economic loss for the mariculture industry. Despite many years of study, virulence factors and the mechanisms of the infection process remain unknown. In this study a comparative proteomic approach through two-dimensional gel electrophoresis and gel free analysis (LC MS/MS) is used to analyse differential protein expression between two *N. perurans* isolates (putatively avirulent and virulent strain) with the goal of identifying proteins associated with virulence. This study is believed to be the first detailed proteomic analysis of *N. perurans.*

Using detergent phase partitioning, putative avirulent and virulent strains of *N. perurans* are enriched into hydrophilic and hydrophobic fractions. These soluble and insoluble fractions are then subjected to two-dimensional electrophoresis separation following protein identification via LC MS/MS. A gel free approach was also undertaken to compare the efficiency of the 2D technique. Samples obtained from 2D gels and samples prepared for gel free analysis were subjected to reverse phase liquid chromatography coupled with tandem mass spectrometry.

Initial results derived by the 2D gels and LC MS/MS suggest that there are significant differences in hydrophillic proteomic profile of the putatively virulent versus avirulent strains of the amoeba. The secretome of *N. perurans* and the membrane proteome will also be targeted in future experiments to fully identify the virulent proteins of the ectoparasite.

MISIDENTIFICATION OF BACTERIA WITHOUT THE USE OF MOLECULAR CONFIRMATION

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Abstract

The development of more sophisticated molecular techniques has highlighted a problem of misidentification of bacteria when relying on phenotypic observations and biochemical testing without the use of molecular techniques as confirmation. This can lead to known fish pathogens being missed during diagnostic investigations.

Recent examples where misidentification of bacterial species would have occurred without the use of molecular methods during Marine Scotland Science (MSS) diagnostic case studies include:- A colony which could not be identified using phenotypic techniques was sequenced and found to be *Vibrio anguillarum*. This isolate displayed non-typical colony characteristics and without the use of molecular techniques would have been misidentified in the past as a non-fish pathogen. In addition, isolates have also been originally identified as more serious fish pathogens and have later been sequenced and confirmed as a different species.

During the development of new qPCR assays to identify known fish pathogens, isolates from the MSS culture collection have been sequenced to confirm their identification to facilitate the validation of the assay. For example, an isolate originally classified as *Tenacibaculum maritime*, was subsequently confirmed as a different *Tenacibaculum* species by 16S rRNA gene sequencing.

To prevent further misidentification of known fish pathogens in the future, molecular techniques can be utilised. The development of specific qPCR assays can be used to confirm the identity of bacterial isolates. A universal bacterial endogenous control has also been development at MSS allowing confidence in negative results. This has been combined with the use of the boil DNA extraction method to enable rapid and inexpensive identification of fish pathogens by molecular techniques.

THE POTENTIAL ROLE OF INVASIVE SPECIES IN THE TRANSMISSION OF PATHEGENS IN THE MARINE ENVIRONMENT

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Abstract

Many tunicate species display rapid territorial expansion when introduced beyond their native range, and as such are often considered successful invaders. To date research into the invasion pathways and impacts of tunicates is well documented, but less is known about their ability to act as carriers, reservoirs or vectors for parasites and pathogens. In recent years culture of oyster species has been impacted by a range of pathogens that have resulted in significant mortality events and losses for the sector – the European flat oyster *Ostrea edulis* has been impacted by the haplosporidian *Bonamia ostreae* and the Pacific oyster *Crassostrea gigas* by the herpes virus OSHV-1µVar and more recently several *Vibrio* species. This study is focusing on the potential impact of an invasive species on the maintenence of these pathogens and their ability to introduce new pathogens and parasites to culture sites.

In July 2018 samples of the invasive tunicates *Styela clava* and *Botrylloides violaceus* were collected from Cork Harbour, at an oyster farm and marina respectively. Samples of the European flat oyster (*Ostrea edulis*) and Pacific oyster *(Crassostrea gigas*) were also collected to assess current status of the oyster stocks. A further sample of the native tunicate *Ascidiella aspersa* was collected from Lough Hyne (Special Area of Conservation) to compare the parasite load of a native species, removed from aquaculture and human activity, with those of the invasive species.

The focus of the work will be on assessing the presence of any parasites in these tunicate species and to screen for oyster pathogens namely *Bonamia ostreae*, herpes virus or *Vibrio* species using Polymerase Chain Reaction (PCR), Quantitative PCR (qPCR) and histology. Laboratory transmission trials between invasive tunicates and commercially important shellfish species will be carried out to determine if transmission of pathogens can occur between these species.

