Wednesday September 5th – Langevin / Cartier
Virology 3
Moderator - Mark Polinski (Dept of Fisheries & Oceans Canada)

3:15 PM	Virology 3	Polinski - Piscine Orthoreovirus Infection Dynamics and Host Interactions Depend on the Strain of Atlantic Salmon Infected
3:30 PM		Wessel - PRV1: Virulence Differences in Atlantic Salmon
3:45 PM		Markussen - Analyses of Genome Sequences and Protein Structure of Strains of Piscine Orthoreovirus (PRV1) with Putative Different Virulence in Atlantic Salmon (*Salmo salar*)
4:00 PM		Siah - Genetic Diversity of Piscine Orthoreovirus 1 Across Geographic and Host Ranges: A Phylogenomic and Historical Analysis
4:15 PM		Gagne - A Survey of Piscine Reovirus (PRV) in Atlantic Canada
4:30 pm		**Vendramin - Piscine Orthoreovirus-3 (PRV-3), a New Pathogen for Farmed Rainbow Trout**
4:45 PM		Di Cicco - The Same Strain of Piscine Orthoreovirus (PRV-1) is Involved with the Development of Different, but Related, Diseases in Atlantic and Pacific Salmon in British Columbia
5:00 PM		**Zhang - Does Piscine Orthoreovirus (PRV) Harm the Respiratory Capability of Infected Atlantic Salmon (*Salmo* salar) Smolts? An Assessment that uses Physiology to Characterize Phenotype**

Piscine Orthoreovirus Infection Dynamics and Host Interactions Depend on The Strain of Atlantic Salmon Infected

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Piscine orthoreovirus (PRV) is the most recently identified member of the orthoreovirus genus. It has a pervasive and global distribution in salmonid species that encompasses both wild and farmed populations. Like many reoviruses, the virulence of PRV appears to be generally low; however, in an aquaculture setting this virus can be the aetiology of disease. Specifically, a variant known as PRV-1 from Norway has been demonstrated by *in vivo* experimentation to cause heart and skeletal muscle inflammation (HSMI) in Mowi strain Atlantic salmon. This disease is currently one of the most impactful transmissible diseases affecting Atlantic salmon production in Norway. Interestingly, experimental challenge studies with PRV from Pacific Canada (a subtype of PRV-1) have routinely caused extreme viremia in Pacific adapted Mowi-McConnell Atlantic salmon but without noteworthy pathology or other manifestations. The disparity for disease outcome between these two regions appears to be linked with host recognition of the virus, and could be a result of virus and/or host specific factors. Here we present our current research into determining if host-specific factors may contribute to the development of PRV associated disease such as HSMI. This work identified that identical challenge of two discrete strains of Atlantic salmon from Canada yielded strikingly different PRV infection dynamics and host responsiveness, suggesting that PRV-associated disease is at least in part conditional on genotypic factors specific to the host organism.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

PRV1: Virulence differences in Atlantic salmon

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Piscine orthoreovirus 1 (PRV1) is a ubiquitous virus in farmed Atlantic salmon (*Salmo salar*) in Norway, and has been shown to be the etiological agent of Heart and skeletal muscle inflammation (HSMI). HSMI is a prevalent disease in Norway; however, PRV1 is also present in apparently healthy Norwegian salmon. Furthermore, in BC, Canada, the virus is prevalent but the presence of HSMI is less evident. Earlier experimental transmission studies in BC using PRV1-containing material showed that PRV1 from BC was transmissible to but failed to induce HSMI in Atlantic salmon. It is apparent that the development of disease is complex, involving viral, host and environment factors. Studies that are able to separate out the impact of the different factors are highly warranted. Recently, we were able to purify PRV1 from blood which enables more standardized studies comparing putative virulence differences between virus strains. In the present study, we compared three strains of PRV1. This included two Norwegian strains; one originating from a severe HSMI outbreak in 2012 while the second strain was revived from archived material dating back to 1988 approximately 10 years before HSMI appeared in farmed salmon in Norway. In addition, a BC strain not associated with HSMI was included. The three different strains were propagated in Atlantic salmon and heparinized blood was collected at peak of infection and used as source for PRV1 purification. Finally, the purified virus was inspected by electron microscopy to confirm presence of virus particles and the batch was quantified by absolute quantification RT-PCR. Atlantic salmon were challenged by ip injection using equal amounts of the three virus strains. Results from the study will be presented, including analysis of viral load, hemoglobin concentration and histopathological lesions.

