Metabarcoding to assess changes in benthos occurring around fish-farms

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Environmental 'metabarcoding' is a multi-step process whereby bulk DNA is extracted from environmental samples and certain genetic markers amplified. These amplified markers are then sequenced, using next-generation-sequencers, and compared to reference-databases to enable DNA-based identification (Lejzerowicz, Esling et al. 2015).

Global aquaculture is expanding rapidly and now supplies >50% fish-protein for human consumption. Scotland produces ~180,000 tonnes of Atlantic salmon per annum and the Scottish Government has set targets to double the economic value of aquaculture by 2030 (Anon Undated).

Salmon culture is associated with a range of changes in the receiving environment including the organic enrichment of the benthic habitat. Organic enrichment is linked to changes in the benthos that have, traditionally, been monitored by taking grab samples and extracting and identifying the macrobenthos. This macrobenthic identification process is expensive, time consuming and reliant on a dwindling number of taxonomists.

In Scotland, the way fish farming is regulated is changing. The intensity of benthic monitoring will increase ~six-fold and will exceed UK analytic capacity. In addition, traditional macrobenthic results are usually only available several months after sampling making adaptive management impossible. The adoption of more intensive monitoring may enable, where demonstrably compliant, the increase in fish-biomass per site.

There is considerable potential for metabarcoding to augment and, ultimately, replace traditional macrobenthic approaches to benthic compliance assessment (Pawlowski, Esling et al. 2014). In order to achieve this change in approach we need to understand (i) how to take samples (~10 g) from grabs for metabarcoding that are repeatable and representative of the grab (~15 kg) (ii) how best to link metabarcoding data to traditional macrobenthic metrics.

We assessed repeatability by taking 5 replicates per grab at various distances (cage-edge to reference) from around three fish-farms (150 samples). For each sample, we examined three gene-markers (16S, 18S and COI to cover bacteria to metazoa). Results show a high degree of consistency between replicates and clear, consistent differences in the bacterial community between locations and between distances within locations.

Comparisons between metabarcoding and traditional approaches will be evaluated using discriminant analysis. This will identify which group of 'species', identified using metabarcoding, are best able to replicate the metrics derived from traditional macrobenthic analysis.

Acknowledgements
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The development of clinical diets to treat farmed fish gill diseases

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Amoebic gill disease (AGD) presents a significant problem for sustainable salmonid aquaculture, causing an estimated annual loss of over 80 million USD in Scotland alone. Commercial treatments rely upon freshwater and chemical baths, however these methods are expensive, time-consuming and often short-lived. Clinical diets that modulate the immune system have been proposed as a potential aid in the maintenance of AGD management, due to the ease of administration within the feed.

The main focus of this project has been to assist the salmon feed industry specialist Cargill in the development of a clinical diet that can mitigate the effects of AGD infection, with an emphasis on increasing the understanding of the molecular pathways stimulated during infection and after conventional bath treatment. Two trials have been completed, with the first focusing primarily on the impact of infection and the second one on the influence of plant-based in-feed additives on host immune responses during AGD infection.

The results from the first trial showed that AGD infection induced a response characterized by inflammation with limited humoral (antibody) involvement. As severe inflammation is a symptom of AGD, this may not be beneficial for the host. In the second trial, the feed containing the plant-based additives actively inhibited the expression of a transcription factor involved in Th1 responses, with fish fed on the experimental diet showing lower gill scores overall. These results show the potential for clinical diets to positively modulate the host immune response and reduce disease severity without the need for conventional treatments.

The biggest knowledge gap regarding AGD is the manner in which the parasite directly interacts with the host. TEM images have shown amoeba pseudopodia creating close but not direct membrane-membrane interactions, suggesting that like other amoeba species the cytopathic effect on the host may be caused by a secreted molecule from the amoeba, that has yet to be elucidated (Nielsen et al., 2016). Therefore, future work should focus on establishing if such secretions exist and whether the pathways they trigger in the host can be modulated, perhaps to induce tolerance (via in feed exposure) in farmed fish prior to the seawater phase grow-out.

