

Strategic Salmon Health Initiative

In the Pacific Northwest, Chinook, sockeye, and coho salmon and steelhead trout have experienced precipitous declines over the past 30 years, with many populations threatened or endangered, and some already extinct. Our program applies a series of novel molecular and ecological approaches to understand the role of infection and disease outbreaks in salmon declines, a difficult task when mortality is mostly unobservable. High throughput pathogen monitoring tools have revealed the distribution and abundance of over 60 potential salmon pathogens (viruses, bacteria, fungal and protozoan parasites) in 28,000 wild, hatchery and cultured salmon.

Models informed by a decade of infection data in juvenile Chinook salmon sampled during early marine residence has revealed that temperature is the most significant driver of infection, with exposure to aquaculture, proximity to freshwater, age at ocean entry, hatchery or wild origin, stock, and latitude associated with a more limited number of pathogens.

Models are also beginning to resolve the pathogens most closely correlated with year-class strength, some transmitted in freshwater, and others in the early marine environment. Climate warming is a major factor for salmon to contend with. Impacts can directly cause stress in salmon, but also indirectly impact prey availability and predator distributions, as well as disrupting the natural balance between pathogens and their hosts.

NAME, RESEARCH AREAS, & AFFILIATION

Dr. Kristi Miller (Saunders) is an aquatic molecular biologist with expertise in genetics, genomics, transcriptomics, immunogenetics, and fish health, with research on Pacific and Atlantic salmon spanning 27 years. Fisheries and Oceans Canada; University of British Columbia (adjunct).

SELECTED PEER-REVIEWED PUBLICATIONS past 5 years (**Trainees*)

In Review or In Press

Lennox, RJ, Eldoy, SH, Vollset, KW, **Miller**, KM, Li, S, Kaukinen, KH, Isaksen, TE, Davidsen, JG. In Review. Abundance, diversity and effects on marine habitat use of infectious pathogens among sea trout (*Salmo trutta*) in Norwegian fjord systems. Journal of Fish Diseases (December 2020)

Chapman, JM, Lennox, RJ, Twardek, WM, Teffer, AK, Robertson, MJ, **Miller**, KM, Cooke, SJ. In Review. Changes in the condition, infectious agents, and transcription profiles of wild Atlantic salmon during up-river migrations. CJFAS Submitted December

Furey, NB, Bass, AL, **Miller**, KM, Li, S, Lotto, AG, Healy, SJ, Drenner, SM, Hinch, SG In Review. Infected juvenile salmon experience increased predation risk during freshwater migration. Biology Letters In Review (October)

Teffer AK, Carr, J, Tabata, A, Schulze, A, Bradbury, I, Deschamps, D, Gillis, C-A, Brunsdon, EB, Mordecai, G, **Miller**, KM. 2020. A molecular assessment of infectious agents carried by Atlantic salmon at sea and in three eastern Canadian rivers, including aquaculture escapees and North American and European origin wild stocks. Facets: In Press

Teffer AK, **Miller** KM. 2020. Infectious agent screening of fish using non-lethal versus lethal tissue sampling and high-throughput qPCR. Trans Amer Fish Soc: In Press.

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- *Thakur KK, 4 authors KM **Miller**. 2019. Infectious agent detections in archived Sockeye salmon (*Oncorhynchus nerka*) samples from British Columbia (1985-1994). *J Fish Dis* 2019: 1-15. <https://onlinelibrary.wiley.com/doi/pdf/10.1111/jfd.12951>
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Innovative Molecular Technologies and Approaches relevant to Dr. Miller's talk at the AAAS

Linking genomic profiling of non-invasive biopsies with acoustic/radio-tracking (first studies: Miller et al. 2011; Jeffries et al. 2014)

Acoustic- and radio-telemetry has been applied broadly in wildlife biology to track movement patterns and migration behaviour. In salmon, due to high fidelity homing to natal rearing areas and the development of genetic stock identification methods sensitive enough to identify individuals to stock of origin, tracking studies can also identify fate (survival). In collaboration with Dr. Scott Hinch at University of British Columbia, in 2005 Dr. Miller started conducting transcriptional profiling of gill biopsy samples taken as salmon were being radio- or acoustically tagged to determine if gene activity at the time of tagging was associated with migratory behaviour and/or fate. This approach has shown across multiple studies that adult salmon dying prematurely en route to spawning grounds are often physiologically compromised well before they entered the river, with a low probability of survival. Similar studies in out-migrating juvenile salmon have revealed pathogens and disease states associated with salmon survival during downstream migration. Interestingly, multiple studies have revealed that adult salmon showing molecular signatures of compromise actually swim faster towards spawning grounds, but often do not make it. Moreover, signatures of wound healing, inflammation, viral disease, and differential immune activation have repeatedly been associated with poor migratory survival.

