Factors Influencing Infectious Agent Communities and Infection Burden in Free-Ranging Migratory Adult Salmonids Across Canada

by

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Dedication

In loving memory of Ursula “Sula” Ward (Grannie) and Dr. Lt. Colonel George Robert Irvin Power (Dindin), both of whom passed during the course of this research. Each shared with me their insatiable curiosity, love of family, and provided unwavering support that I will carry with me always.

Abstract

While the importance of disease-induced mortality in wild fish populations has been purported, little research has investigated infectious agent dynamics in free-ranging fishes. The objective of this thesis was to investigate host-pathogen dynamics in three ecologically, culturally, and economically important salmonid species to characterize biological and environmental factors that mediate host response and survival associated with relative infection burden (RIB). To achieve this goal, a synthesis of the current state of wild fish epidemiology was conducted, with a focus on novel genomic techniques and ways in which they can be used to complement commonly employed fisheries field methods. High throughput qPCR and non-lethal gill biopsies were then used in three separate field studies to investigate RIB of microbial infectious agents and genetic biomarkers of host condition. To characterize the transcriptomic response of a long-lived, iteroparous salmonid to infectious agents and investigate the potential for invasive pathogens in Arctic waters, Arctic char from three separate populations were sampled during the fall migration, revealing site-specific differences in pathogen species richness and RIB, and provided new records for detection of likely endemic infectious agents. To investigate the influence of high water temperatures, Atlantic salmon were experimentally displaced downstream of a barrier and periodically sampled to track changes in infectious agent prevalence, relative infection burden, and host response over a 32 day period. Here, river temperature was significantly related to stress and immune biomarkers, while RIB was negatively associated with body condition. Finally, radio telemetry was used to track post-release survival and behaviour of coho salmon exposed to experimental fisheries stressors to characterize interactions between fisheries stress,

host condition, and RIB. Fishery treatment and RIB were not associated with survival, however RIB was associated with post-release behaviour in that high RIB began up-river migration post-release sooner than fish with lower RIB. The duration of handling alone was significantly related to mortality irrespective of fisheries treatment. Together, these findings demonstrate the diversity of factors associated with infectious agent communities and host response and provide valuable information for the ongoing monitoring and investigation of wild salmonids.

Acknowledgements

Throughout my graduate studies I have been extremely fortunate to work with Dr. Steven Cooke and the incredibly talented students, managers, and technicians that have made up the Fish Ecology and Conservation Physiology Lab over the years. The collaborations and friendships that I have made over the last several years have and will continue to be inspirational and I look forward to working with many current and past colleagues in the future. I am especially grateful to A. Teffer, V. Nguyen, J. Brooks, G. Raby, R. Anderson, K. Cook, J. Brownscombe, and C. Hatry, all of whom provided support, lively discourse, and many laughs over the years. Endless thanks are given to T. Ward, who provided support during all facets of this process.

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Preface

All research presented in this thesis was conducted in accordance with animal care protocols in accordance with guidelines put forth by the Animal Care Council of Canada and approved by committees at Carleton University, the University of British Columbia, and Fisheries and Oceans Canada. All animal collection was done under federal scientific collection permits administered by the Department of Fisheries and Oceans Canada.

Thesis Format

This thesis is presented in manuscript-based format. In an effort to maintain clarity, as there are subtle differences in methods in each data chapter, I have left the detailed description of laboratory methods in each chapter, resulting in repetition of certain sections. Acknowledgements for each data chapter have been included in the thesis acknowledgement section.

Chapter 2: Disease ecology of wild fish: Opportunities and challenges for linking infection metrics with behaviour, condition, and survival.

Chapman, JM, Teffer, AK, Kelly, L, Miller, KM, Cooke, SJ.

The idea for this paper was conceived by Chapman with contribution from Cooke, Teffer, and Kelly. A version of this paper was presented at the American Fisheries Society Conference in August 2018 by Teffer in lieu of Chapman's attendance. Another version of this paper was presented at the International Conference of Fish Telemetry by Chapman in June 2019. This manuscript is in prep for submission to Fisheries Research.

Chapter 3: A survey of microbial infectious agents and characterization of immune response in adult migratory Arctic char (*Salvalinus arcticus*) intercepted during fall migration in three watersheds in the Kitikmeot region of Nunavut, Canada.

Chapman, JM, Lennox, RL, Struthers, D, Malley, B, Harris, L, Miller, KM, Cooke, SJ.

This study was designed by Chapman and Cooke with data collection and field support from Lennox, Struthers, Malley, and Harris. Laboratory analyses were conducted by Chapman with support from Miller. Chapman analyzed the data and wrote the paper, which is currently in prep for submission to the Journal of Fish Diseases.

Chapter 4: Changes in the condition, infectious agents, and transcription profiles of wild Atlantic salmon during up-river migrations

Chapman, JM, Lennox, RL, Twardek, W, Robertson, M, Miller, KM, Cooke, SJ.

This study was designed by Chapman, Robertson, Cooke, and Lennox with data collection and field support from Lennox and Twardek. Robertson provided equipment and logistical support. Laboratory analyses were conducted by Chapman with support from Miller. Chapman analyzed the data and wrote the paper, which is currently in prep for submission to the Canadian Journal of Fisheries and Aquatic Sciences.

Chapter 5: Handling, infectious agents, and physiological condition influence survival and post-release behaviour in migratory adult coho salmon after experimental displacement

Chapman, JM, Teffer, AK, Bass, AL, Hinch, SG, Patterson, DA, Miller, KM, Cooke, SJ. 2020. Handling, infectious agents, and physiological condition influence survival and post-release behaviour in migratory adult coho salmon after experimental displacement. Conservation Physiology 10.1093/coaa033.

This study was designed by Chapman, Cooke, Hinch, and Patterson. Chapman collected the data with significant contribution from Bass and Teffer. Hinch, Patterson and Cooke provided equipment. Bass and Teffer contributed to data processing. Chapman conducted laboratory analyses with support from Bass, Teffer, and Miller. Chapman analyzed the data and wrote the paper.

