

Grant application title: SASG6 Investigating the seasonal microbiome of Atlantic Salmon gills

Recipient: Morag Clinton (PhD candidate)

Address: Scottish Oceans Institute, East Sands, St Andrews. KY16 8LB

Email: mc287@st-andrews.ac.uk

Activities covered by grant: Laboratory supplies (RNAlater solution) for microbiome study.

Overview

Funding from this award was used to purchase essential laboratory supplies, specifically RNAlater solution, as part of the project "Investigating the seasonal microbiome of Atlantic Salmon gills". Funding allocated from MASTS has allowed safe preservation of tissue for transport, analysis and long-term storage as part of the ongoing collection phase of this project. Following completion, we expect this research to provide a picture of seasonal changes to the gill associated microbiome as well as any alterations to bacterial populations associated with on farm activities.

Background

The aim of this work was investigation of the adherent bacterial populations on Atlantic salmon gills during a full annual cycle of production. This was achieved through regular collection of gill samples and next generational sequencing of adherent bacteria to determine the community composition of gill tissue. Funds were requested from MASTS for essentials laboratory supplies.

It is understood that salmon mucosal surfaces are colonised by mixed bacterial populations known as the microbiome, a mixture of commensal and symbiotic bacteria as well as opportunistic pathogens¹. Current thinking is that while microbial populations are affected by environmental bacterial populations, it is also true that substrate environment for growth and modulation by the immune system has a significant effect on bacterial survival and community composition². The majority of research to date focuses on intestinal bacterial populations, with less information currently available regarding the biodiversity and seasonality of the microbiota of skin and gill surfaces of fishes. However it is known that disruption of normal microbial populations can lead to dysbiosis, with proliferation of potentially pathogenic species of bacteria³, and so alterations to the adherent bacterial populations of gills may occur with gill disease or other modulators of microbial populations as well as seasonally with environmental populations.

Benefits of this award

Data collection began May 2017 (with fieldwork sampling due to conclude May 2018) from a Scottish Sea Farm site on the Isle of Mull. Various methods of sample collection were considered, and as flash freezing was not an option due to the farm location and transportation to the sampling site, a preservation media was required. The chosen protocol involved excision of gills from euthanised fish and preservation in RNAlater for transport and later DNA extraction. Purchase of sufficient volume of RNAlater, considered superior to cheaper fixation alternatives such as ethanol or formalin⁴, was made possible by MASTS funding, allowing for immediate sample preservation and improved long-term storage. This financial assistance from MASTS allowed for an increased sample size per visit for improved statistical power as well as more frequent sampling visits.

Outputs and Impacts

Production of Atlantic salmon is important not just within the Scottish economy, but globally. With growing and diversifying production, aquaculture research must progress to thoroughly understand the health of production species. Gill disease is currently thought to be one of the most important causes of production loss in farmed Atlantic salmon⁵, however diagnosis is often confusing, with multifactorial causes of disease^{6,7}. Dysbiosis of the gill associated bacterial community may play an important role in the complicated aetiology of gill disease, and warrants investigation to inform the important industry that is Scottish aquaculture. Existing research focused mainly on the intestinal microbiome of Atlantic salmon suggests that colonizing bacterial communities can be indicators of the health in fish, acting symbiotically for mutual benefit⁸. This way well be the case for gills also, however first some initial investigation is required.

This project is expected to provide a picture of seasonal changes in the bacterial community of gills, providing essential information regarding the 'normal' microbiome of farmed Atlantic Salmon gill tissue as well as the consequences of gill damage.

Increasing our collective knowledge of predisposing factors for and the consequences of gill disease will aid the aquaculture industry in its prevention and treatment, towards the major goals of reduced antibiotic use and improved fish health and welfare.

Acknowledgements

In addition to the financial contribution of MASTS through the small grant scheme, this project is financially supported by Eastbio through the provision of PhD funding. We would also like to thank Scottish Sea Farms for access to farm locations and the provision of fish, without which this research would not be possible.



1. Willey MJ, Sherwood L, Woolverton CJ. *Prescott's Microbiology*. McGraw-Hill; 2011.
2. Lokesh J, Kiron V. Transition from freshwater to seawater reshapes the skin-associated microbiota of Atlantic salmon. *Nat Sci Reports*. 2015;6(19707). doi:10.1038/srep19707.
3. Gomez D, Sunyer JO, Salinas I. The mucosal immune system of fish: The evolution of tolerating commensals while fighting pathogens. *Fish Shellfish Immunol*. 2013;35:1729-1739. doi:10.1016/j.fsi.2013.09.032.
4. Gray MA, Pratte ZA, Kellogg CA. Comparison of DNA preservation methods for environmental bacterial community samples. *FEMS Microbiol Ecol*. 2013. doi:10.1111/1574-6941.12008.
5. Mitchell SO, Rodger HD. A review of infectious gill disease in marine salmonid fish. *J Fish Dis*. 2011;34(411-432). doi:10.1111/j.1365-2761.2011.01251.x.
6. Kvellestad A, Falk K, Nygaard SMR, Flesja K, Holm. Jan Arne. Atlantic salmon paramyxovirus (ASPV) infection contributes to proliferative gill inflammation (PGI) in seawater-reared *Salmo salar*. *Dis Aquat Organ*. 2005;67:47-54.
7. Steinum T, Kvellestad A, Colquhoun DJ, et al. Microbial and pathological findings in farmed Atlantic salmon *Salmo salar* with proliferative gill inflammation. *Dis Aquat Organ*. 2010. doi:10.3354/dao02266.
8. Merrifield DL, Rodiles A. Mucosal Health in Aquaculture: The fish microbiome and its interactions with mucosal tissues. In: *Mucosal Health in Aquaculture*. ; :273-295. doi:10.1016/B978-0-12-417186-2/00010-8.