

**Thursday September 6<sup>th</sup> – Tilly / Tupper**  
**Co-infections in Fish**

**Moderators** – **Bartolomeo Gorgoglione** ( Wright State University ) **Simon Jones** ( Dept of Fisheries & Oceans )

1:15 PM	<b>Co-infections in Fish</b>	<u>Gorgoglione &amp; Jones</u> - Co-infections in Fish
1:30 PM		<u>Adamek</u> - <i>Flavobacterium branchiophilum</i> as a Secondary Pathogen in Koi Sleepy Disease
1:45 PM		<u>Getchell</u> - <i>Pseudomonas mandelii</i> and Viral Hemorrhagic Septicemia Virus Co-Infection in Sodus Bay, NY
2:00 PM		<b><u>Jones &amp; Wargo</u> - Impacts of Vaccination and Genetic Disease Resistance on Transmission in Single and Co-Infections in Rainbow Trout</b>
2:15 PM		Long - Impact of Co-Infections on Gene Expression in Sockeye Salmon <i>Oncorhynchus nerka</i>
2:30 PM		<u>Cabellero-Solares</u> - Analyzing the Molecular Mechanisms Underlying <i>Lepeophtheirus salmonis</i> and Bacterial Co-Stimulation in Atlantic Salmon
2:45 PM		<u>Karlsen</u> - Complex Skin Disease, Co-Infection of Atlantic Salmon by <i>Moritella viscosa</i> and <i>Aliivibrio wodanis</i>



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

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



## Special Session: Co-infections in Fish

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Infection with multiple pathogens is a more typical scenario than single pathogen infections, both in farmed and wild populations. Despite this, our understanding of host-pathogen interactions and disease outcomes is primarily based on knowledge gathered from single-pathogen studies and observations from ecological and disease surveillance programs. A body of knowledge is beginning to emerge that reveals complex and often poorly predicted host interactions occur during concomitant infections in fish. New studies are focusing on the impact and dynamics of heterogenous co-infections affecting teleost fish. Using several model pathogens in salmonid and non-salmonid species, current scientific advances target improvements in the assessment of diagnostic tools during multiple infections and characterise pathological, immunological and disease outcomes. Co-infections may enhance the impacts of disease in farming conditions or increase the ecological consequences of disease in wild fish. The goal of this Special Session is to raise awareness of ongoing research, and to foster new studies focusing on the interaction between infectious agents in fish hosts.

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## ***Flavobacterium Branchiophilum* as a Secondary Pathogen in Koi Sleepy Disease**

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Koi sleepy disease (KSD) is often fatal condition affecting common carp. Therefore, KSD is of increasing importance for global aquaculture. Despite the fact that the carp edema virus (CEV) is most likely the causative agent of KSD, the disease often seems to present itself as multifactorial. Several parasites and bacteria species are present on gills, skin or in internal organs of fish suffering from clinical KSD. In this study, we analysed a possible interaction of flavobacteria and CEV infections in the development of clinical KSD in carp suffering from proliferative gill disease. We examined selected field samples from Germany and Hungary and suggested the presence of CEV and flavobacteria co-infections in subsets of the samples. To confirm this, several infection experiments were performed to study the transfer and dynamics of both infections. We analysed which *Flavobacterium* species could be isolated/identified from KSD affected fish and concluded that *Flavobacterium branchiophilum* is a possible co-pathogen. Antibiotic treatment and studies involving differently KSD susceptible carp strains showed that CEV seems to be the primary pathogen causing an insult to the gills of carp and by this enabling other pathogens including *F. branchiophilum* to establish co-infections. Despite the fact that a *F. branchiophilum* co-infection is not required for the development of clinical KSD; it could contribute to the pathological changes recorded during the outbreaks of this disease.

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## ***Pseudomonas mandelii* and Viral Hemorrhagic Septicemia Virus Co-infection in Sodus Bay, NY**

