Tuesday Sepember 6th – Langeve / Cartier
eDNA / Metagenomics
Moderator – Sascha Hallett (Oregon State University)

9:30 AM	eDNA / Metagenomics	**Bernhardt - Development of a Non-Invasive Method for Concentration and Detection of Salmonid Alphavirus From Seawater**
9:45 AM		**Barry - Rapid DNA Based Detection of Whirling Disease Causing Parasites From Environmental Samples**
10:00 AM		**Shea - Assessing Environmental Microparasites in Relation to Atlantic Salmon Farms in BC**
10:15 AM		Soto - Development of Multiplex Quantitative PCR Assays for the Detection of Invasive Species and Aquatic Animal Pathogens

Development of a Non-Invasive Method for Concentration and Detection of Salmonid Alphavirus from seawater

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Commercial Atlantic salmon (*Salmo salar L.)* farming is a vital industry in the coastal areas of Norway. It is the second most important export product and provide high-value nutrients that represent a valuable part of a healthy diet to humans. Despite these contributions, significant losses of fish during seawater phase production persist. The causes of these high mortalities have yet to be fully unravelled. However, virus-related diseases are thought to constitute the most important causes. Virus transmission and disease control strategies are thus important issues in Atlantic salmon health management. Since Salmonid Alphavirus (SAV) has the ability to spread via horizontal transmission and seawater represent the natural environment of Atlantic salmon, the seawater from the fish environment could be used for evaluation of SAV transmission. Currently, SAV transmission in Atlantic salmon farms is largely surveyed using traditional manual methods. These methods are selective, invasive and are limited to *in vivo* sampling of live fish for identification of the virus. Development of a reproducible non-invasive method to confirm the presence of the virus in fish environment, will serve as an early warning system and may have significant impact on Atlantic salmon health management. The aim of this study is to establish a non-invasive method for detection of SAV in seawater by sampling from the fish environment for the purpose of virus detection without sacrificing live fish, thus satisfying the 3R requirement relating to experimental animals: replace, reduce and refine. The method is based on concentration of SAV in seawater through filtration and adsorption to charged membranes, before detection and quantification of the virus with reverse transcriptase quantitative PCR (RT-qPCR). First external clinical signs were observed on day 12 post cohabitation (lethargic fish and findings of faecal casts in high dosage tank). Pathological signs associated with SAV-PD were observed from day 16 post cohabitation in fish from high dosage tank (enlarged spleen, petechial bleeding on pylorus and ascites). Samples of seawater and Atlantic salmon tissues from both high- and low dosage tanks in the SAV-PD cohabitant challenge trial were analysed. Mid kidney and gill samples from high- and low dosage SAV-PD tanks were positive by PCR on day 20-25 post cohabitation. In addition, concentrated seawater from high and low dosage SAV-PD tanks were positive by PCR on day 20-25 post cohabitation. The correlations of the results from tissue and seawater samples and clinical signs provide evidence that suggest that filtration of seawater can be applied as an early warning system for the presence of virus in Atlantic salmon farms.

Conference Session Designation: **(** Virology or Aquatic Epidemiology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Rapid DNA Based Detection of Whirling Disease Causing Parasites from Environmental Samples

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Whirling disease is a disease of fish caused by an invasive myxosporean parasite, *Myxobolus cerebralis*. It was first detected in Canada in Johnson Lake in Banff National Park, Alberta in August 2016, and little is known about the transmission of this parasite in Canada. It affects salmonid fish and has potential to impact the recreation sport fishing industry due to increased mortality in juvenile fish and waterbody closures to prevent spread. As well, many salmonid species that are currently threatened or endangered in North America are susceptible to whirling disease. Current testing focuses on detection of *M. cerebralis* in fish tissues, requiring lethal testing of both infected and non-infected fish. However, the parasite has an intermediate host, the oligochaete worm *Tubifex tubifex* and two environmental stages found in water and sediment that create other avenues for detection. We propose that using these environmental and *Tubifex* samples are a reasonable alternative to fish sampling and will be especially useful in large scale monitoring programs. In addition, *T. tubifex* susceptibility to *M. cerebralis* is not consistent across the species, with experiments showing some are refractory. Characterization of these worm populations will help target future monitoring and control programs based on the presence or absence of susceptible *T. tubifex*.