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Chapter 1: **General Introduction**

The theory of evolution by means of natural selection put forth by Darwin in 1859 outlined how variation in heritable phenotypes are under selection pressure by the environment, resulting in variable fitness outcomes and, over time, adaptations that maximize fitness. Yet it was not until Leigh Van Valen's proposed antagonistic coevolution as an explanation for linear extinction rates that the reciprocal adaptation in predator-prey or pathogen-host systems was addressed (Van Valen 1973). The proposed "Red Queen Hypothesis" outlines a co-evolutionary arms race, where any advantage one species may gain over another through natural selection leads to reciprocal selection pressure, and so on. While confined by phenotypic limitations, such oscillations in genotype frequencies may have a more important role than environmental selection pressure in certain evolutionary contexts (Marrow et al. 1992). For host-pathogen interactions, these oscillations are believed to have shaped the foundation of many biotic processes, including sexual reproduction where genetic recombination decreases the frequency of maladaptive gene combinations that might otherwise lead to compromised immune function (Hamilton et al. 1990; Jokela et al. 2009).

Parasitism – when an organism derives nutrients by living in or on another organism (the host) to the detriment of the host – is estimated to be the most common life history strategy, evolving independently across multiple taxa (Poulin and Randhawa 2015). Consequently, parasites are ubiquitous and comprise a large percentage of global biodiversity (Dobson et al. 2008), potentially outnumbering free-living organisms (Poulin 1997; Rossiter 2013). Parasitic organisms range in size from chordates (e.g. the sea lamprey *Petromyzon marinus*) to microscopic viruses, bacteria, and protozoa, and are by

definition pathogenic agents of disease (though some parasites may be commensal, leading to discussions on the merit of the term pathogen; see Méthot and Alizon 2014). The negative consequences of disease in wild populations has been recognized as a central factor to consider when investigating population viability (Dobson and May 1986, Plowright et al. 2008). The role pathogens play in shaping wildlife evolution, demographics, and distribution through pathogen-induced selection pressure is well recognized (Van Valen 1973; Grenfell and Dobson 1995; Marcogliese 2004; Eizaguarrie and Lenz 2010), though incorporation of disease in research on wild animal behaviour, ecology, and physiology is comparatively lacking when considering its importance. Similarly, the effect of pathogens on animal behaviour, distribution, fitness, and survival is considered an increasingly important component of wildlife conservation and management (Dobson et al. 2008; Plowright et al. 2008; Kotob et al. 2017), yet parasites have been chronically overlooked in wildlife research (Windsor 1997; Nichols and Gómez 2011; Hellard et al. 2015). In fisheries, investigations of wild fish behaviour and ecology rarely address the potential consequences of parasites (Binning et al. 2017). Because pathogens can have an array of effects on host fitness beyond survival alone, more work on wild fish disease ecology has the potential to directly inform conservation actions (Gómez and Nichols 2011).

The continued decline in global fisheries is of grave concern among researchers, managers, and stakeholder groups (Costello et al. 2012; Pauly et al. 2005), particularly for highly exploited species such as those in the family Salmonidae (Parrish et al. 1998; Price et al. 2008). In many salmon fisheries, population declines are surpassing what is expected based on harvest rates alone (Cohen 2013), and have continued even after

moratoriums on fishing have been put in place (Limburg and Waldman 2009). Such observations have spurred investigations into alternative sources of mortality in wild fishes, including biological, environmental and anthropogenic stressors. While direct mortality has proven difficult to observe, sublethal impacts of stress has been linked with suppressed maturation, potentially reducing spawning success and population recruitment (Jeffries et al. 2012; Baker et al. 2013; Teffer et al. 2018). Because stressors rarely occur in isolation and may combine in unexpected ways (Folt et al. 1999), researchers have begun to investigate how sublethal stressors may reduce an individual's resiliency to additional stressors – i.e., how fish are able to cope with cumulative stress (Miller et al. 2014). For example, substantial evidence has reported the negative effects of elevated temperatures on salmon's ability to respond to and recover from aerobic stress (Wilkie et al. 1997; Eliason et al. 2013), fisheries capture (Raby et al. 2015), and more recently, pathogen infections (Kent 2011; Marcos-López et al. 2010; Teffer et al. 2018).

Given the ubiquitous distributions of pathogens in wild fish populations, characterizing the mechanisms underlying infection dynamics and host response in salmonid populations will clarify the role of current and potential factors contributing to salmonid population declines in the face of cumulative stressors. Because species- and/or stock-specific adaptations indicate that responses may not be consistent across multiple contexts (Cook et al. 2018; Miller et al. 2014), it is important to understand factors underlying such variation in outcomes. This thesis explores the challenges and various methods available to investigate infectious agents in wild salmonids, and uses a combination of research methods to investigate the interactions among infectious agents, host response, and anthropogenic stressors in a variety of biological contexts. Three

different species of salmonids were examined, each representing a unique combination of environmental and anthropogenic stressors, to investigate the variable context of response to infectious agents from biological and environmental perspectives highly relevant for conservation.

1.1 Anadromous Salmonids in Canada

In Canada, commercial, recreational, and Indigenous fisheries harvest fish in all three oceans (i.e. Pacific, Arctic, and Atlantic), and species belonging to the family Salmonidae are of particular economic and cultural importance due to their nutritional value, relative ease of harvest during migrations, and large size. Salmonids are predatory fish species including salmon, char, and trout, among others. Anadromous salmonids – species that reproduce exclusively in freshwater and migrate to and from marine waters to feed – are uniquely important for coastal ecosystems where they transport nutrients between aquatic and terrestrial environments (Cederholm et al. 1999). It is during these migrations that they are targeted by fishers because of the predictable nature of their movements and extremely high densities. As a result, many of Canada's anadromous salmonid populations have experienced significant decline in part due to high exploitation rates (DFO 2010).

The benefits of undergoing diadromous migrations for salmonid species include access to superior feeding grounds in the ocean compared to freshwater, facilitating enhanced energy acquisition for growth and gonadal development (Dingle 2014; Jensen et al. 2014). Moving between marine and fresh waters may also reduce infection through migratory recovery by clearing pathogen species that are sensitive to changes in salinity

(Poulin et al. 2012; Shaw and Binning 2016). However these migrations do not come without great costs; during outmigration, the energetic demands associated metabolic shifts required for smoltification, and increased risk of predation in the marine environment results in high marine mortality across anadromous salmonids (Halfyard et al. 2013; Clark et al. 2016; Daniels et al. 2019; Jensen et al. 2019). In return or spawning migrations, adults cease feeding and so rely on endogenous energy stores to maintain swimming, navigate hydrologically challenging barriers, build nests, defend territories, and spawn (Groot and Margolis 1991; Kadri 1995; Bombardier et al. 2010). Adults are also faced with osmoregulatory challenges as they once again move between hyper- and hypo-tonic waters (Shrimpton et al. 2005).

