

Tuesday September 4th – Archibald / Campbell
Sea Lice 1 & 2
Moderator – Mark Fast (Atlantic Veterinary College / UPEI)

9:30 AM	Sea Lice 1	<u>Nowak</u> - Amoebic Gill Disease – An Emerging Disease in Mariculture?
9:45 AM		<u>Jorgensen</u> - Interactions Between the Skin Parasite <i>Ichthyophthirius multifiliis</i> and a Fish Host <i>Danio rerio</i>
10:00 AM		<u>Dalvin</u> - Morphological and Immunological Changes in Rainbow Trout (<i>Oncorhynchus mykiss</i>) Skin in Response to Salmon Louse (<i>Lepeophtheirus salmonis</i>) Infection
10:15 AM		<u>Overgard</u> - Do <i>Lepeophtheirus salmonis</i> Rhabdoviruses (LSRVs) Dampen the Local Skin Inflammatory Response in Atlantic Salmon <i>Salmo salar</i> ?
10:30 AM		Refreshments
10:45 AM	Sea Lice 2	<u>Poley</u> - Excretory and Secretory Factors of the Salmon Louse: Genomic Characterization, Effects on Feeding, and Impacts on Host Immunity
11:00 AM		<u>Carvalho</u> - Functional Feeds Impact Molecular Responses of Atlantic Salmon (<i>Salmo salar</i>) to Co-Infection with <i>Lepeophtheirus salmonis</i> and Infectious Salmon Anemia Virus
11:15 AM		<u>Skugor</u> - The Effect of the <i>Caligus rogercresseyi</i> Parasite Burden on the Progression of Co-Infection With the Intracellular Bacterium <i>Piscirickettsia salmonis</i>
11:30 AM		<u>Kvamme</u> - Salmon Lice as an Environmental Indicator for Management of Norwegian Salmon Farming – An Overview
11:45 AM		<u>Are Hamre</u> - Development of the Salmon Louse <i>Lepeophtheirus salmonis</i> Parasitic Stages in Temperatures Ranging From 3°C to 24°C.



8th International Symposium on Aquatic Animal Health

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



Amoebic Gill Disease – An Emerging Disease in Mariculture ?

Barbara F. Nowak

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Amoebic gill disease (AGD) was first reported 30 years ago from two salmonid species, Atlantic salmon in Tasmania, Australia and coho salmon in Washington State USA, farmed in marine environment. The causative organism, *Neoparamoeba perurans*, was described more recently and Koch's postulates have been fulfilled even later due to initial difficulties with culturing the causative agent *Neoparamoeba perurans*. Clinical AGD has been observed in cultured fish in fourteen countries across six continents. Atlantic salmon is the main commercial species affected, but the disease has been also seen in cleaner fish and other fish species farmed in the marine environment. While immune response of salmon affected by AGD has been studied, there is no vaccine available. This presentation reviews our current knowledge of this disease, in particular its emergence in mariculture.

This presentation summarises our current knowledge about the known host range, characteristics of the parasite, case definition and host-parasite relationship. While there has been a lot of progress in our understanding of this disease some fundamental questions still remain unanswered. The emergence of AGD appears to coincide with high sea surface temperature and with the intensification of mariculture. In contrast to the situation in Tasmania, in Europe AGD is one of many gill conditions affecting Atlantic salmon farmed in sea cages. This means that a new approach is needed for holistic management of gill health in farmed Atlantic salmon.

Conference Session Designation:

(Ectoparasites / Sea Lice)

Presentation Format:

(Oral)



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Interactions Between the Skin Parasite *Ichthyophthirius Multifiliis* and a Fish Host *Danio Rerio*

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The parasite *Ichthyophthirius multifiliis* is the causative agent of white spot disease and a major problem for the aquaculture and ornamental fish industry. It infects skin and gills of freshwater fish and cause high mortality during outbreaks. The zebrafish has become an important model to study a wide spectrum of vertebrate biological processes and has proven especially valuable within developmental biology and genetics. I have used the zebrafish as a model to study immunological responses during infections with *Ichthyophthirius multifiliis*. Using adult transgenic zebrafish with Green Fluorescent Protein (GFP)-tagged neutrophils a setup was for the first time developed completely immobilizing the tail fin of the live fish. Utilising this setup, the behaviour of neutrophils during a parasite assault was examined with a confocal microscope. This model offers an unprecedented real time view into the interactions between the parasites and the neutrophils at the single cell level. The neutrophil population dynamics were also investigated and within the first day of the parasite infection, the number of neutrophils in the tail fin increased four-fold. However, during the following two days, the number of neutrophils declined even though the size of the parasites increased and the damage to the fish intensified. Video-recordings of the interface between the parasites and the neutrophils at the single cell level revealed how the parasites have a way of evading and fighting the immune system of the host. Using zebrafish as a tool to investigate cellular immunity has expanded our knowledge on this host/parasite relationship and with the many accessible transgenic lines there is potential for new discoveries.

