Wednesday September 5th – Tilly / Tupper
Diseases of Wildfish 1 & 2
Moderator - Roland Cusack (Nova Scotia Department of Fisheries & Aquaculture)

9:30 AM	Diseases of Wildfish 1	de Jourdan - Histopathological Liver Changes and Additional Findings in Inland Silverside *Menidia beryllina* Exposed to Individual Polycyclic Aromatic Hydrocarbons
9:45 AM		**Lynn - Observations of Increasing Prevalence and Intensity of the Fluvial Ectoparasite *Argulus canadensis* on Migrating Outer Bay of Fundy Atlantic salmon.**
10:00 AM		**Cook - Dermal Injuries, Disease, and Immune Responses in Wild-Caught Pacific Salmon**
10:15 AM		**Chapman - Temperature Influences Post-Release Condition and Disease Progression in Adult Atlantic Salmon**
10:30 AM		**Refreshments**
10:45 AM	Diseases of Wildfish 2	Teffer - Incorporating Multiple Infections and Cumulative Stressors in Evaluations of Disease Development in Wild Fish
11:00 AM		**Soto-Davila - Atlantic Cod (*Gadus morhua*) Primary Macrophages Response to *Aeromonas salmonicida* Infection**
11:15 AM		Becker - An Epidemiologic Model of Koi Herpesvirus (KHV) Biocontrol for Carp in Australia
11:30 AM		Borucinska - Selected Biomarkers of Health and Water Quality in Dogfish Sharks *Mustelus canis* from the Long Island Sound in 2000 and 2017 Cohorts

Histopathological Liver Changes and Additional Findings in Inland Silverside *Menidia Beryllina* Exposed to Individual Polycyclic Aromatic Hydrocarbons

Ben de Jourdan[1], William Stubblefied[2], Jerry Heidel[3], Jeff Wolf[4] and Mike Kent[5]

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Histopathology provides links between toxicological effects observed in laboratory studies and responses at the cellular and tissue level, and thus may elucidate health impacts of contaminants that may be related to survivability. However, a limitation of histopathology is that it requires trained individuals to make proper diagnostic evaluations, and the interpretation of borderline pathological changes is to some degree subjective. Here we evaluated over 1,000 Inland silverside (*Menidia beryllina*) exposed to dissolved concentrations of single polycyclic aromatic hydrocarbons (PAHs). The primary endpoints were morbidity, growth, and histopathological changes. The liver was the only organ that exhibited exposure-related histopathologic changes. Two liver findings consistently observed in hepatocytes of exposed fish were increases in nuclear pleomorphism and increases in lipid, but not glycogen vacuolation. For certain PAHs, increases in nuclear pleomorphism were strongly correlated with increased mortality. Given the potential subjectivity of histopathological interpretations, two of the authors with extensive experience in fish histopathology evaluated all the histological slides independently so that the degree of consistency between the pathologists could be compared. There was a high degree of scoring consistency between the two pathologists that was statistically significant. The results of this study demonstrated that histopathology interpretations of fish tissues are repeatable when trained pathologists adhere to specific diagnostic criteria.

Conference Session Designation: (Toxicology / Tox Path)
Presentation Format: (Oral)

Observations Of Increasing Prevalence And Intensity Of The Fluvial Ectoparasite *Argulus Canadensis* On Migrating Outer Bay Of Fundy Atlantic Salmon