There is a vast and well researched diversity in the geno- and phenotypic plasticity of salmonid species, including high variation in life-history and reproductive strategies which vary considerably among anadromous species. While all species begin their lives in fresh water, the duration of freshwater residency and timing of seaward migration varies within and among species (Groot and Margolis 1991; Klemetsen et al. 2003). Most Pacific salmon species in the genus *Oncorhynchus* demonstrate semelparity, returning to their natal freshwater lakes after only 2-4 years feeding at sea for a single reproductive event, dying shortly after. In contrast, Arctic char *Salvelinus alpinus* are iteroparous and can live for up to 28 years, executing numerous reproductive cycles within their life span (Roux et al. 2011). Intermediate to these is the Atlantic salmon *Salmo salar*, where individuals have low post-spawn survival rates and so few individuals reproduce multiple times in their lives (Jonsson, Jonsson & Hansen 1991). The metabolic and physiological processes associated with these spawning migrations are

similar with respect to the onset of anorexia and use of endogenous energy stores during migration, however little comparative work as addressed differences in immunocompetency and pathogen communities across this range of life-history strategies.

1.2 Pathogens of salmonids

Anadromous fishes serve as hosts to a myriad of pathogenic agents (Marcogliese 2004; Miller et al. 2014). The majority of what is known about pathogens in salmonids is based on information garnered from research and operations in aquaculture (Thompson 2001), particularly those that cause significant economic losses or may be transferred to wild populations (reviewed in Olivier 2002, Lafferty et al. 2015). Given that macroparasite species (trematodes, cestodes, and parasitic arthropods) generally do not cause mortality and if they do, the proportion of hosts that perish is generally not high enough to yield population-scale effects (Marcogliese 2005; Miller et al. 2014), the focus of the data chapters in this dissertation will be on microparasite infections (bacteria, viruses, protozoans, and fungi). As research methods proposed in Chapter 2 can be applied to both macro- and microparasites, examples from both groups are considered.

Salmonids are host to numerous microparasite species that vary in virulence and pathogenicity (reviewed in Miller et al. 2014). Recent surveys have demonstrated that individual migratory salmon are most often carrying a multitude of pathogens at a given time (Miller et al. 2014; Teffer et al. 2017; 2019; Bass et al. 2017). Bacteria species can be highly prevalent, for example '*Candidatus* Branchiomonas cysticola', which is observed in as many as 100% of individuals surveyed in some populations (Bass et al.

2017). High prevalence rates such as this suggest low pathogenicity based on infection from this agent alone (Bass et al. 2017). In contrast, viral agents were observed in less than <10% of surveyed individuals, either because of naturally low prevalence rates or because of high pathogenicity (Teffer et al. 2018). Yet investigating the impact of individual pathogens does not address ecologically relevant contexts; transmission, pathogenicity and/or virulence of an infectious agent may be mediated by the presence of other pathogens (Sofonea et al. 2015; Kotob et al. 2017; Karvonen et al. 2019). Consequently it may be more informative to investigate how shifts in pathogen communities within hosts are associated with factors such as host condition and survival.

Like their hosts, pathogens are influenced by biotic and abiotic factors within the aquatic environment, and the magnitude of effect can vary depending on the biology of the pathogen. Firstly, for infectious agents that require an intermediate host for transmission, that intermediate host must be present in adequate abundance to facilitate transmission pathways (Marcogliese 2004). For this reason, pathogen diversity is generally reflective of overall aquatic ecosystem diversity (Chapman et al. 2015). Hydrological dynamics, salinity, and temperatures must also be suitable for transmission of free-living stages of pathogens moving between hosts (Hendrick 1998). Of potential environmental factors, temperature is a central factor associated with host and pathogen physiology. Some pathogens response positively to increases in temperature, for example the globally distributed ciliate *Ichthyopthurius multifiliis*, the causative agent of freshwater ich (Noe and Dickerson 1995), or *Tetracapsuloides bryosalmonae,* the pathogen associated with the highly pathogenic Bacterial Kidney Disease (Bettge et al. 2009). In contrast, several bacterial species are more pathogenic at low water

temperatures, evidenced by the increased proliferation and up regulation of genes associated with virulence in bacterial pathogens in warmer water temperatures (e.g. *Yersinia ruckeri* (Enteric red mouth disease) and *Flavobacterium psychrophilum* (Bacterial cold water disease)*;* Guijarro et al. 2015). Interestingly, transcription of virulence associated proteins is independent of optimal growth temperatures, which are 6-10 °C higher (Starliper 2011, Méndez et al. 2018), demonstrating the complexity of pathogen dynamics and necessity to include context-specific factors when investigating fish disease.

Recent investigations have revealed changes in the prevalence and relative load of pathogens during the spawning migration of sockeye salmon *Oncorhynchus nerka* (Teffer et al. 2017) and Chinook salmon *O. tshawytscha* (Bass et al. 2017). As Pacific salmon are semelparous, immune function is down-regulated as energy is reallocated towards reproduction during senescence (Dolan et al. 2016). Similar evidence was demonstrated in experimental holding studies of coho salmon *O. kisutch* (Teffer et al. 2019), however no surveys have been conducted investigating temporal or site-specific pathogen communities in iteroparous salmonid species in Canada. Given the declines in salmonid stocks, potential consequences of infectious disease on salmon stocks (Miller et al. 2011), and lack of knowledge of infectious agents in wild populations (Mordecai et al. 2019), additional research profiling infectious agent communities is needed.

1.3 Host stress response and immunocompetency

Host defenses have evolved to minimize the negative fitness consequences resultant from infection. Defense mechanisms occur pre-infection, reducing exposure or

infection risk, or post infection, whereby the consequences of infection are reduced (Schmidt-Hempel 2011). Pre-infection defenses include behavioural adaptations that reduce exposure to pathogens, and can include behaviours such as large-scale migrations (Loehle 1995; Altizer et al. 2011; Poulin et al. 2012). Physical barriers such as mucous and integument are also considered components of pre-infection defense. Post-infection defense is executed by the host's immune system, a complex and dynamic integration of cellular processes made up of innate and acquired components responsible for recognizing and responding to aberrant cells within the body (Alvarez-Pellitero 2008). The immune system of salmonids contains the sophisticated components found across vertebrates, including the nonspecific innate or adaptive immune pathway (Woo 1992) Maintaining and up-regulating the immune system is energetically costly, potentially squandering resources from other fitness-related functions, and so limited in its efficacy. For example, it would be of no fitness benefit if all energy was placed towards clearing infection to the complete detriment of gonadal development (Van Baalen 1998). Fish must be able to maintain metabolic processes (e.g. movement, somatic growth, and/or gonadal development) that maximize fitness, and so the level of immune response can be considered plastic in that it is within the confines of this energetic balance (Schmidt-Hempel 2011).