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New York fishery biologists reported dead pumpkinseed (*Lepomis gibbosus*) and bluegill sunfish (*Lepomis macrochirus*) floating and laying on bottom all over Sodus Bay on 2 May 2018. Dead and moribund specimens were collected for necropsy. No significant external lesions were noted. Gill clips of five sunfish examined showed light to moderate presence of piriform ciliates, encysted digenes, and *Dactylogyrus sp.*, and skin scrapes showed few trichodinids. One pumpkinseed had an enlarged spleen and a darkened liver with small white cysts. Examination of fixed tissues revealed necrosis, congestion, and bacteremia in many organs. Kidney loop samples were inoculated onto TSA/5%SB and significant growth was observed after 2 days of incubation. Cornell's Animal Health Diagnostic Center Bacteriology Section identified the isolate with MALDI-TOF as *Pseudomonas mandelii*. EPCs were inoculated with tissue homogenates from sunfish and cytopathic effects were observed after 3 days incubation. VHSV was detected by RT-qPCR from pumpkinseeds brain and pooled organs, with the highest viral copy number near 5000 per 50 ng of total RNA. The diagnosis was complicated by the co-infection with both *P. mandelii* and VHSV. The severe bacteremia observed in histology slides suggests *P. mandelii* was the primary pathogen in this case. Both pathogens were observed in other fish kills documented in New York this spring, though not as co-infections. An outbreak of VHSV in late March 2018 involving thousands of dying gizzard shad (*Dorosoma cepedianum*) occurred in another part of Lake Ontario within Irondequoit Bay, approximately 60 kilometers west of Sodus Bay. Mortality events of sunfish also occurred in May 2018 in Conesus Lake and the Seneca River from which *P. mandelii* was cultured, but VHSV was not detected. In 2015, *P. mandelii* was also associated with a spring outbreak in pumpkinseed and bluegill sunfish from two different water bodies in New Jersey (Lovy et al. JFD 2017). Together, these reports indicate that *P. mandelii* is an emerging bacterial pathogen affecting freshwater fish during spring.

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## Impacts of Vaccination and Genetic Disease Resistance on Transmission in Single and Co-Infections in Rainbow Trout

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Globally, infectious diseases are responsible for major conservation and economic losses in wild and farmed fish populations. Prevention tools, including vaccination and breeding for genetic disease resistance, are used in many systems to prevent mortality by such diseases. Studies are often done to evaluate the efficacy of a preventative method at reducing disease, but the impact on transmission is rarely studied. Protection under diverse field conditions, such as variable pathogen exposure dosages, is also not fully understood. Furthermore, there is little information on how preventative methods alter host-pathogen relationships. For example, it is largely unknown how vaccination impacts non-target pathogens that co-infect the host. These knowledge gaps make it difficult to infer the epidemiological impacts of disease prevention tools. In an attempt to fill these gaps, we investigated the leading pathogens in rainbow trout (*Oncorhynchus mykiss*) aquaculture: infectious hematopoietic necrosis virus (IHNV) and *Flavobacterium psychrophilum*. We evaluated the impacts of vaccination and genetic disease resistance on mortality and transmission across a range of challenge dosages of IHNV and *F. psychrophilum* to accurately reflect field variability. There is evidence of a dose effect; as dose increases, shedding increases and vaccine efficacy decreases. We also evaluated how vaccination and genetic disease resistance impact transmission dynamics during simultaneous and sequential co-infection of IHNV and *F. psychrophilum*. Our results indicate co-infected fish shed more of both pathogens than they do in single infections, but the order that the pathogen infected the host may impact transmission. These studies are aimed at developing a more robust framework for inferring the efficacy of disease prevention strategies. Our results will also help to inform and improve disease management in one of the top aquaculture species in the United States.

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## Impact of Co-Infections on Gene Expression in Sockeye Salmon

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Synergistic co-infections in fish increase disease severity and mortality but there is little information available on the impact of co-infections on host gene expression. Both infectious hematopoietic necrosis virus (IHNV) and the salmon louse *Lepeophtheirus salmonis* are enzootic in marine waters of Western Canada, and there is a high likelihood of co-infection. Transcriptomic studies have suggested infection with *L. salmonis* impairs the host ability to respond to a virus challenge. To gain a better understanding of the host transcriptomic response to co-infection, Sockeye Salmon *Oncorhynchus nerka* smolts were infected with *L. salmonis* (V-/SL+), IHNV (V+/SL-), both (V+/SL+), or neither (V-/SL-). Anterior kidney and skin samples were collected at 3 and 7 d post-lice infection (dpl) for gene expression analysis. Genes of interest (GOI) for this study were associated with acute phase response, cytokines, antigen display, interferon-induced, immunoglobulins, tissue repair, and iron transport and circulation. Expression of genes associated with the antiviral response (*interleukin-1 $\beta$* , *interleukin-10*, *mx-1*, and *rsad2*) was significantly down-regulated in the V-/SL+ treatment, confirming earlier observations. Conversely, there was no significant difference in the up-regulated expression of these genes in the V+/SL- and V+/SL+ treatments. Up-regulation of tissue repair and iron transport genes in response to *L. salmonis* infection occurred in both the V-/SL+ and V+/SL+ treatments, suggesting little impact of the virus. Work is underway to determine whether infection with IHNV affected the previously reported T<sub>H</sub>2 anti-inflammatory response to *L. salmonis*. In summary, despite the significant effect of co-infection on salmon survival, expression of the GOI examined here was not significantly impacted by co-infection. Transcriptomics was not a useful predictor of the host response to co-infection and likelihood of survive. Future studies will require a global assessment of host responses to adequately understand host-pathogen interactions and outcomes in the context of co-infection.