This project utilizes environmental samples collected from 45 stocked ponds and 300 wild sites from Central and Southern Alberta. These include sediment samples, invertebrate worm samples and water samples from stocked ponds. These samples are extracted for DNA using different methods tailored to the sample type and tested in a qPCR assay targeting the 18S gene of *M. cerebralis*. Additionally, worm samples are barcoded targeting the CO1 gene to determine species as identification by morphology is unreliable.

We will use these results to look at infection prevalence in worm samples within and across sample sites. Using the CO1 barcodes of Tubificid-like worms, we aim to create a phylogeny of worms in Alberta to look for cryptic species that may correlate to parasite susceptibility. Species composition of Tubificid-like worms will be analyzed and compared other sites, as well as to highlight connections to parasite presence. DNA extracted from sediment, worms and water will be tested for presence of *M. cerebralis* DNA and to be compared to results from fish samples.

We hope that with this work we can create a reasonable alternative to using fish samples for monitoring whirling disease presence in Canadian fresh water systems.

Conference Session Designation: (Myxozoa)
Presentation Format: (Oral)
Student Presentation: (Yes)

Assessing Environmental Microparasites in Relation to Atlantic Salmon Farms in BC

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British Columbia supports a large Atlantic salmon aquaculture industry in addition to a diversity of wild Pacific salmon stocks. Spatial overlap between farmed and wild populations is not uncommon, as many salmon farms operate along Pacific salmon migration routes. There is a growing concern regarding potentially harmful interactions between farmed and wild populations via their shared environment with an emphasis on the transmission of infectious disease. In fact, it has been found that salmon farms increase the risk of sea lice infection in wild salmon smolts; however, it remains unclear whether transmission of microparasites such as viruses and bacteria occurs between farmed and wild populations. We assessed environmental pathogen transmission by filtering water samples collected nearby and far from active salmon farms. We screened water samples for a diverse group of 37 viral, bacterial, and eukaryotic microparasites using a high throughput qPCR platform. I will present this methodology as well as preliminary results obtained via this sampling method.

Conference Session Designation: (eDNA/Metagenomics)
Presentation Format: (Oral)
Student Presentation: (Yes)

Development of Multiplex Quantitative PCR assays for the Detection of Invasive Species and Aquatic Animal Pathogens

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Batrachochytrium dendrobatidis, Ranavirus, *Ceratonova shasta*, *Myxobolus cerebralis*, *Tetracapsuloides bryosalmonae* and *Ichthyophthirius multifiliis* are important pathogens of cultured and wild fish and amphibians previously reported in the Pacific Northwest. Quagga Mussels, *Dreissena rostriformis bugensis*, Zebra Mussel, *D. polymorpha*, New Zealand Mudsnail, *Potamopyrgus antipodarum*, and the Asian clam, *Corbicula fluminea* are important invasive species currently monitored in the state of California, USA. Environmental DNA (eDNA), defined in this study as "genetic material obtained directly from substrate samples," has the potential to be a powerful tool for evaluating the presence of organisms, of which direct observation is impossible, and for assessing biodiversity in aquatic environments. In this study, the presence of 10 different organisms, including important amphibian and fish pathogens, as well as important invasive species to California, was investigated using eDNA analysis of river sediment samples collected in areas affected by recent fire activity in Plumas National Forest, California, USA. Extracted DNA from sediment samples collected in 2017 and 2018 from 38 different watersheds were used as template for recently developed and validated TaqMan probe quantitative polymerase chain reaction multiplex assays. The most consistent fish pathogens detected were *C. shasta* and *I. multifiliis*. None of the targeted invasive species DNA were detected. Future efforts to genotype the detected organisms is warranted to clarify the pathogen diversity detected in environmental samples.

Conference session designation: (eDNA / Metagenomics)
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