The general definition of a stressor is any stimulus that displaces an organism from homeostasis thus eliciting a cascade of metabolic and behavioural responses (Selye 1973; Barton 2002). Stressors can include environmental (e.g. temperature), physical (e.g. wounds), or perceived (e.g. startle response) stimulus. The stress response in fish consists of primary, secondary, and tertiary responses that have evolved to respond to,

mediate the effects of, and overcome potential threats to ultimately return to homeostasis (Barton and Iwama 1991; Barton 2002). In cases where the stress response cannot be resolved, organisms can be pushed to their physiological limits, leading to sublethal impairments and if severe, mortality (Barton 2002).

As mentioned within the context of migration, elements associated with the stress response can reduce immunocompetency in host species across all taxa (Hing et al. 2016). The following components are a simplified description of the stress response, an allostatic process driven by fluctuations of reactive homeostasis (Romero et al. 2009) to provide a general context for the interplay between stress and host condition. Initial exposure to a stressor initiates a "fight or flight" response based on signal cascades from the hypothalamo-pituitary-interrenal axis resulting in the release of catecholamines and corticosteroids (Barton 2002). This primary response induces secondary changes to osmoregulatory, cardiac, metabolic processes that propel the organism into a heightened state of preparedness for response (Barton and Iwama 1991). Cellular processes are a component of the secondary stress response and includes changes in expression of genes associated with glucose metabolism (Wendelaar Bonga 1997), oxidative stress (Pickering and Pottinger 1995), cellular maintenance (Iwama 2004), and immune function (Dhabar 2002). Finally, the tertiary phase of the stress response is characterized by whole-organism shifts in performance and behavior, and can include sustained decreases in immunocompetency (Tort 2011), maturation (Baker et al. 2013), and survival (Mazeaud et al. 1977). Fish must be able to return to homeostasis through the clearing of cellular metabolites and ions, an additionally metabolically expensive process (Wendelaar Bonga 1997). The energetic demands and suppression of immune function associated with the

stress axis result in increased susceptibility to disease outbreaks under conditions of severe or chronic stress (Snieszko 1974; Tort 2011; Pickering and Pottinger 1989).

Phenological shifts associated with migration often use the stress response as part of the internal cue to initiate and sustain unidirectional movement (Lennox et al. 2017). Consequently, changes to host immunocompetency and pathogen exposure is a major component of migrations. Migrating adults have a marked increase in hormones associated with stress and gonadal maturation, specifically cortisol, testosterone, and estradiole (McCormick et al. 2013; Zwollo 2018). These hormones have been implicated in reduced immunocompetency through reduction in B lymphocyte and IgM+ antibody response (Zwollo 2018), and recent work has demonstrated increases in pathogens corresponding to the duration of spawning migration in Pacific salmon (Bass et al. 2017). Migrating salmonids also school and move through heterogenous environments, likely interacting with a diversity of habitats and species that may act as vectors for pathogens while in dramatically higher density, elevating exposure risk from conspecifics. In contrast, evidence suggests out migrating smolts may increase expression of genes associated with certain components of innate immunity, perhaps to prepare for exposure to novel pathogens (Yada 2007; Sutherland et al. 2014). In contrast, down-regulation of cellular immune associated interferons has been observed in Pacific salmon species (

Similarly, it is only beneficial to have evolved defense to pathogen species likely to be encountered within a lifetime. It could then be expected that discrete populations of the same species living in different habitats may have unique immunological profiles associated with regional pathogen species. This is supported by substantial evidence demonstrating the heritability of both behavioural (Kortet et al. 2010) and immunological

(Eizaguarrie and Lenz 2010) defense processes, resulting in what is known as localized adaptation (Grandstaff et al. 2003).

1.4 Human and climate change associated stressors

The stress response is an adaptation allowing animals to respond maximally to threats that imposed selection pressures over the course of their evolution. Human and climate change associated factors, however, are changing the natural dynamics of stressors that fish face in aquatic environments. With respect to host defense and/or pathogen infection dynamics, stressors may act independently or cumulatively or synergistically depending on the ecological context in which they are experienced (Miller et al. 2014). Yet given the high probability of an individual harbouring infection from at least one pathogen (Sofonea et al. 2015; Kotob et al. 2017), it is reasonable to assume any additional stressor is cumulative in the context of wild fishes.

Fisheries stressors include exhaustive exercise, air exposure, and physical injury from contact with fishing gear (hooks, nets) and handling (reviewed in Raby et al. 2015). These interactions can leave released fish physiologically compromised (reviewed in Davis 2002; Wilson et al. 2014) and potentially vulnerable to pathogen infection and disease progression (Lupes et al. 2006). How a capture-and-release interaction may influence disease-related mortality is termed “capture-mediated-pathogen-induced mortality”, and while estimates of post-release mortality in general have been determined for many commercial harvest salmonids (reviewed in Patterson et al. 2017), the level of pathogen-induced mortality that occurs in the wild is unknown. Stress and injury associated with fisheries capture may provide opportunity for pathogens present within

the captured fish to increase in virulence (Sofonea et al. 2015), or reduce physical barriers to entry for pathogen transmission (Kanno et al. 1990).

Another human associated impact is global climate change, which has resulted in increased temperatures in both marine and freshwater environments and is expected to continue to impact global fisheries for the foreseeable future (IPCC 2019). Thermal stress acts directly on migratory salmonids, particularly in the freshwater environment (Wilkie et al. 1997; Crossin et al. 2008; Eliason et al. 2011), which can have substantial metabolic consequences when temperatures approach or exceed thresholds of thermal tolerance (Fry 1971). A vast body of research has addressed the potential impacts of increased water temperatures on migratory salmonid physiology and survival (Crossin et al. 2008; Jonsson and Jonsson 2009; Martins et al. 2011, 2012; Fenkes et al. 2016). Thermal stress can reduce swimming capacity (Eliason et al. 2013), and has been linked with substantial increases in pre-spawn mortality in semelparous species (Martins et al. 2012) and post-spawn mortality in iteroparous species (Jonsson et al. 1997; Berg et al. 1998).