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Morphological and Immunological Changes in Rainbow Trout (*Oncorhynchus Mykiss*) Skin in Response to Salmon Louse (*Lepeophtheirus Salmonis*) Infection

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Infestations with the salmon louse (*Lepeophtheirus salmonis*) is a challenge in North Atlantic salmonid aquaculture industry, where both Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) are farmed. The salmon louse damages the skin of the host by feeding on skin and mucus. In addition to these mechanical damages and resulting host responses, there is also evidence, mainly from Atlantic salmon, indicating that salmon lice can reduce the immune response of the fish. The mechanisms and timing of such a process is however yet to be elucidated. Here we have infected rainbow trout with salmon lice and followed development from attached copepodids and subsequent stages leading to mobile adult lice. The objective of this study was: 1) to describe the morphological changes in fish skin at infestation sites, 2) to compare transcriptomic changes in fish skin in uninfected and infected individuals (both at and away from the site of attachment) and 3) to localize immune cells at the infestation site using immunohistochemistry. Our results indicate large morphological changes in the skin of the fish during maturation of the sea lice as well as associated transcriptomic changes and occurrence of immune cells in the skin. The outcome of this work will enhance our understanding of the interaction between salmon louse and salmonid hosts.

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Do *Lepeophtheirus Salmonis* Rhabdoviruses (Lsrvs) Dampen the Local Skin Inflammatory Response in Atlantic Salmon *Salmo Salar* ?

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Rhabdoviruses are a family of enveloped negative-sense single-stranded RNA virus infecting a variety of hosts. Recently, it has been shown that the salmon louse (*Lepeophtheirus salmonis*) is commonly infected by one or two vertically transmitted *L. salmonis* rhabdoviruses (LsRVs). Their prevalence was close to 100 % along the Norwegian coast, and it is challenging to obtain material for studies on host impact and infection routes due to the present lack of suitable cell lines to propagate these viruses. Hence, virus free louse strains were established from virus infected lice carrying both LsRVs by treating them with N protein dsRNA twice during development. We could then analyze how these viruses transmit among lice, whether the viruses affect louse biology and study the interaction between the lice and the salmon host. The viruses had limited effect on louse survival, developmental rate and fecundity. The LsRVs were present in the louse salivary glands, and interestingly, LsRV free lice induced a higher local skin expression of IL1 β and IL8 than the LsRV infected lice. The inflammatory response is important for louse clearance, and the present results suggest that the LsRVs can be beneficial for the louse by dampening inflammation. However; it is not known whether this is a direct modulatory effect of secreted virions, or if virus replication is altering the level of louse salivary gland proteins.

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Excretory and Secretory Factors of the Salmon Louse: Genomic Characterization, Effects on Feeding, and Impacts on Host Immunity

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Virulence is a property of the host-parasite (HP) relationship that can be measured by the consequent effects on host fitness and parasite success. With respect to the salmon louse the HP relationship involves the feeding response by the louse and the subsequent immune response by the salmon. In resistant species (e.g., coho salmon) the host response dominates, while in susceptible species (e.g., Atlantic salmon) the feeding/parasitic response generally suppresses the host response. This is achieved by producing immunosuppressive molecules that directly affect the ability of the hosts immune system to recognize the attached parasite while facilitating nutrient acquisition (i.e., virulence factors, VFs). Here, we have employed a multi-pronged approach to describe a group of putative VFs in the salmon louse. A cluster of VF genes associated with feeding were identified using correlation analysis of gene expression across six different studies. These VFs were annotated by predicting ORFs, adding accessions from UniProt, GenBank, and LiceBase, applying conserved protein domain layouts (ranges + accessions), determining the presence of signal peptides, and determining orthologs to known virulence factors in *Ixodes* spp. Using LC-MS/MS we identified 18 virulence-associated proteins in the secretions of *L. salmonis*, 9 of which were concordant with putative VFs in the transcriptome. We then characterized the pattern of expression of a select group of VFs (*hypodermin-B*, *carboxypeptidase B*, *cathepsin L*, *legumain*, and *neprilysin*) during feeding on resistant or susceptible hosts by qPCR. *In situ* localization and RNA interference experiments indicate these molecules may be important mediators of the HP interaction.

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Functional Feeds Impact Molecular Responses of Atlantic Salmon (*Salmo Salar*) to Co Infection with *Lepeophtheirus Salmonis* and Infectious Salmon Anemia Virus.

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Infectious salmon anemia virus (ISAv) is a highly contagious aquatic pathogen that causes considerable economic losses in farmed Atlantic salmon (*Salmo salar*). Sea lice (*Lepeophtheirus salmonis*) infestation also represents a major challenge in the salmonid farming industry and various treatment protocols have been put in place to manage and prevent sea lice outbreaks at farm sites. Immunostimulant additives to functional diets aid in strengthening the host immune response and are becoming an integral part of disease management practices in aquaculture. This study aims to characterize biomarkers and molecular responses in Atlantic salmon during a co- infection with *L. salmonis* and ISAv during administration of four blinded functional feed diets. A highly virulent ISAv strain was IP injected into donor fish which were cohoused (ca. 10-15% of tank density) 10 days after injection with experimental tanks (4 tanks per feed group) to achieve peak shedding rates at time of stocking. By 46 days post infection, mean lice abundance ranged from 6.1 – 9.2 lice per fish, with two diets showing significantly lower sea lice infections (ca. 27-29% reduction). However, under co-infection with ISAv, the diets with lowest louse abundance had the poorest survival, 37-46%, and highest viral load. Host transcriptional responses to infection were assessed in multiple tissues and across low, medium and high lice counts and viral loads. Biomarkers associated with functional feed background and infection level were identified and their significance will be discussed.