GENETIC DIVERSITY OF PISCINE MYOCARDITIS VIRUS IN ATLANTIC SALMON IN IRELAND

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Abstract

Piscine myocarditits virus (PMCV) is a double stranded RNA virus which has been linked to cardiomyopathy syndrome (CMS) in Atlantic salmon (*Salmo salar* L.). The first recorded outbreak of CMS in Ireland occurred in 2012. Samples were collected in the current study from farmed Atlantic salmon from various sites in Ireland and the open reading frames (ORFs) 1 and 3 were amplified in order to examine the genetic diversity of PMCV in Irish samples. Results showed PMCV to be largely homogenous in Irish samples, showing little genetic diversity. However several amino acid positions of the ORF3 amino acid sequence showed uniquely Irish variations, with all Irish samples in this study containing a VQQ motif having a lysine at position 4, a threonine at position 222 and most had a leucine in place of a proline at position 241, and all those with an IKR motif showing a valine at position 77. The phylogeny generated in the present study showed all current PMCV sequences, from 2016 to 2018, are descended from two of the sequences first identified in 2012, which were both linked to CMS. In the current study however, over three quarters of the PMCV isolates which were sequenced came from healthy fish, suggesting that either PMCV is evolving to become less virulent in Ireland, or that Irish Atlantic salmon are developing immunity.

AQUACULTURE FROM THE INSIDE-OUT: CHARACTERISING THE GUT MICROBIOME IN THE CULTIVATION OF THE EUROPEAN LOBSTER (*HOMARUS GAMMARUS*)

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Abstract

The gut microbiome can markedly impact host fitness and survival. As such, environmental pressures on gut community composition may correlate with differential growth rates of the host and susceptibility to disease. Investigation into microbiome composition and the potential of its manipulation could, therefore, substantially benefit future aquaculture production.

Using a large-scale mariculture project, we investigated compositional differences in the gut microbiota of the European lobster (*Homarus gammarus*), comparing hatchery vs sea-based animals over a one year period. Analysing this community in relation to growth and individual health states, through means of a histology-lead disease assessment, we highlight the impact of environmental factors on bacterial taxa within the gut and discuss its subsequent impact on the host.

High-throughput amplicon sequencing demonstrates how bacterial assemblages are significantly different between hatchery and maricultured lobsters; with species richness and species diversity increasing in sea-based individuals. Bacterial communities also undergo substantial compositional shifts as the host ages. This is particularly true of hatchery-reared individuals, with a reduction in the dominance of *Vibrio* species in older animals. Furthermore, size variation in lobsters of the same age correlates to significant differences in gut diversity and the distribution of bacterial taxa.

Our data provides the first extensive characterisation of the gut microbiome in the European lobster and indicates how the better understanding of this complex community could be applied to aquaculture planning and management in order to maximise the cultivation of the host. Furthermore, this information could form the foundation of targeted probiotic development, further benefiting an ever-growing aquaculture sector.

EMERGENCE OF *YERSINIA RUCKERI* BIOTYPE 2 IN NORWAY

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Abstract

Yersinia ruckeri is the causative agent of enteric redmouth disease in salmonid fish and is found throughout the world in areas where salmonid fish are farmed. Two biotypes (BT) of *Y. ruckeri* are recognized; BT1 is motile with peritrichous flagella and secretes lipase, while BT2 is non-motile and lacks lipase secretion. Loss of motility in bacterial pathogens has been attributed to avoidance of the host immune system, where the flagellum becomes a liability during infection due to its antigenic properties. Vaccination may thus represent a selective force favouring evolution of pathogenic strains lacking, or with altered, flagella.

Five independent, and presumably vaccination-driven, emergences of *Y. ruckeri* BT2 have previously been described due to single mutation events in the otherwise conserved genes encoding the flagellar export apparatus. With the exception of a single report in 1985, BT2 has not been detected in Norwegian aquaculture. However, we have recently identified BT2 amongst clinical isolates from farmed Atlantic salmon in Norway in 2017, belonging to the clonal complex dominating the disease situation in Norwegian aquaculture. Whole genome sequencing revealed a novel mutation in the flagellar export apparatus that is the likely cause of the BT2 phenotype and which, together with MLVA genotyping data, points to yet another case of BT2 developing independently of previously known BT2 strains from the US, Australia, UK and mainland Europe.

METAGENOMIC CHARACTERISATION OF THE CLEANER FISH MICROBIOME

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Abstract

The global salmon farming industry has been blighted by sea lice infections, costing the industry €300 million a year. Cleaner fish, namely Ballan wrasse and lumpsuckers, are deployed to salmon farms to eat the lice off the salmon, this has proved an effective strategy for reducing the disease burden. The use of wild caught cleaner fish is unsustainable and there is the risk of spreading infection to salmon farms, hence significant investment is needed to develop farming practices for cleaner fish. Cleaner fish species are relatively new to aquaculture so research is critically needed to improve their health status, pre- and post-deployment to salmon farms.