Temperature impacts aquatic environments directly through physiological mechanisms as described above, but also indirectly by facilitating range expansions in species previously excluded from environments based on the lower ranges of thermal tolerance (Cheung et al. 2013; Fossheim et al. 2015). When considering pathogens, incoming species may act as vectors, bringing new pathogens in to naïve host populations that have limited immune response (Crowl et al. 2008). While some research has demonstrated invasive species "leave their enemies behind" and so are a limited source for pathogen transmission (Torchin et al. 2003), the likelihood of transmission between endemic and invasive species increases among closely related groups such as salmonids,

where many agents are shared among taxa (Miller et al. 2014). Consequently, human induced climate change can have far-reaching ramifications beyond the immediate effect of local thermal tolerance, and it is crucial to have a baseline understanding of pathogen dynamics in endemic populations.

1.5 Hypotheses and objectives

The objective of my dissertation is to investigate the context-specific dynamics associated with infectious agent communities by using three species of wild salmonids as models representing different life-history strategies and environments (Figure 1-1). The overarching hypothesis of this thesis is that host response is associated with species specific phenology and infection burden, and that external stressors also mediate host-pathogen dynamics (Figure 1-2). Moreover, individual infectious agent communities are expected to vary across species, and within species across individuals and/or locations when applicable. Different sampling methods and experimental techniques were used to address species- and context- specific research objectives. By integrating multiple field sampling methods with molecular genomic techniques, this work contributes to the understanding of interactions among salmon phenology, infectious agent communities, and host response to stress and infection in wild, free-ranging salmonids.

The work presented herein addresses several unique objectives. The second chapter provides a detailed synthesis of the context of etiological research in wild fishes, focusing on novel research methods and how they can be deployed in tandem with commonly used fisheries research methods to address complex ecological questions. The specific challenges and limitations of attempting to characterize the effect of pathogens

on wild fishes are outlined in detail, providing context for the following data chapters. The first data chapter (Chapter 3) investigates the individual context of host-pathogen dynamics and localized adaptation in a long-lived iteroparous species. To do so, I sampled three populations of Arctic char *S. alpinus* in the high Arctic and characterized infection burden and immune response profiles, providing the first comprehensive pathogen screening for microbial infectious agents in this species. The next data chapter (Chapter 4) incorporates temperature as a natural stressor to investigate how additional stressors may change host-pathogen dynamics using Atlantic salmon *S. salar*, an iteroparous species that demonstrates low post-spawn survival, as a model. Here I was able to also include temporal shifts in host immunocomptency and pathogen communities over time to monitor disease progression and changes to immune function. Rather than using telemetry, this chapter demonstrates how migratory behavior can be harnessed to create free-ranging holding scenarios in an experimental setting. Finally, the effect of anthropogenic stressors and potential for fisheries-mediate-disease-induced mortality was experimentally investigated (Chapter 5). Here, the role infectious agent communities play in the context specific responses to fisheries stressors was investigated by combining experimental fisheries treatments and molecular screening techniques with radio telemetry, using coho salmon *O. kisutch* as a model. This research also characterizes individual and temporal variation in host immunocompetency and pathogen communities that occurs during senescence and spawning migrations in a semelparous species. In the general conclusion (Chapter 6), I synthesize findings and provide a comparative summary of data collected as part of this research and discuss future directions for relevant research. Collectively, the research presented through this work provides further evidence

of host- and context- specific responses to infectious agents and enhances current understanding of infectious agent distributions in wild salmonids across Canada.