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Antiviral response of rainbow trout (*Oncorhynchus mykiss*) gill epithelial cell line RTgill-W1

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Aquaculture has grown steadily in the last few decades, playing an increasing role to meet the growing demand for fish in an increasing world population. One of the biggest challenges in aquaculture is disease caused by viruses, bacteria or fungi. Among these pathogens, viruses are the most dominant agents that cause very significant aquaculture losses. Of the ten fish diseases listed in 2016 by the OIE as notifiable, eight are viral. Salmonid alphavirus (SAV) is one of the viral disease causing pancreas disease and sleeping disease in farmed Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*) in Europe. In the present study, we have used a trout gill epithelial cell line (RTgill-W1) as a model to characterize the initial response of Rainbow trout gills to SAV.

Our results indicate that SAV-2 is be able to multiply in RTgill-W1 cells. The cellular integrity of the gill epithelium was found to be disturbed at an early stage of SAV-2 infection. The loss of barrier function was found at the early stage of infection with SAV-2 while rebuilding of the barrier was observed at the late stage of infection. However, the virus did not induce any visible changes (cell morphology) suggesting a low level of virus multiplication. At molecular level, RT-gill-W1 cells were found to launch a strong antiviral response against SAV-2, which was also observed upon stimulation with poly(I:C).

To study in further detail the mechanisms involved in the molecular antiviral response the phosphoproteome of RTgill-W1 cells in steady state and poly(I:C) stimulated conditions was conducted. A higher number of phosphoproteins (360 unique phosphoproteins) were identified in poly(I:C) stimulated cells. Some of these are known to be involved in antiviral response. Other phosphoproteins identified, covered a wide range of functions from transcription, translation to intracellular trafficking and cytoskeleton maintenance. Several unique signaling pathways were also found to be activated by poly(I:C). These results provide an untargeted view of the key signaling pathways that are quickly activated in response to viral PAMPs in fish gill cells.

Findings of the study suggest that trout gill epithelia have the ability to mount a defense against SAV-2 infection. Overall, the findings of this study could facilitate developing antiviral drugs/vaccine which is conducive to the aquaculture production.

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Cumulative effects of deltamethrin treatment on health status in cultured rainbow trout (*Oncorhynchus mykiss*)

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The objective of the work undertaken was to study the cumulative effect of deltamethrin (AMX®) treatment on blood biochemistry and tissue histopathology in commercial trout to determine its effect on the trout health status. The fish were exposed to a chemical bath at 0.2 mL of the product per 1 m^3 sea water (0.2 µg deltamethrin/L sea water) for a period of 30 minutes. Blood samples were taken before the treatment, immediately after, 48 hours after and 10 days after the delousing treatment was being applied. The treatment and samplings were repeated after 35 days. Tissue samples for histopathological study were collected before the start of a treatment and 10 days after the second treatment.

Fish serum biochemical analysis showed significant increase concentrations (p<0.05) in total bilirubin, total protein, creatinine, potassium and iron, recorded immediately after the first treatment. Levels of total bilirubin, LDH activity and iron concentrations were significantly increased 48 hours after the treatment. Iron concentration stayed significantly higher 10 days after the treatment. In the second treatment, markers of liver damage, such as ALP and ALT, showed increased activity in all time points after the treatment together with high levels of total proteins and albumins. During the 48h after the treatment and 10 days after the treatment, a notable increase in creatinine and iron concentration was seen.

Histopathological evaluation of gills showed highest damage to the secondary lamellas in form of blood congestion, telangiectasia, excessive bleeding and edema after the treatment. Liver damage was visible after series of delousing treatments as mild necrosis of hepatocytes with severe dilatation of sinusoids. The kidney tissue damage after delousing treatments showed increased number of melano-macrophage centers (MMC) and blood accumulation in interstitial area. Splenic tissue showed excessive hemosiderin deposits when compared to fish from before treatment.

These results indicate the possible impact of sea lice treatment through direct toxicity of the chemical to liver tissue. Blood biochemistry has a good potential to approach fish heath status and was correlated with organ/tissue damage. Liver was significantly affected and weakened after the treatments showing a more sensitive response than the other studied organs. We conclude that organ injury resulting from deltamethrin should be considered as a cause of decreased organ function after sea lice treatments.