The fish microbiome plays an important role in the protection against pathogens, the development of the immune response and the overall health of the animal. This project aims to characterise the composition and functionality of the cleaner fish microbiome. Wild Ballan wrasse were caught from the south-west coast of England, DNA was extracted from the gut wall and the gut contents, to characterise the adherent and non-adherent microbiome, respectively. The DNA was sequenced using shotgun metagenomics and the reads were taxonomically classified using a dedicated software package, *Centrifuge*. The gut microbiome is dominated by Proteobacteria, followed by Firmicutes and Bacteroidetes and the microbiome is well conserved between individual fish, down to the species level. This points to an important conserved functionality, and the specific functions of the wrasse microbiome will be elucidated in the next phase of work.

Future work will focus on characterising the microbiome in the early life stages of cleaner fish, including eggs, fry and juveniles. Understanding the microbiome will allow the development of dietary and probiotic interventions to improve the health status and effectiveness of farmed cleaner fish, helping to tackle sea lice infections in salmon farms.

CHARACTERISATION OF THE ATLANTIC SALMON MICROBIAL COMMUNITY ON GILLS DURING AN AMOEBIC GILL DISEASE OUTBREAK: FIRST STEP OF AN EARLY AGD DETECTION

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Abstract

Amoebic Gill Disease (AGD) is associated with marine farmed Atlantic salmon (*Salmo salar* L., 1758) and has been observed to reach over 50% in some stocks in Tasmania [1]. Its presence was first confirmed in Ireland in 1996 [2], becoming a primary salmonid health concern in Europe. At present, the only way to reduce AGD impact on fish stocks is to rigorously control *Neoparamoeba perurans* (AGD first aetiological agent) presence and development in an advanced stage, preventing early diagnosis. As described previously [3, 4], the microbial community present on a biological tissue plays an important role in the development many diseases in all species. Therefore, characterizing the microbial community of farmed Atlantic salmon gills throughout different AGD stages may allow a correlation to be observed between microbial community and early AGD symptoms. In the present research, a new method is under development to isolate the microbial community of salmon gills, and to extract total microbial DNA and RNA using AGD affected and non-AGD affected gill samples. This would allow us to characterize the abundance of the microbes present and to correlate them with different AGD stages during an 2017 outbreak. Preliminary results show a clear change in the microbial community on gills during the AGD outbreak. All microbiome development is currently under analyses to correlate it with the AGD severity. This new DNA and RNA extraction protocol can be used for various sample types to characterize superficial microbial communities.

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COCKLE HEALTH, DISEASE CONNECTIVITY AND TROPHIC INTERACTION DYNAMICS

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Abstract

Parasites represent a substantial portion of the species diversity and biomass in food webs and ecosystems, being able to directly or indirectly alter the structure of the community by impacting specific physiological host functions and host population dynamics that, in turn, interact with non-hosts populations. Consequently, their effects may also trigger parasite-mediated trophic cascades. In turn, the infectious disease dynamics is affected by the food web structure. On the other hand, the presence of hyperparasites acting as parasites of other parasites not only affects the parasite's consumption, but it can also induce trophic cascades, playing a significant role in conducting or suppressing diseases. Particularly, the presence of microsporidian pathogens as hyperparasites of fish and shellfish pathogens, such as digeneans, proposes a potential vector role for these hosts to higher invertebrates and vertebrates over evolutionary timescales.

The impact of major parasites and diseases in fisheries and aquaculture as part of the trophic network is not fully understood, but is fundamental to achieving ecologically sustainable management of marine resources. In this study the disease connectivity between environmental media, such an ecological and commercially important species as European cockle populations and their trophic interactions in the Irish Sea are being investigated. Our objective is to evaluate the trophic transmission of pathogens within the ecosystem to determine the abiotic and biotic drivers or inhibitors of infection under a climate change context and provide a better understanding of how the climatic change will affect cultured and non-cultured cockle population structure and their potential influence on the trophic web and ecosystem functioning. During summer 2018 cockles were evaluated for health status; and site influence on cockle health is being assessed. The cockles are being processed and analysed by molecular techniques (PCR and qPCR), haemolymph cell monolayers and lysozyme activity assays and histopathology for a complete health screening, focussing in the detection of our target pathogens: Trematode, Microsporidia, Haplosporidia and *Vibrio aestuarianus*, which are important pathogens affecting the cockle populations. Results of screening to date and pathogen diversity in the populations screened will be presented.

INVESTIGATING THE PREVALENCE AND TRANSMISSION OF A NOVEL *ENDOZOICOMONAS*-LIKE ORGANISM (ELO) INFECTING KING SCALLOP (*PECTEN MAXIMUS* L.) POPULATIONS IN A MARINE PROTECTED AREA

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Abstract

The king scallop (*Pecten maximus* L.) is the fastest growing fishery in the United Kingdom, with landings by UK vessels generating £58.3 million in 2014. The majority of landings are from wild stocks. Several marine protected areas (MPAs) have been established along the UK coastline, aiming to protect the marine ecology. In June 2013 and May 2014, two king scallop mass mortality events were recorded in Lyme Bay marine protected area (MPA), the largest area of protected water in the UK. Histopathological and molecular study identified an *Endozoicomonas*-like organism (ELO) infecting the gill epithelial tissue of all animals sampled[1]. In this study, prevalence and severity of the ELO infection were monitored, outside of mortality events, between August 2016 and June 2017. In parallel, survival of the ELO outside the host and horizontal transmission routes were investigated.