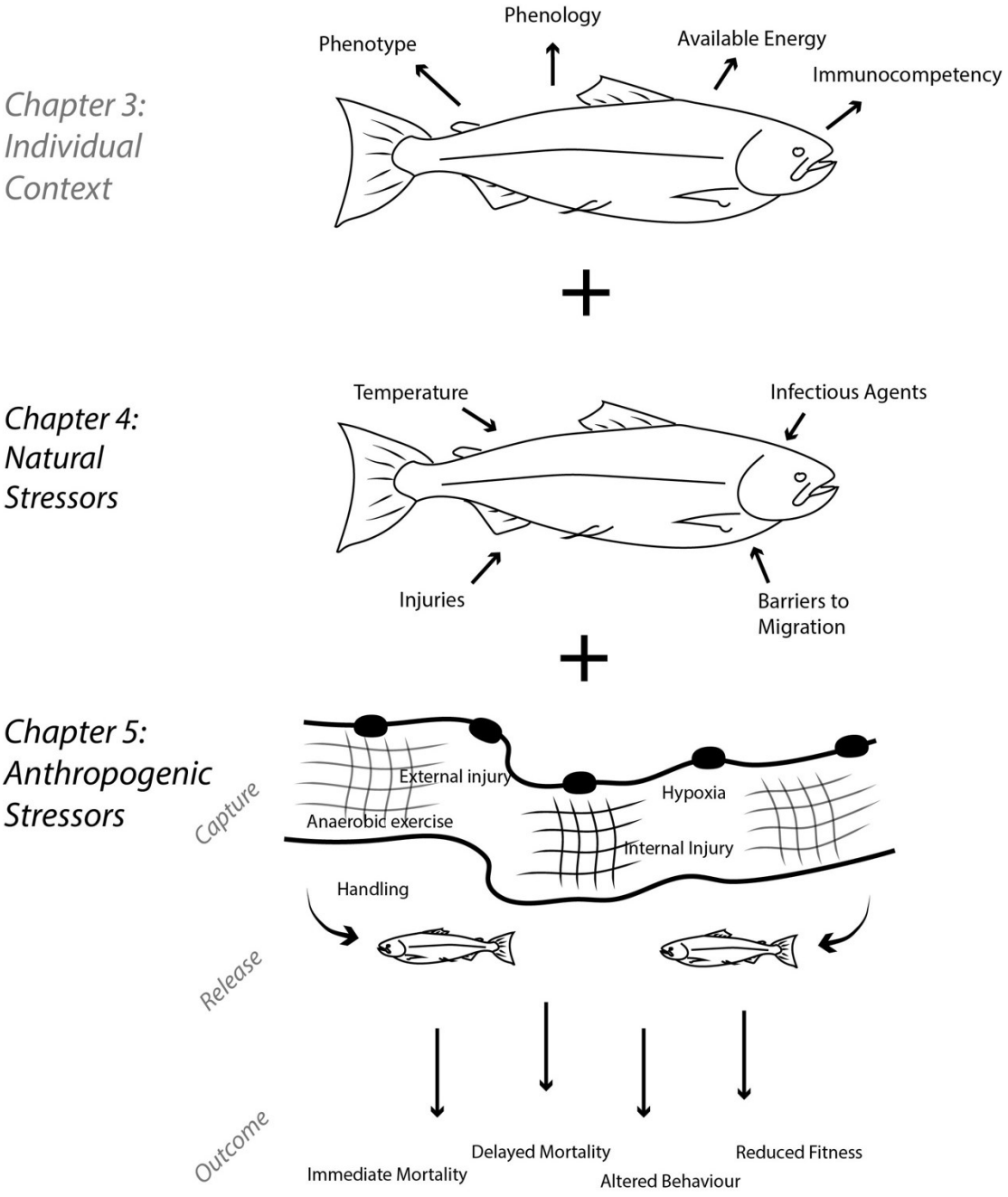

Figure 1-1 Conceptual diagram outlining potential factors mediating host-pathogen dynamics in salmonids, specific aspects of which are addressed within each data chapter.

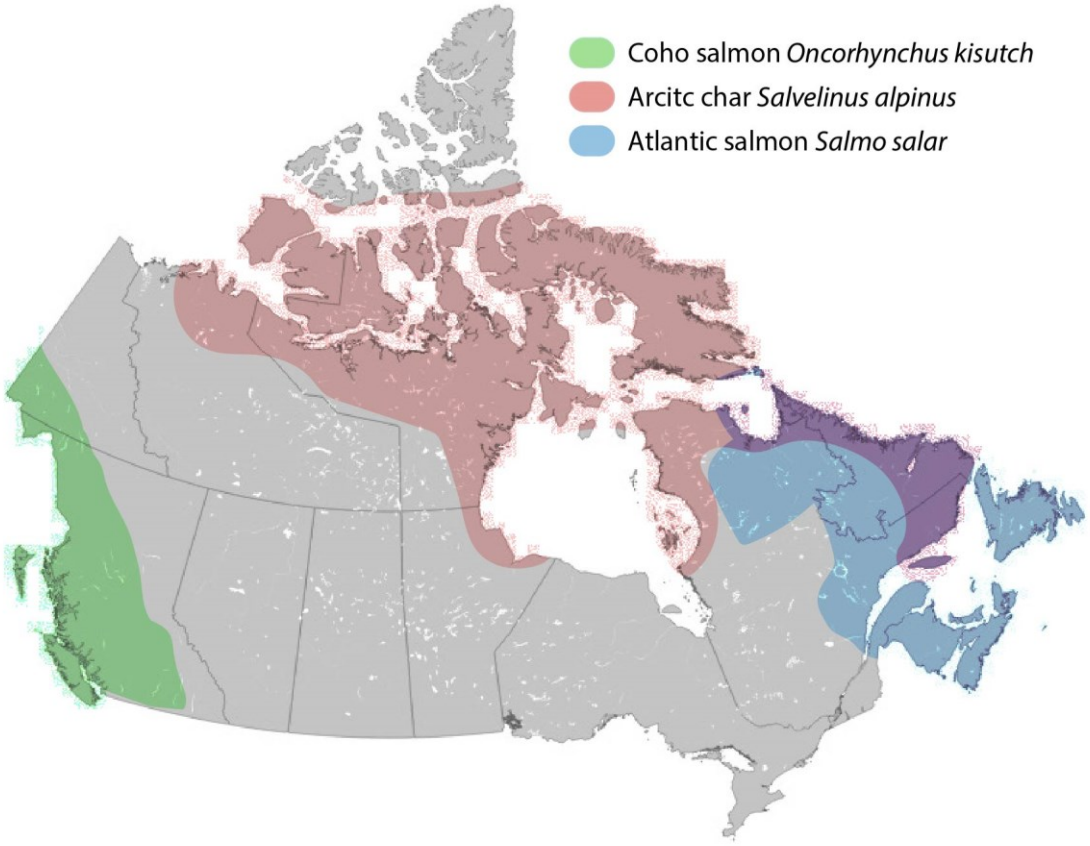

Figure 1-2: Distribution map of salmonid species sampled in this thesis to investigate the context-specific nature of host-pathogen infection dynamics and host response. Purple region indicates overlap between Arctic char and Atlantic salmon distributions.

Chapter 2: **Disease ecology of wild fish: Opportunities and challenges for linking infection metrics with behaviour, condition, and survival.**

2.1 Introduction

Aquatic ectotherms such as fishes are especially vulnerable to infectious agents; environmental factors such as elevated water temperatures can reduce host resistance (Jeffries et al. 2012) and increase infectious agent abundance (Paull and Johnson 2014) or virulence (Thomas et al. 2003, Teffer et al. 2019), enhancing the likelihood of disease development (Snieszko 1974). Infectious diseases have already been implicated in the decline of some wild fish populations (Steinbach Elwell et al. 2009; Gibson-Reinemer et al. 2017), but the mechanisms and scales by which they reduce these populations remain dubious (Hellard et al. 2015). This is perhaps because a great deal of the current understanding of aquatic disease ecology is garnered from experimental studies (Hellard et al. 2015) or observations of disease from aquaculture, particularly for microparasite species (Austin and Austin 2016). Fisheries epidemiology is unlike that of terrestrial systems due to its greater host species diversity, spatial complexity, speed of transmission, and the lack of observational data (McCallum et al. 2004). It is extremely challenging to collect individuals that are experiencing disease in aquatic ecosystems; moribund fish are likely vulnerable to predation, while mortalities are scavenged, sink to depths challenging to sample, or decompose quickly and thus of little use for examination (Herman 1990). These challenges have led fish disease ecologists to rely heavily on knowledge of infectious agents and host response garnered from cultured fish, where mortality, morbidity, and disease states are more easily observable, isolated, and examined. Application of these findings to wild populations, however, is precarious given

that human intervention has caused substantial geno- and phenotypic divergence of cultured fish from their wild ancestors. Indeed many artificial breeding programs have created phenotypes that are completely absent from wild populations (Blanchet et al. 2008; Christie et al. 2012) which greatly reduces the relevance of these data in wild contexts.