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## Analyzing the Molecular Mechanisms Underlying *Lepeophtheirus salmonis* and Bacterial Co-Stimulation in Atlantic Salmon

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Co-infection by sea lice and pathogenic bacteria occurs naturally at Atlantic salmon sea cages, and causes multi-million dollar losses to the aquaculture industry. Under laboratory conditions, two experimental feeds (Cargill Innovation) supplemented with two different functional ingredients (CpG and Boost) were tested on lice (*Lepeophtheirus salmonis*)-infested Atlantic salmon (initial weight 290 gr) in comparison with a commercial grower diet (Control). Four weeks after copepodid exposure, with the parasites having developed to the pre-adult stage, salmon were injected intraperitoneally with either phosphate buffered saline (PBS) or a suspension of formalin-killed *Aeromonas salmonicida* (ASAL). Twenty-four hours post-injection, fish were euthanized and dissected for dorsal skin samples. Lice were counted before injection and at the sampling time, which revealed a significant reduction of lice infestation by CpG and Boost diets. For each fish, an area of the skin with a louse attached and an adjacent area with no signs of previous lice attachment, were collected. Dorsal skin RNA was qPCR-analyzed for transcripts involved in inflammatory response (*il1b*, *saa5*), eicosanoid synthesis (*cox2*), antibacterial response (*stlr5a*), and wound-healing (*mmp13*). ASAL induced the transcription of *stlr5a*, *il1b*, and *saa5* significantly. Also, ASAL treatment showed effects on *il1b*, *saa5*, *cox2*, and *mmp13* mRNA levels that differed among dietary groups. Feeding Control and Boost diets resulted in a significantly stronger ASAL-induction of *il1b* and *mmp13* compared with CpG diet. Conversely, *saa5* was significantly ASAL-induced in the salmon fed the CpG diet and not in those fed Control and Boost. Curiously, *cox2* was not ASAL-induced among salmon fed Control and Boost diets, and was significantly repressed by ASAL in the CpG-fed fish, although only slightly (-1.6 ASAL/PBS fold-change). Interaction of louse attachment/ASAL was significant for *mmp13*, the transcription of which was more intensely louse-induced in the PBS-injected/Boost-fed fish than in the PBS/Control and the ASAL/Boost groups. Interestingly, CpG diet significantly increased *stlr5a* induction by louse attachment compared with Control diet. The present qPCR data will be used in the selection of representative samples from each treatment/dietary group for 44K microarray profiling. Relations between transcriptomic and phenotypic (i.e., lice counts, growth, histology) data will also be investigated. It is anticipated that the findings arising from this study will aid in the development of improved feeds to protect farmed Atlantic salmon against sea lice and bacterial co-infection.

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## Complex Skin Disease, Co-Infection of Atlantic Salmon by *Moritella Viscosa* and *Aliivibrio Wodanis*

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The Norwegian aquaculture of Atlantic salmon (*Salmo salar* L.) is hampered by ulcerative disorders associated with bacterial infections. However, the etiology of skin disorders is complex and mechanical injuries, environmental factors, and nutrition are central factors that could effect susceptibility to infections. *Moritella viscosa* is the causative agent of classical winter-ulcer disease. Other contributing bacteria to occurring field outbreaks may be *Aliivibrio wodanis* and *Tenacibaculum* spp. This presentation will be focused on *M. viscosa* and *A. wodanis* interactions. A co-infection model reproduced field observations confirming that both bacteria co-infect Atlantic salmon. It is further hypothesized that *A. wodanis* colonization might influence the progression of a *M. viscosa* infection. From *in-vitro* co-cultivation studies *A. wodanis* impedes the growth of *M. viscosa*. Using bacterial implants in the fish abdomen it is evident that the presence of *A. wodanis* is altering the global gene expression of *M. viscosa*, and that the inhibitory effect is not contact-dependent. Outbreaks of ulceration are associated with salmon reared in marine waters at temperatures below 8°C. Ongoing studies are focusing on RNAseq data to explore relationships between temperature reduction and the effect on the global transcription profile to help improve understanding of putative virulence mechanisms.

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