Over one year, four sample groups of ten adult king scallops were collected from West Bay (Lyme Bay MPA). Animals demonstrated histopathology consistent with intracellular microcolonies of bacteria (IMC) infections. ELO 16S rDNA-specific primers confirmed the presence of ELO in gill epithelial tissue, by PCR, in all animals sampled. Histological analysis and *in situ* hybridisation (ISH) using ELO 16s rDNA-specific probes, identified presence of bacteria in gill, adductor muscle, and digestive tract tissue. Transmission electron microscopy (TEM) also identified intracellular ELO cells within haemocyte and epithelial cells as well as extracellularly. Furthermore, TEM highlighted colonies of bacteriophage virus within ELO cell membranes.

Quantification of the infection severity was achieved using a novel Taqman qPCR assay for the ELO 16S rRNA gene. No significant difference in infection prevalence was observed across four seasons. To investigate bacterial survival, infected host animals were incubated in static water, and environmental RNA (eRNA) samples were extracted and quantified using the Taqman qPCR probe. ELO RNA was prevalent in the absence of the host, over an eight-day sample period.

Epidemiological survey identified ELO infection as almost ubiquitous within the adult king scallop population, throughout the year. Histopathological analysis identified, for the first time, ELO are capable of systemic infection. Furthermore, histology highlighted IMCs rupturing and releasing bacteria. Like many *Endozoicomonas* species, which are facultative endosymbionts, viable ELO is shed from highly-infected animals into the water column with bacteria transferred between proximal host organisms by horizontal transmission. However, the possibility of alternate host vectors involved in the dissemination of ELO infections outside the MPA is possible. This information will aid in designing a surveillance programme, based on eDNA monitoring.

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CATHELICIDIN AND β-DEFENSIN:ANTIMICROBIAL PEPTIDE EXPRESSION IN ATLANTIC SALMON

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Abstract

Introduction: Amoebic gill disease (AGD) is a parasitic infection that affects an increasing number of marine fish species including Atlantic salmon. While AGD triggers a pro-inflammatory immune response in gills, the host immune response in other organs including the expression of antimicrobial peptides (AMPs; the cathelicidins and the defensins) in swim bladder is largely unexplored. In view of the emerging evidence for AMPs as novel anti-infectives, the aim of this study was to investigate the expression of AMP genes (one cathelicidin gene: *asCATH2* and three beta defensin genes: *BDEF1*, *3* and *4*) in the swim bladder tissues of AGD-affected fish 10 days post challenge compared with uninfected fish.

Method: Ethical approval for the experimentation was obtained by the HPRA and GMIT. Sea-water acclimatised Atlantic salmon (n=23) were divided into two groups (control fish and challenged fish) and maintained in recirculating water tanks for 10 days where water quality, temperature and total ammonia nitrite were monitored. The challenge group were infected with approximately 2,750 amoeba per litre of seawater. Swim bladder sampling was performed. RNA was then extracted, converted to cDNA and RT-PCR was performed using specific primers for amplification of *asCATH2* and *BDEF1*,*3* and *4* salmon genes.

Results: All control and AGD-affected swim bladder tissue samples were positive for β-actin and within the AGD-affected fish, infection with the amoeba parasite was confirmed using qPCR. In the unchallenged control fish, constitutive expression of *asCATH2* and each of the beta defensin genes (*BDEF1*, *3* and *4*) was detected in a small number of fish (<20%), and there was no increase in the % positive at day 5 and day 10. Within the AGD-affected fish, an increase in the % expressing *asCATH2* and the beta defensin genes (*BDEF1*, *3* and *4*) was observed at each time point. In particular, expression of *asCATH2* was found in 100% of AGD-affected tissue samples 10 days post challenge, with a higher % also showing expression of *BDEF1* (80%) and *BDEF4* compared with unchallenged controls.

Discussion: The data from this study shows that the swim bladder is a novel site for expression of AMP genes in salmon. While further studies are warranted, the results suggest that cathelicidin and beta defensin genes are likely important components of the innate immune response to the causative agent of AGD.

