Project Report for SG338: A novel experiment to test Atlantic salmon's immuno-competence to the parasite *Anisakis simplex* under increased temperature

Alex Kent, Edinburgh Napier University, Edinburgh, EH11 4BN.

Contact: a.kent@napier.ac.uk

Project Rationale

The emergence of Red Vent Syndrome (RVS) in *Salmo salar* (Atlantic salmon) in 2005, has subsequently been associated with the parasitic nematode *Anisakis simplex* with large numbers found within the urogenital-papilla region, also known as the vent. Although *Anisakis* infestations in fishes are common, the parasite had not previously been reported within the vent region. RVS is only prevalent in *S. salar* and the definitive cause of this condition is still unclear.

The award of this grant aided the run of a pilot study to experimentally infest *S. salar* with live *A. simplex*. The overall aim of this study was to assess both host health and susceptibility to infection of *S. salar* through the following objectives:

i) Determine differences in location preference and migration times of encapsulated and non-encapsulated *A. simplex* within *S. salar*,

ii) Clarify leukocyte abundance within peripheral blood through flow cytometry.

In case of successful infections, a second trial with the inclusion of treatment groups under different temperature regimes was planned.

Methods

The pilot trial was run at the Ellis Aquarium at Marine Scotland – Science (Aberdeen, UK) during August 2016 in compliance with Home Office regulations. A total of 66 Atlantic salmon were allocated to two treatment groups (TG1 and TG2), and one negative control group (NC1) each comprising 22 individuals. Live *Anisakis simplex* was obtained from fresh Atlantic herring (*Clupea harengus L.*) landings from Denholms fisheries Ltd. (Peterhead, Scotland). Prior to administration, nematodes were checked for viability and encapsulation status. 20 encapsulated *A. simplex*, 20 unencapsulated *A. simplex*, and feed pellets were administered to anaesthetised salmon via oral gavage in TG1, TG2 and NC1 respectively. (Fig. 1.) Three fish from all groups were terminated using anesthaesia at 2 and 7 days post infection. Due to low recovery rates of

nematodes from these fish and ethical considerations, all other fish were destructively sampled at 14 days post infection.

Peripheral blood was removed from salmon using a syringe from the caudal vein and kept on ice. Some of the whole blood, was centrifuged using Percoll gradients to remove erythrocytes leaving only peripheral blood leukocytes to be analysed by flow cytometry at the Iain Fraser cytometry Centre (Aberdeen).

Figure 1. Equipment used to perform the oral gavage to administer live *Anisakis simplex* to anaesthetised Atlantic salmon

Outputs

Although all nematodes administered were alive and moving, their potential to migrate through tissues may not be apparent using these factors. Out of a possible 880 *A. simplex* administered, only 3 were recovered. Infestation of *S. salar* with *A. simplex* therefore, was not successful. The flow cytometry performed on both whole blood and peripheral blood leukocytes did not show any intra-specific differences in immune response, which was expected after the fish dissection. Although unsuccessful, the use of experimental challenges within parasite-host interactions remains an important tool. The use of *A. simplex* infested crustaceans, the first intermediate hosts of the nematode, might increase the likelihood of successful infestation in future trials, as it would mirror part of the life-cycle within the marine environment.

Expenditure Summary

The MASTS small research grant award (£490) was used to cover the following expenses for the project:

Project Expense	Cost
Flow Cytometry Training	£250
Analysis of Samples	£240
Total	£490