Introduction

FishProBio is a Marie-Curie actions international outgoing fellowship (IOF) between the Marine Fisheries Genetics Laboratory, Bangor University, UK, and the Institute de Biologie Integrative et de Systemes, Universite de Laval, Quebec, Canada. The project explored the microbiomes (the commensal bacterial flora) of commercially exploitable teleost species in healthy and diseased states. Improved understanding of the role microbiota play in predicting, defining and possibly preventing disease, will be vital as aquaculture increasingly provides our principal source of seafood. This publishable summary details the progress towards fulfilling the project objectives, the work undertaken over the full 36 months of the project and the potential impact of the results

The Project Aims as described in Part B, Annex I, covered in the first 12 months of the project, of the Grant Agreement are described as follows.

A) **Identify community shifts in the surface mucous microbiome of an economically important teleost species in healthy and diseased states**
B) **Identify key changes in microbial community structure that precede disease or define resistance to disease**
C) **Understand the genetic mechanisms that underpin competitive exclusion between an invasive opportunistic pathogen and a proven resident probiotic bacterium**

Progress and work undertaken

The first phase of the project involved the testing of a proven probiotic *in vivo*, as an effective preventative and curative measure to treat infection with the fresh water salmonid pathogen *Flavobacterium psycrophilum*. According to the predicted progress and milestones of the project. Completion of the probiotic *in vivo* portion of the experiment was expected for completion at the end of month 8. 454 amplicon sequencing was predicted to begin by month 10.

Figure 1 - *Salvelinus fontinalis* - the brook charr, an important fresh water aquaculture species in Quebec

As planned the *in vivo* challenge was undertaken, slightly modified to involve three different probiotic candidates, as well as untreated controls. The experiment involved over 1500 individual fish. We selected only susceptible *Salvelinus fontinalis* for the study from as single pedigree. Improving on the described experimental design described in the original proposal, replicates were maintained in isolated aquaria to minimize cross-contamination. In total fifteen aquaria were employed, including five triple replicated treatments: stressed, unstressed, probiotic 1, probiotic 2 and probiotic 3.

Figure 3 - Experimental aquaria used to test probiotics on *S. fontinalis* fingerlings

Skin mucous samples and environmental samples were collected and stored as detailed in the work plan. However, we encountered major difficulties during the stress phase of our experiment. The aquaculture supplier who provided the Rupert pedigree susceptible *S. fontinalis* fingerlings, through lack of phenotypic control, supplied a resistant population. Thus, despite repeated temperature and anoxic stressing, we were not able to induce *F. psycrophilum* infection, identified either by PCR detection, or via observed symptoms and mortality. The experiment was therefore terminated at month six. Subsequently, new fish were ordered from a separate supplier, supposedly from a susceptible Rupert phenotype. We, therefore, restarted the experiment. However, once again, repeated stresses failed to induce infection. Finally, we halted the *in vivo* experimental work at the end of month 11.

Figure 2 - *L. salmonis* is a salmonid parasite the causes significant damage to aquaculture

Once the first unsuccessful trial of the experimental system was completed at the end of month 8, it was decided that there was potential to shift the focus to another teleost system, in the event that the second *in vivo* trial was similarly unsuccessful. Atlantic salmon, *Salmo salar* are globally the most import salmonid in aquaculture in economic terms. Fortunately, there is ongoing research at the Derome lab to examine the interaction between a salmonid parasite - *Lepeophtheirus salmonis* and the *S. salar* microbiome. It was agreed that this strategy would

simultaneously allow the researcher to fulfill their training objectives in a parallel teleost-pathogen system, while adhering to the overall aims of the project.