DEVELOPMENT OF LABORATORY-BASED PROTOCOLS FOR CHALLENGING *CRASSOSTREA GIGAS* WITH OYSTER HERPESVIRUS (OsHV-1 µVar)

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Abstract

The Pacific oyster (*Crassostrea gigas*) is a globally valuable aquaculture species with annual production of >625,000 tonnes in 2014. Ostreid herpesvirus (OsHV-1) has been observed in farmed Pacific oysters and has been associated with sporadic mass mortality events for over 30 years, but the emergence of a new variant (OsHV-1 µVar) in the late 00's was reported as the cause of high mortalities resulting in large loses within the *C. gigas* aquaculture industry worldwide. During initial µVar outbreaks, mortalities in excess of 80% were regularly observed, and infection with Oyster herpesvirus still poses an issue for Pacific oyster production around the world. However, it has been demonstrated that some oysters carry an inherent level of resistance to infection when challenged with the pathogen, and that this resistance has an element of heritability. This study developed bath and injection disease challenge systems for juveniles, and a bath challenge system for larvae using cryopreserved isolates of OsHV-1 µVar. Results were validated by qPCR and *in situ* hybridisation.

Individual bath challenges on juveniles were performed in 12-well cell culture plates in triplicate with 5-fold dilutions of cryopreserved virus. Dose-dependent mortality (up to 60%) was observed in challenged animals, and high viral copy numbers were quantified in DNA extracted from mortalities. Control animal mortality was low. A bath challenge was performed on 1,000 juvenile *C. gigas* in a flow through tank system. Animals were exposed to cryopreserved virus by static immersion and held in flow through conditions at 20°C for 20 days, during which mortality reached 19.4%. Significant differences were detected in viral copy numbers in oyster tissue between animals that died in the challenge and animals that were alive at the end of the 20 days. Water samples were taken throughout bath challenges and demonstrated peaks in viral copy numbers in seawater prior to mortalities occurring. Individual injection challenges were carried out in triplicate with a defined concentration of cryopreserved virus in 12-well cell culture plates for 10 days. Mortalities ranged from 87.5 to 100% and high viral genome copy numbers were detected in DNA extracted from oyster tissue. *In situ* hybridisation showed presence the of large quantities of viral DNA in gill tissue. No mortalities or viral DNA were seen in control animals. Larval challenges were performed in small volumes using 96-well plates. Three-to-five-day old larvae were challenged with defined concentrations of cryopreserved virus for 5 days, over which dose-dependent mortality was observed.

The results demonstrate that the laboratory-based challenge systems using cryopreserved virus seed stocks are reliable and repeatable. These highly controlled challenge systems can be utilised in the determination of susceptibility of *C. gigas* stocks to OsHV-1 µVar. The ability to test for resistance in early life-stages could allow for rapid identification of resistant stocks for selective breeding.

EVALUATION OF NEW CHEMOTHERAPEUTIC APPROACHES TO TREAT AMOEBIC GILL DISEASE

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Abstract

Amoebic gill disease (AGD) is one of the most important diseases affecting the gills of marine fish including Atlantic salmon. The aetiological agent of AGD is *Paramoeba perurans*, an amoeba which is notably characterized by the presence of an intracellular kinetoplastid endosymbiont. This disease can cause mortality of up to 50% in sea cages and estimated economic losses to AGD in salmon farming were US$12.55 million in Norway in 2011 and US$81 million in Scotland in 2013. The current treatments for this disease include freshwater bathing and hydrogen peroxide but these methods are relatively impractical, effective against only mild cases and can represent 10-20% of present production costs. As a result, there exists an opportunity to develop a new chemotherapeutic intervention to treat AGD, as such an approach could be beneficial in terms of cost-effectiveness and efficiency for the rapid removal of the parasite and long-lasting protection. This present study aimed to identify and prioritise compounds that warrant further evaluation as potential new chemotherapeutants for treating AGD. To this end, literature searching was performed to identify diseases of humans and animals caused by amoebic and kinetoplastid parasites and the drugs used for treatment. In total, we identified six major relevant diseases caused by amoebae and kinetoplastid parasites, including amoebiasis and trypanosomiasis. A list of more than 100 drugs and targets were identified and these have been prioritized for subsequent evaluation against AGD. This new bibliographic-based approach has highlighted numerous potential chemical candidates to assist in the development of new cost-effective and practical solutions for the fish farming industry to mitigate against AGD.

UNDERSTANDING PAST AND CURRENT PERFORMANCE OF THE COMMON COCKLE *CERASTODERMA EDULE* TO BETTER UNDERSSTAND ITS FUTURE

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Abstract

The common cockle *Cerastoderma edule* is a valuable bivalve species both commercially and ecologically in Europe, however, commercial landings of *C. edule* have decreased in recent years. Efforts are required to restore the European cockle fishery to ensure its future sustainability, however, in order to plan for the future it is first necessary to better understand the past and current characteristics of cockle populations. This study is part of the COCKLES Project, an Atlantic Area Interreg Project with partners from five European countries, and consists of two parts (a) a desk study to assess past population dynamics and (b) an eighteen month field survey to determine current cockle populations at two sample sites in Ireland, the UK, France, Spain and Portugal. In the past survey, "Marine Historical Ecology" techniques were employed to create datasets of cockle population dynamics including abundance, distribution, age structure, growth, sex ratio and health (reproduction and parasites) in the Atlantic area. The data sets were created from a variety of sources including fisheries data, archaeological records, grey literature and published literature, which were analysed descriptively and meta-analytically. In the current field survey, cockle samples have been collected every two months since April 2008 to evaluate population dynamics. The findings from this study will provide a better insight of the current status of European cockle populations and their drivers and/or inhibitors, which will inform future management and restoration of those fisheries.