Microbiome diversity in blue mussel (*Mytilus edulis* L.) larvae relates more to time than diet

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Global demand for the common or blue mussel (*Mytilus edulis*) continues to increase. One barrier to increased production is the reliable availability of high quality mussel spat, the stage where the juvenile bivalves start settlement. To this end, the SAIChatch consortium was formed to develop a pilot-scale blue mussel hatchery, supporting the optimization of various production traits via targeted research. This effort includes investigations on feeding regimes and the monitoring for harmful bacteria. Disease outbreaks due to bacterial pathogens, especially from the genus *Vibrio*, are a risk in all intensified aquaculture systems (Kesarcodi-Watson et al. 2009) but especially within bivalve hatcheries, as larvae have a relatively underdeveloped immune system. Despite being an essential primary food, marine algae are also an important source of bacteria within a hatchery system (Dubert et al. 2015). Thus, the aims of this investigation were two-fold; to evaluate the effects of selected microalgae species on growth and survival of the mussel larvae and describe the evolution of the total *M. edulis* microbial community abundance and diversity during larval ontogeny and between the different microalgal feeds with particular reference to potentially pathogenic *Vibrio* ssp.

Five different algal diets (*Chlorella vulgaris*, *Cylindrotheca fusiformis*, *Monodopsis subterranean*, *Nannochloropsis oceanica*, and a mix of *Chaetocerous calcitans f. pumilus* and *Isochrysis galbana* mix) were fed to *M. edulis* larvae, that otherwise were reared under common conditions. Samples from each dietary group were aseptically taken before feeding, at 2 days post fertilization, and then weekly for four weeks, until animals reached settlement. Total DNA was extracted, and 'universal' PCR primers used to amplify a hypervariable region of bacterial 16S ribosomal DNA (rDNA) in each sample. These amplicons were indexed, pooled and mass sequenced on the Illumina MiSEQ platform. Sequence data revealed that, based on diversity at the family level, bacterial populations were not significantly different among the diet groups, except for those larvae fed with *M. subterranean*. However, diversity did increase significantly with time, suggesting microbiome development is largely endogenous. Furthermore, the proportion of bacteria from the *Vibrionaceae* family decreased with time ($p < 0.05$) and there was no significant correlation between any feed and presence of this bacterial group.

To our knowledge this is the first report detailing bivalve microbiome diversity during ontogeny and will further our understanding of microbe/host interactions during early development. Furthermore, the workflow developed here can be applied to investigate *M. edulis* bacterial community structure in other environments and at different stages in development.

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Effect of temperature during broodstock conditioning in the Blue mussel (*Mytilus edulis*)

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Blue mussels (*Mytilus edulis*) are, by volume, among the main products of the UK aquaculture sector, second only to salmon farming. However, despite its importance, mussel aquaculture production is not growing at the same rate compared with other aquaculture sectors. According to the latest Scientific, Technical and Economic Committee for Fisheries (STECF) one of the main constraints for bivalve production in Europe is the variability in recruitment and production of larvae and spat for commercial on growing (STECF, 2016). The establishment of an industrial mussel hatchery might improve the Scottish mussel production, by providing a continuous input of high quality juveniles all year round. Nevertheless, hatchery technology is an expensive procedure and most of the established protocols have been developed for other species of bivalves rather than on blue mussels (Helm and Bourne, 2004).
In this study we tested two different conditioning programs on commercial size *M. edulis* adults: a single stage conditioning (SSC) and a cold and hold (C&H) treatment. The conditioning process was monitored by evaluating the broodstock's spawning competence, condition index, gonad histology and lipid biochemistry. Lipid biochemistry was evaluated at lipid class level during the conditioning process via normal phase high performance liquid chromatography (NP-HPLC). The results suggest that increasing the temperature might reduce the ability to control spawning in the conditioned mussels. As SSC demonstrated lower spawning competence and CI after 80 days of conditioning compared with C&H. The histological examination of gonads evidenced that in C&H ripe mussels reached ≈80% of the total after 40 days of conditioning; whilst in SSC evidences of spawning and redeveloping occurred also after 40 days of conditioning.

This study suggests that maintaining mussels at low temperature during the conditioning process, permits to keep their condition for longer periods, with interesting application for hatchery production of mussels. By keeping mussels ripe throughout the year, hatchery production could be extended also outside the reproduction period of blue mussels.