One of the major knowledge gaps present in research on disease in wild fish is the incidence of co-infection (Sofonea et al. 2015; Kotob et al. 2017). In the wild, fish are chronically exposed to a heterogenous composition of infectious agents and are rarely burdened with a single pathogen at a given time, which can directly mediate host-pathogen dynamics (Hellard et al. 2015). In experimental work, presence of infectious agents that are not the focus of study is often viewed as a nuisance or inconsequential and thus ignored (Kotob et al. 2017). However, co-infection is a natural characteristic of wild animals, and incidence of co-infection has been identified in virtually all host taxa comprehensively examined (e.g. Pacific salmon *Oncorhynchus* spp.; Miller et al. 2014; Bass et al. 2017, Thakur et al. 2018), European grayling *Thymallus thymallus* (Pylkkö et al. 2006) and rainbow trout *Oncorhynchus mykiss* (Bandilla et al. 2005)). Bacterial species have been shown to opportunistically infect fish after the epithelial layer is compromised by the entry of other pathogens (Kanno et al. 1990), often termed 'secondary infection' (Pylkkö et al. 2006). Theoretical modelling suggests within-host pathogen interactions determine virulence, recovery, and transmission rates for individual agents (Sofonea et al. 2015), yet few empirical studies have demonstrated such phenomena (reviewed in Kotob et al. 2017).

The aforementioned constraints have contributed to major gaps in the research body addressing disease ecology in wild fish. However, advances in research technologies in both fisheries science and wildlife epidemiology have opened the door for comprehensive studies to investigate multiple scales of fisheries epidemiology in the wild. By strategically combining research methods and fostering collaboration among disciplines, I argue that infectious agent dynamics should be incorporated into investigations of fish ecology and behaviour. In the present article I briefly outline the current application of several novel research methods in fish epidemiology and behavioural ecology, present areas of uncertainty and knowledge gaps that may be addressed through interdisciplinary collaboration, and highlight research opportunities in the context of free-ranging wild fishes. Parasites are defined here as species that have the capacity to negatively impact host fitness, the severity of which is termed the parasite's virulence. I use the terms parasites and pathogens interchangeably throughout to refer to any disease-causing infectious agent (though some parasites may be commensal, leading to discussions on the merit of the term pathogen; see Méthot and Alizon 2014). The focus of this review is endoparasitic infections of both micro- and macro- parasites, however concepts within can be applied to an array of species in both aquatic and terrestrial environments.

2.2 Context of wild fish epidemiology

Wildlife disease research is often focused on pathogens that hold high risk factors for human infection. Helminth parasites - trematodes, cestodes, and nematodes - are the most common etiological agents of human infection from fish borne pathogens (Sangaran

et al. 2016). Humans act as either the definitive host, where sexual reproduction of the parasite occurs, or more often as incidental hosts that are not typical for the parasite's life cycle. Tapeworms of the genus *Diphyllobothrium* are transferred to their definitive host, including humans, through consumption of larval stages found in numerous species of freshwater fish (Kuchta et al. 2013). The nematode *Anisakis simplex,* found in marine and diadromous species including salmonids, infect humans as an incidental host, causing gastrointestinal disturbance through questing behaviour in the gastrointestinal lamina (Audicana and Kennedy 2008). Both *Diphyllobothrium* spp. and *A. simplex* cause significant pathology in humans and continue to be studied extensively. Comparatively, microbial agents (viruses, bacteria, fungi, and protozoans) of fishes are generally less likely to spill over in to human populations. Low contact rates between humans and fish reduce exposure, and host specificity prevents transmission from fish to human. While 75% of emerging human disease are zoonotic in origin (Slingenbergh et al. 2004), the vast majority of such transfers are from terrestrial species. Most human microbial infection traced back to aquatic organisms are associated with harvest from water bodies contaminated with human sewage (Iwamoto et al. 2010). In such cases, infectious agents are those for which humans are the main reservoir (e.g. *Salmonella* and *Shigella* spp.) and aquatic organisms are involved as incidental vectors (Iwamoto et al. 2010). Regardless, an understanding of disease ecology in aquatic and marine systems can aid in the development of theoretical frameworks and hypothesis testing regarding pathogen transmission and disease dynamics in terrestrial systems.

Parasitic helminths rarely cause mortality in their hosts and are much less likely to result in population-scale mortality in the wild, so while they may be of interest from a

human health perspective, they are typically not of great concern for wildlife conservation (Lafferty and Gerber 2002). From a conservation perspective, microbial pathogens (viruses, bacteria, protozoans, and fungi) are far more likely to negatively impact fish populations at a large scale (Bakke and Harris 1998). Yet because humans are generally not directly impacted by aquatic microbial pathogens that commonly infect fish, the limited knowledge of microbial pathogens in wild fish (specifically excluding aquaculture) is relatively constrained to research on agents that cause large-scale die offs, or those that may transfer to wild fish from aquaculture (Lafferty et al. 2017). Fisheries scientists often focus on infectious agents only after "fish kills", when mortality rates exceed consumption by predators and scavengers, sometimes resulting in high numbers of observed carcasses in waterways and on shorelines. Such events tend to be easily identifiable, can be catastrophic for isolated or small populations, and are of great public concern due to their shocking appearance. Unlike studying wild fish when pathogens are more typically distributed within the population, fish kills allow researchers to collect large numbers of mortalities to identify the causative agent (e.g. epizootic haematopoietic necrosis virus in Australian redfin perch *Perca fluviatilis;* Langdon et al. 1986; *Ichthyophthirius multifiliis* in freshwater species in the southern US; Allison and Kelly 1963; infectious hematopoietic necrosis virus in sockeye salmon *Oncorhynchus nerka* in Western Canada; Williams and Amend 1976; herpes virus in Australian pilchard *Sardinops sagax*; Murray et al. 2003). Factors that cause large scale outbreaks (e.g. thermal stress, introduced pathogens), which push disease rates over a threshold of visibility, are complex and difficult to identify or predict in many cases (Langdon et al.

1986; Herman 1990; Adlard et al. 2015). This is particularly true when previous abundance and prevalence rates are not known for the affected population.

Aquatic animal epidemiology is unlike that of terrestrial systems due to high spatial complexity in a three-dimensional environment, speed of transmission, and the lack of observational data (McCallum et al. 2004). An excellent example is viral haemorrhagic septicaemia virus (VHSV), a pathogen that is responsible for large scale die offs in over 30 species of fish from both freshwater and marine ecosystems globally (Escobar et al. 2016). VHSV is arguably one of the best studied wild infectious agents and may be present in as many as 140 species based on ecological niche modeling (Escobar et al. 2018). Research has identified susceptible species (Bowser et al. 2009; Gadd et al. 2011; Moreno et al 2015; Escobar et al. 2018), diversity in host specificity from VHSV genotypes (Ogut and Altuntas 2014), and risk areas based on geographical characteristics (Escobar et al. 2018). However, despite such an extensive body of knowledge, little is known about VHSV in the context of wild fish ecology, including consequences on behavior, phenology, and fitness. Furthermore, co-infection with other pathogens may modulate host susceptibility to and transmission of VSHV; co-infection was therefore recently highlighted as another major knowledge gap in the understanding of the role VHSV plays in aquatic ecology (Escobar et al. 2018).