TRANSCRIPTOME ANALYSIS VIA RNA-SEQ OF THE ACUTE IMMUNE RESPONSE IN ATLANTIC SALMON CHALLENGED WITH *NEOPARAMOEBA PERURANS*

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Abstract

Amoebic gill disease results from an infection of the gills caused by the ectoparasite *Neoparamoeba perurans.* This pathogen has led to significant economic losses to the Atlantic salmon aquaculture industry worldwide and it is the subject of ongoing research. However, little attention has been given to the Acute Phase Response (APR) of the host, which is the immediate physiological reaction to infection playing a central role in the innate immune system.

The aim of this study is to utilise high-throughput RNA sequencing (RNA-Seq) to study transcriptome profiles during the acute immune response phase of Atlantic salmon exposed to *Neoparamoeba perurans*.

This experiment was designed with the goal of identifying candidate genes which can eventually be developed as bioindicators. These genes will have the potential to improve our knowledge in the areas of animal production and welfare.

POSTERS

FROM DETECTION TO REGULATION – THE EMERGENCE OF CARP EDEMA VIRUS DISEASE (CEVD) IN FRESHWATER FISHERIES IN ENGLAND

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Carp edema virus disease (CEVD) is a serious emerging disease of common carp and its ornamental variants. CEVD was first detected in the wild in England in 2012 following Environment Agency led mortality investigations at a still water fishery in southern England. Since 2012, a total of 11 outbreaks of CEVD have been recorded in still water fisheries. The clinical and histopathological characteristics of CEVD are described along with details of the fisheries affected, scale of losses and fishery management practices. The detection of CEV in combination with other pathogens and environmental factors is used to highlight the need for comprehensive and broad spectrum investigations during national incident response. Due to the risk posed to carp fisheries - an industry worth over £1billion annually, fish movement controls have been placed on all CEV-positive waters in England under Section 27a of the Salmon and Freshwater Fisheries Act, 1975 and the Keeping and Introduction of Fish (KIF) regulations. Comparisons are made between CEVD, Koi herpesvirus disease (KHVD) and the condition known as Spring Carp Mortality Syndrome (SCMS). A review of archived material from unexplained mortalities investigated in the late 1990's provide evidence of CEV involvement. The importance of prompt responses to the detection of new and emerging diseases is discussed.

USE OF ARTIFICIAL EGG-LAYING STRUCTURES TO MONITOR AND CONTROL *ARGULUS* INFECTIONS IN STILL WATER FISHERIES – REALITY OR PIPE-DREAM?

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The fish louse Argulus causes serious disease problems in still water fisheries resulting in mortality, poor fishery performance and economic loss. Here we describe the use of 110mm diameter black plastic pipes, positioned vertically in the margins of fisheries as a cheap and simple way to monitor, harvest and remove parasite egg-strings. Since 2016, trials have been conducted at 4 still water trout fisheries in England ranging from 1 to 25 acres in size. Egg deposition was recorded from May to November at temperatures between 8.4ºC and 20.2ºC. Pipes were raised, checked and dried every 2 weeks, with up to 4,600 egg strings successfully harvested from each pipe and up to 20 million individual eggs destroyed annually from each fishery. Three of the four trial sites recorded a decline in egg string numbers throughout the year with light lice burdens on the resident fish and an improvement in fishery performance. Argulus problems persisted at one trial site which was attributed to fishery management practices that promoted parasite survival and development. The benefits and limitations of this approach is discussed in the context of existing options for the management of Argulus in still water fisheries.

ATTEMPTS TO CULTURE CARP EDEMA VIRUS ON *IN VITRO* GILL PRIMARY CELL EPITHELIA IN ASYMMETRICAL CONDITIONS

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Abstract

Carp Edema Virus (CEV) is a poxvirus which infects carp (*Cyprinus carpio*). First described in Japan, it has since been identified across Europe, Asia, North America and South America. Outbreaks can lead to mortalities of up to 75-100% in juvenile koi, while lower mortalities have typically been observed in common carp. In the UK CEV has recently been linked with Spring Carp Mortality Syndrome[1]. Despite attempts in over 30 different cell lines CEV is non-culturable, and not isolated during routine screening, thus currently infections are diagnosed using nested conventional PCR or qPCR assays. The lack of virus culture inhibits full characterisation of and further research on this disease. We developed an *in vitro* model of the carp gill epithelium on Transwell permeable membrane inserts using a double seeding technique[2] with purified carp gill primary cell fractions to attempt to culture CEV. The *in vitro* epithelium enriched with chloride and goblet cells can withstand exposure to apical freshwater, which results in a polarised epithelium that more resembles the natural physiological environment.