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Parasites and diseases are a high concern threat affecting Outer Bay of Fundy (OBoF) Atlantic salmon, a species not listed federally under the Species at Risk Act, but assessed as endangered in Canada (COSEWIC, 2010). OBoF Atlantic salmon were examined for ectoparasites at two sites near Fredericton, NB; the Nashwaak River Counting Fence (NRCF), and the Mactaquac Generating Station (MGS). The MGS is a 55-metre high hydroelectric dam located in the main stem of the SJR. The NRCF is located downstream of the dam and ~25 km from the main SJR stem. Both sites are located ~150 km from the estuary at the Bay of Fundy. Salmon are subject to observation of ectoparasites at each location. Ectoparasites collected 2013-2017 were distinguished morphologically with >99.9% (n = 4301) identified as *Argulus canadensis* (Branchiura), and <0.1% (n = 3) identified as the sea "louse", *Lepeophtheirus salmonis* (Copepoda). *Argulus* spp. infect fishes from freshwater, marine, and estuarine waters and so it is unknown where migrating OBoF salmon acquire *A. canadensis*. However, 90-100% of adult *A. canadensis* survive off their host (*in vitro*) for 60 hours in freshwater or estuarine conditions (17 ppt) whereas 100% of parasites die within 48 hours in seawater (34 ppt). Furthermore, additional *in vitro* studies reveal that *A. canadensis* eggs do not develop in seawater (34 ppt), while 59.3% (38-78%) develop to metanauplii in estuarine conditions (17 ppt) and 95.7% (95-96%) develop to metanauplii in freshwater. Whereas 58% (48.8-73.4%) of eggs hatched to metanauplii in freshwater, only 8.7% (3-16%) hatched in estuarine water. These results suggest that *A. canadensis* is transmitted to salmon exclusively during their migration within the SJR system. This parasite is known from the SJR system prior to completion of the Mactaquac dam in 1968 but was first observed on OBoF salmon at the dam in the mid-1990's. The year 2017 marks the highest prevalence of infection observed to date at both sites, with 91.8% and 81.2% of migrating salmon infected at the MGS and NRCF sites, respectively. We see a dramatic increase in the proportion of fish with high intensity infections (>50 parasites/fish) at Mactaquac from 2009 (1.5%) to 2017 (49.4%). Conversely, the proportion of infected fish with high intensity infections (>50 parasites/fish) remains static at the NRCF during this same time period (0-4%) suggesting differences in parasite transmission associated with fish migration to these two sites. The reason for the increase in ectoparasite prevalence and intensity remains unknown, but is of interest given tissue damage observed at the site of infection on some individuals and the critically low Atlantic salmon numbers.

Conference Session Designation: **(** Diseases of Wild Fin-Fish)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Dermal Injuries, Disease, and Immune Responses in Wild-Caught Pacific Salmon

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Commercial fisheries targeting wild Pacific salmon capture a mixture of co-migrating species, and those of conservation concern must be released. Among released fish, injury severity has been identified as an important predictor of mortality. Typically capture-induced injuries affect the dermis (e.g. skin, scale and mucous loss, net abrasions). Not only are the mucus and scales the primary line of defense against invading pathogens, but the stress of capture may also influence subsequent immune function, potentially interacting with physical injury to accelerate vulnerability to disease. Working alongside commercial Pacific salmon fisheries, non-lethal gill samples were collected from chum salmon, a species commonly released from these operations. Using HT-qPCR on the Fluidigm Biomark Dynamic Array™ microfluidics platform, fish were screened for the presence and relative abundance of 44 microparasite taxa identified as potentially infectious agents in the region. In addition, biomarkers of salmon immune function were examined to identify changes in expression profiles associated with infection dynamics. Severity of dermal injuries was estimated in captured fish and we conducted at-sea holding studies for up to 10 days to monitor changes in microparasite communities and immune response. The Fluidigm Biomark platform has been used with success to monitor the health of wild pacific salmon in BC. Here we expand on these efforts to understand how the latent effects of a fisheries interaction may influence disease progression and host immune function post-release.