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)

Analyses of Genome Sequences and Protein Structure of Strains of *Piscine Orthoreovirus* (Prv1) with Putative Different Virulence in Atlantic Salmon (*Salmo Salar*)

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Piscine orthoreovirus 1 (PRV1) causes heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon (*Salmo salar*) in Norway. The virus is widespread, especially in the marine production phase, but is increasingly also found in the fresh water phase. PRV1 is present in farmed populations with or without a history of HSMI. The virus is also present in migrating wild Atlantic salmon, but at a lower prevalence.

PRV1 replicates in various cell types, and the erythrocyte is the major target cell early in the infection cycle, but the virus also replicates in various cell types including myocytes and macrophage-like cells, including melanomacrophages. The ability of a virus to cause disease is directly related to its target cells.

In lack of susceptible cell lines or functional reverse genetics, virulence studies are performed by experimental infection trials in the target species. In this study, the genome sequences and protein structures of strains of PRV1 with putative different virulence in Atlantic salmon, including a revived Norwegian isolate from 1988, i.e. from more than ten years before HSMI was observed, were compared. Additional information of the strains, like HSMI or no disease record in the population were obtained. The results indicate that the historical Norwegian PRV1 strain represents a precursor form, for several genomic segments, for current virulent strains causing HSMI in Norway, and for the BC strain. Available full genome data suggest higher genome diversity among Norwegian PRV strains, particularly for the S1 genomic segment (σ3 protein). Substitutions in BC strains for this segment have been found in Norwegian wild salmon. The segmented genome of PRV makes gene segment reassortment a likely evolutionary mechanism. We cannot yet firmly link single gene segments or amino acid motifs to virulence. However, the high number of amino acid substitutions make the two gene segments encoding σ3, p13 and µ1 prime candidates. An association between genomic segment linkage and virulence should not be ruled out.

Conference Session Designation: (Virology / PRV)
Presentation Format: **(** Oral)

Genetic Diversity of Piscine Orthoreovirus 1 Across Geographic and Host Ranges: A Phylogenomic and Historical Analysis

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Piscine orthoreovirus (PRV) is a double-stranded, non-enveloped RNA virus which is found globally in farmed and wild salmonids. Currently, there are three genogroups that have been identified for PRV: PRV1 which infects Atlantic Salmon, Pacific salmon, Trout and some non-salmonid finfish; PRV2 which infects Coho Salmon in Japan; and PRV3 which infects Rainbow and Brown Trout in Europe. Each genetic subtype of PRV has a 10 segmented genome consisting of 3 large (L1, L2, L3), 3 medium (M1, M2, M3) and 4 small (S1, S2, S3, S4) segments. Due to its sequence variability, segment S1 has been commonly used to infer phylogenetic relationships and has demonstrated two main sub-genotypes within PRV1: PRV1a and 1b. However, although highly variable, segment S1 alone does not always provide enough genetic variation to discriminate between closely related PRV genotypes.

We have now begun using full genome sequencing to determine the genetic diversity of PRV1 across broad geographic and host ranges. Our goal is to develop information and tools to better study the epidemiology of PRV1 in the North Pacific. To this end we have sequenced PRV1 genomes from different species of salmonids collected in Eastern and Western North America and compared these using a phylo-dynamic analysis alongside publically available PRV1 genome sequences from Norway, Chile and Canada.