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AN *IN VITRO* MODEL TO STUDY SALMONID INNATE IMMUNE RESPONSE TO *NEOPARAMOEBA PERURANS*

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Abstract

Amoebic gill disease (AGD) is a serious disease affecting the marine-farmed Atlantic salmon *Salmo salar* L. industry[1]. The causative agent of AGD is *Neoparamoeba perurans* a free-living protozoan which colonises the gills[2].

RTgill-W1 cells seeded onto Transwell permeable membrane inserts in 12 well plates were inoculated with either a laboratory attenuated or a recent isolated clone of *N. perurans*. Inoculated cells were harvested in triplicate at 0, 1, 3, 6, 24 and 48 h post inoculation and the expression of 12 immune related genes was compared between infected and uninfected cells and between both amoebic clones by Taqman qPCR.

Both clones induced the up-regulation of host cell proinflammatory cytokine IL1β, complement C3, cell receptor MHCI, Th2 transcription factor (GATA3) and Th2 signed cytokines IL10, IL6 and IL4/13A. Genes involved in cell proliferation, PCNA and AG-2, were also up-regulated.

An *in vitro* model using a derived rainbow trout gill epithelium seeded in Transwell inserts has been shown to be a promising tool to study host response to *N. perurans*. The Th2 subset and key genes involved in cell proliferation identified in *in vivo* studies were significantly up regulated in the inoculated cells. This *in vitro* platform could be a valuable tool to test disinfectant compounds to control AGD in marine aquaculture and has a potential to test host resistance to AGD in selective breeding programmes.

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LOOP MEDIATED ISOTHERMAL AMPPLIFICATION (LAMP) FOR THE RAPID IN-FIELD DETECTION OF *NEOPARAMOEBA PERURANS*

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Abstract

Loop mediated isothermal amplification is proposed to be a rapid, cost effective tool for molecular detection on crude DNA extractions thus suitable for in-field diagnostics. We report the development and partial validation of a LAMP assay to detect *Neoparamoeba perurans*, the causative agent of amoebic gill disease (AGD) in Atlantic salmon (*Salmo salar*). A total of five primer sets designed to amplify a partial fragment of the either the 18S or 28S ribosomal RNA gene of *N. perurans* using the Genie realtime LAMP apparatus (Optigene, UK) were compared for specificity and sensitivity. The optimal assay was taken forward into a comparison of 5 "quick and dirty" field appropriate extraction methods. Combining sample collection by swab (Isohelix) and crude rapid extraction (QuickExtract) and amplification in Genie III, consistently gave sample to result in just under 50 minutes, with a detection limit of less than a single amoeba.

CGIAR RESEARCH PROGRAM ON FISH AGRI-FOOD SYSTEMS (FISH)

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Abstract

Sustainable aquaculture flagship

This flagship aims to enable sustainable increases in gender and socially equitable livelihood returns from, aquaculture production without creating adverse socio-economic or environmental impacts.

Fish breeds and genetics: FISH disseminates recent generations of improved breeds from WorldFish's long-established tilapia breeding programs. We continue to further develop improved and more resilient strains of tilapia and carp.

Feeds, fish nutrition and health: FISH develops fish disease and health management measures and improved feeds that will enable women and men farmers to harness the productive potential of improved fish breeds.

Aquaculture systems: FISH develops improved fish farming practices and farming systems for sustainable instensification of aquaculture, and business and enterprise models for smallholders and value chain actors. That can deliver improved performance from healthy, improved seed and sustainable feeds.

THE IMPACT OF UV-B RADIATION ON PACIFIC OYSTER PERFORMANCE AND DEVELOPMENT OF THE PATHOGENS HERPES VIRUS AND *VIBRIO AESTUARIANUS*