Conference Session Designation: (Diseases of Wild Fin-Fish)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Temperature Influences Post-Release Condition and Disease Progression in Adult Atlantic Salmon

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Catch-and-release is a common practice in recreational fisheries, so understanding how fisheries interactions – including exhaustive exercise, air exposure, and handling – impacts post-release condition and survival is necessary for developing appropriate management strategies and inform angler best practices. The impacts of fisheries stress may vary with changing environmental conditions, such as increasing temperature. Here, an in-river holding study was used to understand how exercise, air exposure, and handling in warm and cool waters influence the post-release health and condition of wild Atlantic salmon. In the Campbellton River, Newfoundland, adult salmon migrating back to fresh water were collected at a counting fence and subject to experimental exhaustive exercise, air exposure, and handling, biopsied for gill and blood, then placed in an in-river holding pen for monitoring. RNA extracted from gill biopsies were used to screen for pathogen loads and Atlantic salmon immune and osmoregulatory gene expression. By combining in-situ fisheries simulations and gene expression technologies, we explore the relationship between fisheries related stressors, salmon condition, microbial pathogen productivity, and post-release survival.

Conference Session Designation: (Diseases of Wild Fin-Fish)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Incorporating Multiple Infections and Cumulative Stressors in Evaluations of Disease Development in Wild Fish

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As constituents of aquatic ecosystems, infectious agents inherently influence the survival of wild fish. However, empirical evidence demonstrating links between infections and mortality of wild fish is scare, especially regarding multiple infections and stressors. We conducted a series of laboratory and field studies to characterize the disease-associated mechanisms of early mortality of adult Pacific salmon (*Oncorhynchus* spp.) during freshwater migration in the context of cumulative stressors (high river temperature, fishery non-retention). Individuals were collected prior to or following river entry and transported to cool or warm freshwater tanks, or radio-tagged and released to evaluate migration behavior. A subset was also exposed to a fishery non-retention treatment. Held fish were biopsied weekly while tagged fish were biopsied at release. Physiology, immune activity and multiple infections were measured using high-throughput qPCR of gill tissue and chemical analysis of blood. Ecologically relevant high temperatures increased mortality and infection development and reduced the capacity of individuals, especially females, to resolve stress. Fishery stress also reduced survival but was context-dependent and mortality was generally delayed by more than a week. Fish with heavy infections migrated faster in the river but had reduced migration success, while gillnetting and air exposure reduced migration rates. River exposure was associated with more severe infections and increased mortality relative to fish that bypassed the lower river (marine-collected), supporting a causal influence of river-derived infections in early mortality. Our results suggest that multiple infections influence adult Pacific salmon survival in fresh water by affecting host physiological and behavioral responses and overall resilience to cumulative stressors.

Conference Session Designation: (Diseases of Wild Fin-Fish)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Atlantic Cod (*Gadus morhua*) Primary Macrophages Response to *Aeromonas salmonicida* Infection

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In contrast to other teleost, Atlantic cod (*Gadus morhua*) has an expanded repertoire of MHC-I components, but lacks the MHC-II and CD4, which are essential for antibodies production and prevention of infectious diseases. The mechanisms underlying fights against bacterial infections in *G. morhua* are not understood. *Aeromonas salmonicida* subsp *salmonicida* is a recurrent infection in cultured and wild fish, and has been reported in Atlantic cod. Macrophages are some of the first responders to bacterial infection and the link between innate and adaptive immune response. Here, we evaluated the viability, production of reactive oxygen species (ROS), cell morphology, and gene expression of cod primary macrophages in response to *A. salmonicida* infection. We found that *A. salmonicida* infects cod macrophages without killing the cod cells in contrast to *Salmo salar* infected macrophages. Cod infected macrophages upregulated key genes involved in the inflammatory responses (IL-1β, IL-8, IL-10, MHC-I, LECT-II, G-type L) and bacterial pathogen recognition (BPI/LBP). These results suggest that *A. salmonicida* trigger immune mechanisms that allow cod infected macrophage survive during infection in contrast to *S. salar* infected macrophages.