Our preliminary phylogenetic analysis of concatenated genome sequences shows that PRV1 from the West Coast of North America clusters separately with high bootstrap credibility from PRV1 from Eastern Canada, Norway and Chile regardless of host species. Within this cluster, a monophyletic group was suggested for PRV1 from farmed Atlantic Salmon. Interestingly, PRV1 from Eastern Canada which forms a separate monophyletic group clusters separately from PRV1 from Chile and Norway. Phylo-dynamic analysis of these data along with historical records of fish movements within and between countries will inform hypotheses of how PRV1 spread among regions.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

A Survey of Piscine Reovirus (PRV) in Atlantic Canada

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The piscine reovirus (PRV) is a recently identified virus (Palacios et al 2010) that has been linked to Heart and Skeletal Muscle Inflammation (HSMI) in Atlantic salmon (Wessel et al 2017). PRV is often detected without symptoms of HSMI (Garver et al 2016) and viral culture cannot be done. Although the host range of this virus appears primarily restricted to salmonids, it has been occasionally detected in a few non-salmonid species such as capelin *Mallotus villosus*, Atlantic horse mackerel *Trachurus trachurus*, Atlantic herring *Clupea harengus*, and great silver smelt *Argentina silus* (Wiik-Nielsen et al 2012).

In Norway, high loads of PRV have been suggested as a requirement for the development of HSMI in Atlantic salmon. In western North America (N-A), PRV is detected in both wild Pacific salmon and farmed Atlantic salmon, with a high prevalence in farmed fish (Marty et al 2015). In N-A, observation of lesions typical of HSMI by histology has only recently been made in one BC farm infected with PRV, with lesions more pronounced in individuals with higher load of PRV. No typical clinical signs associated to HSMI (i.e. change in behaviour, mortalities, etc.) were observed in parallel (DiCicco et al 2017).

Determination of the PRV situation on the Eastern coast of N-A is ongoing, as part of collaboration with the industry initiated in 2016. Work completed in the past 2 years include a PRV survey in wild fish, hatcheries and sea farms. Prevalence, sequencing of PRV and phylogeny, and *in vivo* challenge results will be presented.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Piscine Orthoreovirus-3 (PRV-3), a New Pathogen for Farmed Rainbow Trout

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Piscine orthoreovirus – PRV have emerged as relevant pathogens for salmonid aquaculture worldwide. Currently three different subtypes with specific host are described for this viral species.

PRV-1 is the causative agent of heart and skeletal muscle inflammation (HSMI) in Atlantic salmon and and is associated with jaundice syndrome in farmed Chinook salmon

PRV-2 causes erythrocytic inclusion body syndrome (EIBS) in Coho salmon.

- PRV-3 causes heart pathology resembling HSMI in rainbow trout.

PRV-3 was firstly discovered in 2013 in Norway during disease outbreaks affecting farmed rainbow trout. A first series of experimental trials conducted in a joint project involving DTU, NVI and NMBU were performed to assess its pathogenicity and pathogenesis in *O. mykiss* and *S. salar*. The Norwegian PRV-3 isolate has been further characterized analyzing its genome and antigenic features. An experimental infection study with purified virus demonstrated that PRV-3 infects rainbow trout and induces pathological heart lesions similar to HSMI, and thus fulfill Koch's postulates. Furthermore, the infection upregulates IFN production, and induces specific antibody response in later phases. In late 2017 the presence of PRV-3 was also reported in different countries in Europe including Scotland, Germany, France, Italy and Denmark. Interestingly, these viral isolates appear to be genetically distinct from the Norwegian isolate leading to proposition of two separate clades within PRV-3 viral type (PRV-3a and PRV-3b).

In Denmark the virus has been associated with severe disease outbreaks in recirculating aquaculture systems. Clinical signs are represented by reduced appetite followed by uncoordinated swimming behavior and increased mortality; necropsy findings include severe anemia and ascites. Such outbreaks are complex disease cases where different bacterial (including *Flavobacterium psychrophilum* and *Renibacterium salmoninarum*) and viral pathogens (IPNV) are present at the farm. Notably PRV-3 load increases in the target organs (heart, spleen) before the clinical disease appear, whereas the other pathogens are not detected in a systematic pattern. In 2018 in cooperation with the Danish Aquaculture industry a project mapping the prevalence of PRV-3 in the country, investigating its virulence and the risk for vertical transmission, was funded and initiated. An overview of the results will be presented.