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Abstract

Since 2008 oyster cultivation sites around Europe have been experiencing increasing incidences of mass mortality events, generally occurring over the summer months. Osterid herpesvirus and variants (OsHV-1 μVar) and *Vibrio aestuarianus* are oyster pathogens which have been associated with increased oyster mortality, and a range of environmental factors can impact on the levels of infection. Medium length ultraviolet radiation (UV-B λ280 – 320) is highest during summer months and can fluctuate significantly between years. UV-B is known to cause damage to DNA through the formation of mutagenic lesions which interrupt DNA translation and can lead to apoptosis. UV-B has been shown to reduce the performance of aquatic animals and increasing the rate of mortality. The role of UV-B radiation in the seasonal mass mortality events of *C. gigas* has not been investigated. The aim of this research is to investigate the biological impact of UV-B on the health of *C. gigas* and the development of OsHV-1 μVar and *V. aestuarianus* pathogens. To date, two lab trials have been carried out exposing oyster seed and adults to UV-B radiation in both sea-water submerged and aerially exposed experiments. Field trials are currently underway in Dungarvan Bay, Ireland with oysters held at different intertidal heights thus exposed to naturally different levels of solar UV-B. In the lab trials, it was found that exposure to UV-B radiation significantly increased the rate of mortality in oyster seed but not in adult individuals. OsHV-1 μVar was not detected in oysters in the lab trials although it has been detected in the oysters held at both intertidal heights of the field trial. *V. aestuarianus* was detected at very high levels in the lab trial oysters and infection prevalence was reduced after exposure to UV-B. In the field trials, *V. aestuarianus* was more prevalent in low shore oysters than in those held at the high shore during period of high UV-B and temperature. Further results from the field trials are still being obtained although preliminary results seem to match those of the lab trials. Exposure to UV-B (artificial or natural) increases the rate of mortality in *C. gigas*, while also hindering the development of *V. aestuarianus* bacteria.

PROTEOLYTIC ACTIVITY IN EXTRACELLULAR PRODUCTS OF *IN VITRO* CULTURED *PARAMOEBA PERURANS*

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Abstract

Paramoeba perurans, Feehan *et al*. 2013 is the causative agent of amoebic gill disease (AGD), one of the main concerns for the marine Atlantic salmon industry worldwide. Despite more than 30 years of extensive research, the relationship between host and parasite remains unclear. The aim of the current study is to characterise proteolytic activity in extracellular products (ECPs) of *P. perurans* and to investigate its relevance to the development of AGD. Extracellular proteases are known to be an important virulence factor in a number of human as well as fish infections and, in some cases, can also be used as a tool to distinguish between infectious and non-infectious strains of a parasite.

An in-gel zymography method (Vandooren *et al.* 2013) was used to analyse the secretions from *P. perurans* culture. Briefly, medium from *in vitro* cultured *P. perurans* containing ECPs was harvested and subjected to electrophoresis under non-reducing conditions, in an acrylamide gel co-polymerised with gelatin. The gel was washed and incubated in a developing buffer to encourage substrate digestion by enzymes present in the sample. To reveal 'clearance zones' of proteolytic activity the gel was stained with Coomassie Brilliant Blue™.

Three temperatures of incubation for zymogram development were tested: 10, 15 and 37ºC. While multiple gelatinolytic enzymes were observed following incubation of the gel at the highest temperature, one distinct 'clearance zone' between 37 and 50 kD was present in gels incubated at 15ºC as well as 10ºC. The results suggest that some proteases secreted by *P. perurans* are active at temperatures corresponding to those observed in the natural environment of the parasite.

Further work will be carried out to identify the protease class which the enzyme showing activity at 15ºC and 10ºC belongs to and to investigate its relevance to pathogenesis. The results will contribute to the identification of virulence factors involved in the development of AGD. Characterisation of the digestive activities of ECPs from *in vitro* cultured *P. perurans* will potentially contribute towards development of new tools for disease control, including in-feed immunostimulants, vaccines and targeted drugs.

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ECOLOGY OF THE THREE-SPINED STICKLEBACK IN THE BURRISHOOLE CATCHMENT

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Abstract

The post glacial colonisation of freshwater by marine three-spined stickleback has been well studied, and indicates thatthe biology and ecology of these fish in novel contact zones is complex . In Lough Furnace, a 141ha brackish lake located on the transition between the freshwater Lough Feeagh and the sea on the west coast of Ireland, there exist three distinct and reproductively isolated three-spine stickleback ecotypes, which are associated with freshwater, brackish and marine habitats, previously distinguished phenotypically by plates and genetically by both neutral and adaptive genetic markers. A new project in respect of the potential mechanisms underlying the apparent reproductive isolation of these fish has commenced recently. This project includes an investigation of the spatial and temporal distribution of spawning activity by mapping aquatic habitats, undertaking isotopic analysis and by strategic trapping of sticklebacks in the sea, in the brackish lake and in the waters connecting both ecosystems. Ecologically significant traits pertaining to the three putative ecotypes are being characteriesd bytargeting variations that differentiate the marine form on the basis of metabolic potential i.e. standard metabolic rate, maximum metabolic rate and aerobic scope in addition to the identification energy phenotypes linked to gut microbiome community profiles and feeding preferences that are likely to effect micro- and macro-parasite community diversity associated with each ecotype. Here, results from the first breeding season investigations are presented.. This project is part of the Burrishoole Ecosystem Observatory Network 2020 programme (BEYOND2020), which is seeking indicators of ecosystem responses in a changing global environment.