

**PHYSIOLOGICAL, BIOCHEMICAL AND
HISTOLOGICAL APPROACHES TO EXAMINING
SEA LICE-SALMON INTERACTIONS**

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Sea lice infections are a major concern for salmonid aquaculture operations. Laboratory models have been established to examine interactions between sea lice (*Lepeophtheirus salmonis*) and Atlantic salmon (*Salmo salar*) as part of an effort to develop alternate strategies for sea lice control. We have developed methods to grow sea lice from eggs and culture them to the infective stages in tanks under controlled conditions. Sea lice have been maintained on salmon in our re-circulation system for as long as 7 months. Experiments conducted to date include 4 major areas: 1) production of sea lice eggs and assessment of their viability, including production of maximum numbers of infective copepodids, 2) effects of sea lice on the development of chronic stress and suppression of host salmon defence mechanisms, 3) identification of factors (eg. enzymes) in mucus that change during the course of infection and characterization of their source and roles, and 4) examination of resistance of recovered and naive Atlantic salmon to sea lice. A review of our research to date is presented.

Development of standardized methodology enabling consistent and repeatable infections of salmon with sea lice under laboratory conditions

Salmon for physiological and pathological studies are obtained as smolt from certified hatcheries and smoltified in the Atlantic Veterinary College Aquatic Animal Facility by increasing salinity over a 1 week period to sea water strength (30 ppt) and maintained at 10EC under controlled conditions. A system of obtaining *L. salmonis* eggs from aquaculture sites and culturing them to the infective copepodid stage in the laboratory has been developed. Consequently, sea lice have been maintained on salmon in a re-circulation system for as long as 7 months. The numbers of surviving adult females sea lice and their egg-strings decreased over the experimental period (Mustafa et al., 1999). Eggs from these parasites also lost their hatching ability and ability to develop into infective copepodids. Currently, attempts are being made to increase culture viabilities by examining potential areas of stress and trying various modifications to the culture system.

Effects of sea lice on the development of chronic stress and suppression of host salmon defence mechanisms; identification of factors (eg. enzymes) in mucus that change during the course of infection and characterization of their source and roles

Infection of Atlantic salmon with sea lice in a laboratory setting induces a chronic stress response with a resultant increase in plasma cortisol and a suppression of non-specific defence mechanisms (Bowers et al., in press; Mustafa et al., 2000). The fish were not stressed during the larval stages of lice development, but plasma cortisol levels were elevated and non-specific immune responses depressed when the sea lice became mobile pre-adults and adults. The stress response could be measured well before any physical damage, such as skin lesions, was observed.

Biochemical changes in the mucus were examined since the mucus is the primary site of interaction between salmon and *L. salmonis*. Mucus protease activity was observed to increase over the course of a sea lice infection in Atlantic salmon (Ross et al., in press). Low molecular weight, 17 to 22 kDa, trypsin-like proteases were of particular interest and were determined to be derived from the sea lice (Firth et al., submitted). A cDNA library has been constructed from whole pre-adult *L. salmonis* and several clones have been partially sequenced to yield an expressed sequence tag (EST) library. Comparisons of ESTs with sequence databases resulted in 56% of the ESTs having similarity to known genes from other organisms. Chymotrypsin and trypsin genes have been identified and a number of full-length trypsin clones have been obtained (Johnson et al., submitted). Comparison of the sequence of the trypsin found in the salmon mucus with the above genes will be carried out in the future. It is proposed that the trypsin is being secreted by the sea lice into the mucus to either aid in feeding and/or to help the sea lice in avoiding the host immune system by digesting humoral immune

factors.

Changes in protein glycosylation state were observed in plasma samples from infected Atlantic salmon. Two plasma proteins were identified in 2-D gel electrophoresis as having an increased number of sialic acid side chains in infected fish. Sialic acid moieties on glycoproteins are important in the regulation of protein and cell turnover and changes in the sialylation state of these plasma proteins may be an important trigger or indicator of the health status of the fish. Identification of the sialylated proteins in plasma is underway.

At present we are comparing the responses of rainbow trout, Atlantic and coho salmon to infections with *L. salmonis*. It is known that coho salmon, in comparison to Atlantic salmon, are particularly resistant to sea lice (Johnson and Albright, 1992), but the mechanism for this resistance is not known. Rainbow trout are also being included due to their increasing commercial potential (partly because they are relatively resistant to the ISA virus). The development of chronic stress, suppression of host defence mechanisms, and changes in host mucus and serum biochemistry are being studied. Results from this study are being presented at this Congress (Fast et al., this volume).

Rainbow trout were just as susceptible to sea lice infections as Atlantic salmon and exhibited a similar stress and immune response. Fish with *L. salmonis* infections had a significant suppression in macrophage respiratory burst activity and phagocytic capacity once all sea lice had reached the pre-adult and adult stages. Macrophage function remained suppressed for an additional 60 days. Trout were exposed to spores of the gill microsporidian pathogen *Loma salmonae* after sea lice reached the adult stage. Gill xenoma counts revealed that sea lice infected fish had 2.5 times more xenomas than did non-infected fish. This study supported the hypothesis that sea lice infections decrease non-specific immune responses such that fish have increased susceptibility to subsequent infections (Mustafa et al., submitted).

Morphological studies were carried out based upon a novel technique for preserving carbohydrate complexes for light and electron microscopy which we developed at the Atlantic Veterinary College (Horne and Sims, 1998). There is a thinning and >washing out= of mucus on adult Atlantic salmon when they have a significant burden of sea lice. This may lead to increased osmoregulatory stress, which is observed with sea lice challenge. A detailed study of the light and electron microscopy of mucus distribution on Atlantic salmon is currently being analyzed: Five Atlantic salmon were carefully sampled at 13 sites each for epidermis and its mucous coat. The resulting 65

sites are being compared for thickness of skin and mucus, abundance of mucus-producing cells, and the ultrastructural features of the mucus.

Resistance of recovered and naïve Atlantic salmon to sea lice

During the establishment of a reproducible sea lice infestation model, it was observed that Atlantic salmon experience decreased numbers of sea lice following an initial infection. Based on this observation, an experiment has been designed to investigate the response of Atlantic salmon to sea lice by determining the degree of parasite rejection and susceptibility of recovered fish to subsequent infections. Results to date show that salmon have significantly lower intensities of infection compared to controls during the subsequent infections, indicating that salmon may develop some protective immunity in reinfested fish.

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