Conference Session Designation: (Virology - PRV)
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Student Presentation: **(** Yes)

The Same Strain of *Piscine Orthoreovirus* (PRV-1) is Involved with the Development of Different, but Related, Diseases in Atlantic and Pacific Salmon in British Columbia

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Piscine orthoreovirus Strain PRV-1 is the causative agent of heart and skeletal muscle inflammation (HSMI) in Atlantic salmon (*Salmo salar*). Given its high prevalence in net pen salmon, debate has arisen on whether PRV poses a risk to migratory salmon, especially in British Columbia (BC) where commercially important wild Pacific salmon are in decline. Various strains of PRV have been associated with diseases in Pacific salmon, including erythrocytic inclusion body syndrome (EIBS), HSMI-like disease, and jaundice/anemia in Japan, Norway, Chile and Canada. We examine the developmental pathway of HSMI and jaundice/anemia associated with PRV-1 in farmed Atlantic and Chinook (*Oncorhynchus tshawytscha*) salmon in BC, respectively. In situ hybridization localized PRV-1 within developing lesions in both diseases. The two diseases showed dissimilar pathological pathways, with inflammatory lesions in heart and skeletal muscle in Atlantic salmon, and degenerative-necrotic lesions in kidney and liver in Chinook salmon, plausibly explained by differences in PRV load tolerance in red blood cells. Viral genome sequencing revealed no consistent differences in PRV-1 variants intimately involved in the development of both diseases, suggesting that migratory Chinook salmon may be at more than a minimal risk of disease from exposure to the high levels of PRV occurring on salmon farms.

Conference session designation: (Virology)
Presentation format: (Oral)

Does Piscine Orthoreovirus (PRV) Harm the Respiratory Capability of Infected Atlantic Salmon (*Salmo Salar*) Smolts? An Assessment that uses Physiology to Characterize Phenotype.

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Piscine orthoreovirus (PRV) replicates in red blood cells (RBC) and is the etiologic agent of the heart and skeletal muscle inflammation (HSMI) of Atlantic salmon. Consequently, PRV clearly has a potential to impact cardiac pumping, oxygen binding or the oxygen consumption in skeletal muscle, each of which independently or collectively could result in impaired oxygen uptake in normoxia and escalated harm in hypoxia. Hence, we applied an integrated respiratory assessment paradigm (IRAP) to test if experimentally induced infection with a Pacific strain of PRV would physiologically compromise the *in vivo* respiratory capability of Atlantic salmon smolts domesticated on the Pacific coast (Mowi-McConnell) over a 21-week period post-injection. The IRAP assessments were time-matched with assessments of cardiac histopathology, oxygen carrying capacity of RBC and viral load (as determined by qPCR) to provide a broad assessment of harm, which were all intended to coincide with early infection (week one), peak viral load (week 3), highest prevalence of physiological symptoms (week 9), late persistence (week 18), and respiratory response following a hypoxic challenge (week 21). IRAP assessments revealed that saline-injected control fish did change their respiratory physiology over time. However, time-matched comparison of the blood-injected control (BC) fish and the PRV-injected fish revealed no appreciable and sustained differences in respiratory capability over the first 18 weeks of PRV infection, despite the generation of substantial viremia and a significantly elevated presence of minor heart inflammation in PRV-infected fish. Normal oxygen affinity and maximal oxygen carrying capacity of RBC were also maintained. Thus, fish that were infected with high loads of PRV maintained their respiratory performance in normoxia and hypoxia over the first 18 weeks of infection. At week 18, the fish administered a hypoxia challenge (O_2 reduced to 15 % sat.) were physiologically reassessed at week 21. While PRV infected fish had a 12% lower absolute aerobic scope (AAS) at week 21 than BC fish, this was in part because BC fish increased their AAS by 7% post hypoxia. Therefore, PRV infection and a high PRV load alone had a negligible effect on respiratory capability of hosts in normoxia and hypoxia, but in conjunction with hypoxia reduced its aerobic phenotypic plasticity. In summary, despite the presence of severe PRV viremia and mild heart inflammation, functional harm at RBC, anatomical harm at heart and physiological harm at whole-animal have been demonstrated to be absent in Pacific adapted Mowi-McConnell Atlantic salmon. Consequently, these results question the suitability of using PRV load as the sole predictor of harm to PRV-infected Atlantic salmon.

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