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The Effect of the *Caligus Rogerresseyi* Parasite Burden on the Progression of Co- Infection with the Intracellular Bacterium *Piscirickettsia Salmonis*

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In salmonid aquaculture worldwide, co-infection by multiple species of parasites and microbial pathogens is the norm rather than the exception. Yet, not much is known about the burden of coinfection on fish health and the differences between infections by single pathogen species and coinfection. The aims of the study were to identify gene expression profiles specific for the co-infection of ectoparasitic *Caligus rogerresseyi* and intracellular bacterium *Piscirickettsia salmonis*, the causative agent of SRS, and delineate the effect of the parasite burden on the progression and severity of the ensuing condition.

Atlantic salmon were infected with 15 (Low) and 50 (High) number of *C. rogerresseyi* infective copepodids, and half of the fish from each group was I.P. injected with the LF89 strain of *P. salmonis* 16 days post-infection (dpi) with *C. rogerresseyi*. In addition, the study included non-infected fish and one group infected only with *P. salmonis*.

Lice numbers were counted at three time points (13, 28 and 36 dpi), and mortalities were followed until 36 dpi. The average number of lice was 11 in the Low and 31 in the High group at 13 dpi while the numbers rose to 81 and 136 by 36 dpi as a consequence of lice becoming sexually mature and reproducing in our tank system. Mortalities started at day 11 post *P. salmonis* injections, and the group co-infected with 50 copepodids and bacterium showed highest number of dead fish by 36 dpi.

Profiling of plasma revealed the effect of the treatments on urea and iron levels, with most pronounced differences seen in the High co-infected group. Results from microarray profiling of head kidney samples taken prior to lice infection, 13 dpi and at two time points during the co-infection (28 and 36 dpi) will be discussed.

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Salmon Lice as an Environmental Indicator for Management of Norwegian Salmon Farming – An Overview

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Norway is the largest producer of farmed salmon in the world, with an annual production of more than 1.2 million tons. The growth of the salmon farming in Norway may continue, but only if the environmental impact of the industry can be considered sustainable. At present, the mortality salmon lice inflict on wild salmonids are the only indicator chosen to indicate this impact. As such, the salmon louse may have a substantial impact on the billion-dollar salmon farming industry in Norway. Recently a novel management system for the fish farming industry was ratified, dividing the Norwegian coast into 13 production areas where high levels of sea lice would induce reduction of production in the entire area, whereas low lice levels would induce growth in production. This system relies on tools and results from the Institute of Marine Research (IMR), varying from field surveillance of sea lice infestations on wild salmonids and salmon louse biology to modelling of salmon louse dispersal and infestation levels.

Traditionally, salmon lice on wild salmonid populations has been monitored by a large scale surveillance program along the coast of Norway. This has provided data on salmon louse levels on wild salmonids in areas with and without salmon farming, effect of fallowing and protected areas, and salmon louse induced mortality on wild salmonids. However, as it is not possible to survey the entire Norwegian coast, a model system describing the dispersal of salmon louse has been developed. This modelling system has been used to divide the coast into semi-isolated production areas, describe the dispersal of salmon louse from all fish farms, an operational near real time dispersal model of louse, create salmon migration models as well as infestation models. Here a brief overview of the Norwegian management system will be presented together with results originating from IMR during the development of the system.

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Development of the Salmon Louse *Lepeophtheirus salmonis* Parasitic Stages in Temperatures Ranging from 3° C to 24° C.

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The development rate of the salmon louse *Lepeophtheirus salmonis* is greatly influenced by sea water temperature. This study describes how the growth rate of *L. salmonis* change with temperature and identify the extreme high and low temperatures at which development to adult is compromised. Atlantic salmon was infected with copepodids and development was monitored in 8 temperature groups spanning from 3°C-24°C until the lice were adult. Development was severely compromised at 3°C and 24°C, while the lice developed normally without severe mortality in the range 6°C-21°C. At 6 °C most female lice had become adults at 67 days post infection, or c. 400 daydegrees, but at 21°C development was significantly quicker and most females were adults after 14 days, at only c. 300 daydegrees. The lice developed almost five times faster at 21°C than at 6°C and required c. 25% fewer daydegrees to become adult. Body size also decreased significantly while temperature increased. After infection the lice grow through five stages before reaching the adult stage: the copepodid stage, two chalimus stages and two preadult stages, all of which, with a few exceptions, appeared to last approximately equally long for males and females respectively. Thus, a simple model describing the mean daily growth rate (stages pr. day) as a function of temperature was made for each sex. The relationship between mean daily growth rate and temperature was best described by a second order polynomial. The term relative age is introduced and used to describe the pattern of development in terms of percent of total development time to adult, and applied to calculate the timing of developmental events as a function of temperature.

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