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Plasma and liver proteome of an unwanted phenotype of seawater-transferred rainbow trout (*Oncorhynchus mykiss*)

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The production of seawater-transferred rainbow trout (*Oncorhynchus mykiss*) has recently shown an increase in production, reaching 87,000 tones in Norway alone in 2016. However, an unwanted phenotype of the species is causing major economic losses, especially during the summer season, when the problem is most prevalent. After seawater transfer, a significant portion of the fish (approximately 10%) become growth-stunted and extremely lean, often presenting injuries, acute fin damage, and high abundance of melatonin in their skin, indicative of severe stress. This developmental anomaly is likely related to a lack of understanding of vital intrinsic (*e.g.* critical size, genetically determined phenotypic plasticity) and extrinsic (light, temperature, salinity, etc.) factors governing the physiological development of the fish during smoltification and seawater transfer.

To investigate the functional phenotype of this anomaly, the plasma and liver proteome of growth-stunted and fast-growing rainbow trout were compared using two mass spectrometry (MS) platforms: gel electrophoresis coupled with liquid chromatography tandem MS (GeLC MS/MS) and isobaric tandem mass tag (TMT) multiplexing in LC MS/MS. These were used to characterize, respectively, the blood plasma proteome and the liver proteome.

In blood plasma, 1474 proteins were successfully identified and quantified (based only on peptides exclusive to each protein) and, of these, 291 were found to be significantly different (q-value < 0.05, fold change > 2) between growth-stunted and fast-growing fish. The roles of these proteins were related to fish growth, stress, anabolism, catabolism, cell differentiation, Na^+, K^+–ATPase activity, and immune response, among others.

In liver, 213 were identified and of those, 78 showed significant differences (q-value < 0.05). In this case, a general decrease in proteins related to anabolism, catabolism, immune response and gas transport (*i.e.* globins was observed in growth-stunted samples.

This study represents the first effort to understand the underlying physiological condition of growth-stunted rainbow trout and provides the first proteome dataset of the species.

Integrating aquaculture and marine renewable energy generation into a single platform: the INNO-MPP project

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Abstract

Sustainable global growth in marine and maritime sectors will require improved efforts to reduce costs and impacts associated with offshore marine structures, whilst maximizing socio-economic benefits. The concept of a Multi-Purpose Platform (MPP), integrating multiple industries, is therefore inherently attractive, for example involving aquaculture and marine renewable energy generation. Studies to date have, however, mainly focused on large-scale systems with little attempt to investigate smaller-scale systems suitable for individual fish farms or island communities.

MPP development poses numerous cross-disciplinary challenges, particularly at smaller scales, in terms of techno-economic feasibility and assessment. The ongoing collaborative 'Innovation-MPP' (INNO-MPP) project, involving partners from the UK and China, seeks to investigate the particular synergies and tensions that might result from integrating aquaculture and marine renewable energy generation within the same platform structure. To date, the project has sought to define the main cross-disciplinary questions (CDQs) by means of an in-depth assessment process, involving one-on-one interactions between multiple systems aboard an MPP (e.g. wind turbines, floating platform structures, aquaculture cages) and outside 'services' required (e.g. water quality, power take-off infrastructure, marine planning processes). Independently, the potential synergies and tensions resulting from the MPP's interactions with the wider marine environment (mobile species, benthic invertebrates, invasive non-native species, etc.) have also been assessed using a similar framework. This assessment process has also improved cross-disciplinary appreciation of differences in terminology and underlying paradigms amongst project partners, who represent very different scientific disciplines (engineering, social and environmental science).

These efforts have resulted in the identification of a wide range of potential MPP-associated interactions (both positive and negative) which may come about through close integration of aquaculture and marine renewable energy within a single platform. These include technical interactions between different platform elements (e.g. wind turbines vs. aquaculture cages) as well as the MPP as a whole interacting with different components of the marine environment (e.g. seabirds). Each interaction is also underpinned by an assessment of uncertainty. The most important interactions will now be investigated further to assess their significance to practical future MPP deployments.

Having identified the various synergies and tensions, the potential of small-scale MPP development is now being investigated more thoroughly through two case studies, one focused on remote fish farm operations off western Scotland and the other on small isolated island communities in China. Initial results from both case studies will be presented.

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