High throughput infectious agent monitoring platform (Miller et al. 2014, 2016)

At the disease evidentiary hearings during the Canadian Federal Cohen Commission of Inquiry into declines in Fraser River Sockeye salmon in 2011, it became clear that there was limited understanding of the pathogens and diseases negatively wild salmon largely owing to the inability to observe mortality, especially in the ocean, as predators quickly remove compromised animals from the water column. As well, many emerging pathogens discovered in cultured salmon around the world had never been assessed in salmon from North America. Dr. Miller's laboratory sought ways to utilize molecular approaches to fill these gaps in knowledge. Her developed a high throughput infectious agent monitoring approach, based on microfluidic quantitative (q)PCR, for simultaneous quantitation of 47 infective agents (viruses, bacteria, fungi, and protest parasites). Included on the platform were assays to most of the infective agents known or suspected to cause disease in salmon worldwide. Dr. Miller's lab analytically validated the performance of the assays and the platform to ensure the results were reliable (sensitive, specific, repeatable, and reproducible on other platforms). This platform has now been applied in her lab to conduct pathogen surveillance in over 28,000 fish, including wild, hatchery, and farmed salmon, and several species of marine fishes. Many papers (listed above) coming out of Dr. Miller's laboratory are based on data from this platform and have shed new light on the spatial and temporal distribution of pathogens in BC salmon (wild, hatchery and aquaculture), and variations between hatchery and wild salmon (Thakur et al. 2018; Nekouei et al. 2019), Chinook life-history types (Tucker et al. 2018). For Sockeye, Chinook and Coho salmon, infectious profiles gleaned from over a decade of early marine samples are currently being explored in models to identify environmental correlates with infection and pathogens associated with annual variations in marine survival (year-class strength). Several papers are in development. Dr. Miller is currently collaborating with scientists in Norway, Alaska, California, Washington, and eastern Canada to expand applications of this powerful tool around the world.

Molecular viral disease development diagnostics (Miller et al. 2017)

The detection of an infective agent does not mean an animal is diseased, as it is common for organisms to carry a range of opportunistic infections at background levels with no ill health. As we expect that predators will remove individuals that are even at early stages of

diseases if they show compromised swimming performance, visual acuity, or shifts in behaviour, the probability of randomly sampling fish in a late stage of disease is low. Traditionally, histopathology is the gold standard to diagnose disease, but is generally applied to diagnose the cause of death, and is not highly sensitive to reveal early stages of disease. Dr. Miller followed the lead of some literature in human medicine where researchers were developing biomarker panels (highly specific gene activity) to differentiate viral and bacterial respiratory diseases. Her team discovered dozens of biomarkers in salmon that were activated consistently across infections by RNA viruses, and showed that with as few as 7 of these biomarkers, one could predict the presence of a viral disease state very early in disease development. Furthermore, it could differentiate viral from bacterial diseases, and viral carrier states from active diseases. And all this could be done using a non-destructive gill biopsy sample. This tool was applied to great effect to study the disease development pathways of piscine orthoreovirus (PRV) in farmed Atlantic and Chinook salmon (see Di Cicco et al. 2018). Moreover, it led to the discovery of over a dozen previously uncharacterized viruses in salmon, three of which were published in Mordecai et al. 2019, with more to come in a paper that will be posted next week on BioRxiv, also led by Dr. Mordecai.

Salmon Fit-Chips (Akbarzadeh et al. 2018; Houde et al. 2019a/b/c; first application in Lennox et al. In Review)

While infectious diseases are highly likely to contribute to salmon declines, salmon are also exposed to a multitude of environmental stressors that could cumulatively or synergistically impact their survival. While environmental monitoring can provide information on seasonal and annual variations in potential environmental stressors such as temperature, salinity, oxygen, and harmful algal bloom events, salmon may behaviourally respond in a way to avoid exposure to stressors, especially in the ocean, making it difficult to ascertain the degree to which environmental stressors may affect them. However, Dr. Miller's team recently developed a new technology, just rolled out in 2019, to detect variability in stressor *impacts* on the salmon themselves. Similar to the viral disease biomarker panels explained above, Dr. Miller's team developed biomarker panels to specifically identify the presence of different classes of stressors (e.g. thermal, hypoxic, and osmotic stress), physiological states indicative of poor health (e.g. inflammation, and state of immune stimulation), and signatures repeatedly associated with mortality (imminent mortality [within 72 hours], and mortality-related [over longer timeframes]). These host gene panels can be combined with assays to pathogens and harmful algal bloom species, and monitored using non-destructively sampled gill biopsies, using the same microfluidics platform as for infectious agent monitoring. Collectively, this technology has been termed Salmon Fit-Chips, and is the first tool of its kind (in any species) to assess overall health and condition, and to study cumulative effects of stressors and disease, with no need for lethal sampling. This tool is currently being applied to determine the spatial and temporal variation in environmental stressors and diseases in the early marine environment, and to determine which stressors, or combination of stressors and diseases, are associated with year-class strength in salmon.