Conference Session Designation: (Diseases of Wild Fin-Fish)
Presentation Format: (Oral)
Student Presentation: (Yes)

An Epidemiologic Model of Koi Herpesvirus (KHV) Biocontrol for Carp in Australia

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Native to parts of Eastern Europe and central Asia, the common carp (*Cyprinus carpio*) is the third most farmed aquatic species in the world. In 2015, 4.3 million tonnes were produced, representing 8.3% of global farmed fish production. Despite their popularity for farming and recreational fishing, carp are considered an important invasive species in North America and Australia. Except for the Northern Territory, carp are established throughout Australia and numerous large populations are found in south-eastern Australia. Despite several introductions in Australia from the 1850s onwards, it was the introduction of the 'Boolara' strain in the early 1960s that led to a dramatic invasion of this pest species. Carp dominate fish communities in some areas of Australia's largest river catchment, the Murray-Darling Basin, comprising of 80 to 90% of the biomass. In 2016, the Australian Government announced the National Carp Control Plan to undertake research and stakeholder consultation to develop a plan for the potential release of *Cyprinus herpesvirus 3* (CyHV-3) to control carp populations.

Since emerging in 1997, koi herpesvirus disease (KHVD) has caused high mortality in common carp affecting all age classes of both wild and farmed fish. KHVD is notifiable to the OIE. Natural infections with CyHV-3 have only been detected in common carp, and varieties (e.g. koi carp). The disease is characterised by irregular patches on the skin and severe gill necrosis and inflammation. CyHV-3 infections occur in water temperatures between 16 to 28°C with optimal transmission and development of viremia between 22 to 24°C. Surviving carp develop anti-CyHV-3 antibodies and may have enhanced resistance to the disease, but may also become persistent carriers and shed virus. KHVD is exotic to Australia as no outbreaks have been recorded.

The purpose of this paper was to review the current knowledge of transmission factors for CyHV-3 and discuss the potential for recurring epidemic-level mortality events in carp found in the Murray-Darling Basin. Case studies will be presented comparing KHVD outbreaks in wild and farmed carp in Japan and *Epizootic haematopoietic necrosis virus* (EHNV) outbreaks in Australia. First emerging in the early 1980s, EHNV is only found in Australia and infections with this virus are notifiable to the OIE. Clinical outbreaks of EHN have only been observed in the introduced species, redfin perch and rainbow trout. EHNV has spread through several catchment areas in the Murray-Darling Basin with the last recorded outbreak in 2010. Host and environmental transmission factors for EHNV will be compared with CyHV-3. The model from this related virus will inform the potential impact of CyHV-3 as a biocontrol for carp.

Conference session: **(** Diseases of Wild Fish)
Presentation format: (Oral)

Selected Biomarkers of Health and Water Quality in Dogfish Sharks *Mustelus Canis* from the Long Island Sound in 2000 and 2017 Cohorts.

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The aim of this pilot study was to compare selected biomarkers of fish health and environmental quality in Long Island Sound in the western Atlantic. We examined 50 smooth dogfish sharks, *Mustelus canis,* common predatory bottom dwellers in the Long Island Sound. The fish were collected in June of 2000 (30 fish) and 2017 (20 fish). Autopsy was performed following cervical dislocation. All macroscopic abnormalities were noted and fork length, body weight, and liver weight were taken. In addition, gut content samples were collected aseptically from the 2017 fish for future microbiome analysis. Standardized to size and location, sections from gonads and liver were collected for histopathology. After routine processing, tissues were stained with hematoxylin and eosin and examined by bright-field microscopy. Additional staining was done as needed and included PAS, PTAH, Pearl's and Fontana Masson's. Morphometric analysis of hepatic melanomacrophages (MMC) was done using SPOT software. The studied biomarkers included condition factor (CF), hepatosomatic index (HIS), the numbers of MMC and % hepatic surface covered by MMC, levels of follicular atresia, and histopathology of liver and gonads. Apparent differences in the biomarkers between the two years of collection were noted in regards to CF, HIS and MMC and were suggestive of declining fish condition and/or water quality. The microbial gut community will be characterized using 16S rRNA gene sequencing on an Illumina platform from the 2017 cohort in order to compare gut flora with fish health and water quality. Although the small sample size precludes general conclusions, the data are inviting more studies to validate our findings and to continue to monitor trends in water quality in the Sound.

Conference session designation: (Diseases of Wild Fin-Fish and Shellfish)
Presentation format: (Oral)