To evaluate the microbiome of *Salmo salar* in a 'healthy state,' we collected 96 *S. salar* from across the Atlantic encompassing both freshwater and marine phases. Dramatic differences between environmental and gut bacterial communities were observed. Furthermore, community composition was not significantly impacted by geography. Instead lifecycle stage strongly defined both the diversity and identity of microbial assemblages in the gut, with evidence for community destabilisation in migratory phases. Mycoplasmataceae phylotypes were abundantly recovered in all lifecycle stages. Patterns of Mycoplasmataceae phylotype recruitment to the intestinal microbial community among sites and lifecycles stages support a dual role for deterministic and stochastic processes in defining the composition of the *S. salar* gut microbiome. This work was published this year (2015) in the ISMEJ (Impact factor 9.3)

Figure 4 - Wild *S. salar* smolt captured to define the 'healthy state' microbiome

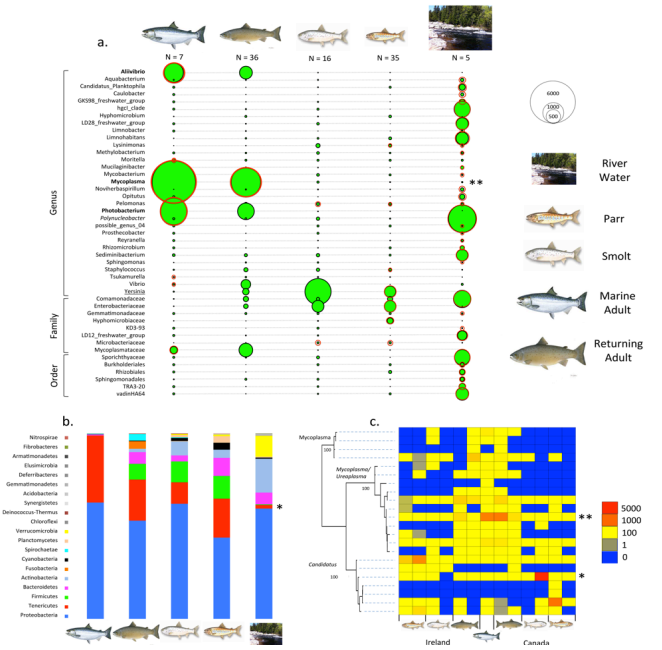

Figure 5 – Summary of wild S. salar gut microbioal diversity. For more details see Llewellyn *et al.*, 2015 10.1038/ismej.2015.189

The experimental challenge with *Lepeophtheirus salmonis* on *S. salar* post-smolts ran in July and August 2014. *L. salmonis*, are naturally occurring parasites of salmon in sea water and cause multi-million dollar losses for aquaculture. Unfortunately, salmon farming provides ideal conditions for L. salmonis growth and transmission (high host density, optimum salinity and temperature) compared with natural conditions, creating problems for the salmon farming industry and, potentially, wild salmonids.

We established six salt water tanks containing a total of 1200 post- smolts from 40 different families (approx. mass 120g/fish) and exposed four to infection with L. salmonis copepodids (two were left as controls). Fish were euthanized 30 days post infection and lice loads calculated. Mucous sabs, mass, length, skin pH and blood cortisol measurements were taken at four time points across all tanks. To assay bacterial abundance and diversity, 208 mucous swabs were subjected to deep sequencing of the hypervarable V4 domain of the 16S rRNA gene array.

Over 4 million sequence reads were generated across the samples. Clear differences in bacterial identity between control and test tanks were evident, especially at the final sampling point (R2=0.3492, p=0.0001). However, the frequency of putative pathogenic bacterial genera was not increased among test tanks. Crucially, pair-wise beta-diversity indices indicated destabilization of microbiome composition among fish sampled at 16 and 30 days post infection (p=0.000438, p=1.04e-15).

Data analysis are ongoing to establish a link between louse load, fish condition and microbial diversity. However, preliminary results suggest an important potential role for sea lice in reducing colonization resistance of the skin to secondary pathogens by destabilising the endogenous mucosal microbiome.

Objective 3 – to understand the genetic mechanisms that underpin competitive exclusion between an invasive opportunistic pathogen and a proven resident probiotic bacterium – was abandoned

Summary and outputs of the project

After initial difficulties were encountered inducing infection with *F. psychrophilum* in the selected teleost-pathogen system, a modification to the experimental strategy were made. Subsequently efforts to establish the natural diversity of *S. salar* gut microbiota progressed exceptionally well, with a report published this year in a high impact journal. Efforts to explore changes in the microbiota associated with salmon skin during sea louse infection successfully identified a possible mechanism by which opportunistic bacterial infection emerge in affected fish. In short, we overcome early difficulties to provide genuine insight onto the microbial ecology of a commercially exploited teleost fish.