2.3 Methods for identifying pathogens

Traditionally, screening for infectious agents in fish has been done using lethal sampling to assess clinical signs of disease, conduct histopathological assessment of diseased tissues, and identify macroparasites and microbial agents using microscopy

and/or culture (Austin and Austin 2016). Histopathology - the study of tissues for diagnostics – remains the gold standard of diagnostics, but is labour intensive, lacks sensitivity during early infection stages, and requires extensive specialized expertise that is typically reserved for veterinary doctors specializing in diseases (Figure 2-1). To assign an agent as causative of a disease, one must fulfill Koch's postulates, a cornerstone of epidemiology; suspected etiological agents are isolated from diseased tissue, cultured, demonstrated to cause the same disease in naïve hosts via inoculation/exposure, and again extracted and cultured from diseased hosts (Grimes 2006). To ensure high quality, accurate histopathology for disease research, fisheries researchers must therefore seek out such advanced training, engage in collaborations, or pay for diagnostic services. This is extremely prohibitive for many fisheries scientists based on availability of training, access to willing collaborators, and budget constraints.

More recently, molecular-based examination of tissues has vastly increased the speed and specificity of infectious agent detection in wildlife (Mendonca and Arkush 2004, Miller et al. 2016, Kralik and Ricchi 2017, Sana et al. 2018). Since the introduction of Polymerase Chain Reaction (PCR) in 1985 (Saiki et al. 1985; Mullis and Faloona 1987), DNA amplification and analysis has revolutionized methods in microbiology and is the foundation of a myriad of rapidly developing genomic technologies. To apply genomic techniques to research on infectious agents, host tissue samples are reduced to only the genetic material present (i.e., RNA or DNA). The result is a mix of host and pathogen DNA and RNA that is used in analysis. From here, several different molecular techniques can be applied independently or complementary, depending on the research question.

2.3.1 Metagenomic sequencing

Rapid advances in gene sequencing technology facilitated a cascade of molecular techniques and platforms that have greatly reduce time and cost of data acquisition. Next-generation sequencing (NGS) is now the most common high-throughput sequencing method that sequences many samples independently and simultaneously (Mardis 2008). It can be applied to any organism containing genetic material and is therefore a useful investigatory tool when investigating microbial pathogens (e.g. RNA viruses; Benton et al. 2015). Traditional NGS platforms produce millions of short sequences with read lengths between 50-300 bp (Mardis 2008); however, NGS technologies producing long reads (10 kb - >100 kb) such as MinION and PacBio have more recently been developed. There are many commercial NGS platforms available (e.g. Illumina, Life Technologies/Ion Torrent, PacBio, etc.) however for simplicity, the Illumina Whole Genome Shotgun sequencing (WGS) and 16S ribosomal RNA (rRNA) gene sequencing workflows will be referenced here. Both methods are useful for the identification and characterization of pathogenic agents, and can be used independently or in tandem. The WGS approach typically works by sequencing sample isolate (genetic material), and sequencing libraries are constructed from sheared DNA that is bound to sequencing adapters that interact with complementary regions on the surface of the Illumina flow cell. Illumina uses cluster generation to amplify the library, which is then sequenced in both directions (termed paired-end sequencing; Head et al. 2014). 16S rRNA gene sequencing differs in that sequencing libraries are not constructed from sheared DNA, rather, they use a targeted approach using primers specific to the 16S rRNA gene that is

then amplified within a sample to create the sequencing library and sequenced as described for WGS. The 16S rRNA gene is used here as a genetic marker as it is a highly conserved hypervariable region within the bacterial ribosome, and so present in all bacterial species, and can therefore be used for bacterial taxonomy-related studies (Woese 1987). Following sequencing and quality filtering, sequence data is then aligned to a reference genome, if available, or assembled *de novo* using contig construction for WGS sequencing (Mardi 2008). Contigs can then be searched through public genetic sequence databases, such as GenBank, using the Blast Local Alignment Search Tool (BLAST; McGinnis and Madden 2004). 16S rRNA sequence data is typically assigned to an operational taxonomic unit (OTU) based off a 97%-99% similarity of sequences and then assigned taxonomy using a reference database such as SILVA, therefore may have a more difficult time differentiating closely related bacterial species (Fox et al. 1992, Acinas et al. 2004).

The molecular resolution of 16S rRNA sequencing is lower than WGS, therefore it is not always useful in detecting pathogenic species or strains that have little differentiation (Grutzke et al. 2019). It is, however, a useful tool for characterizing complex microbial communities and can offer insights into how communities shift in response to disease expression in an organism (Bartram et al. 2011, Li et al. 2016), or for determining phylogenetic relationships between bacteria models (Joung and Cote 2002). WGS is a useful investigatory tool in pathogen outbreaks, as the whole genome of the microbial pathogen is characterized and therefore can provide in-depth information on virulence factors, resistance genes, strain characterization and other features related to pathogenesis (Sridhar et al. 2012, Tyagi et al. 2019). Duchaud et al. (2007) published the

first complete genome of a fish pathogen, *Flavobacterium psychrophilum*, providing a functional analysis of virulence mechanisms employed by the pathogen. A caveat to this method is that it is difficult to assemble large genomes and so this method is largely limited to relatively small genomes such as bacteria and viruses, though nematode genomes have also been sequenced (Ghedin et al. 2004).

RNA-sequencing is another application of NGS technologies and is a useful molecular tool for the study of pathogens and host-pathogen interactions that looks at transcriptome profiling (Ozsolak and Milos 2011). Here, libraries are constructed from complimentary DNA (cDNA) before sequencing (Wang et al. 2009). Following sequencing, transcripts are assembled either using reference transcript annotations or *de novo* assembly and then gene expression can be estimated use qPCR, and downstream applications such as differential gene expression can be compared (Kukurba and Montgomery 2015).

Genetic based molecular methods have evolved rapidly and provide a practical way for researchers to investigate pathogens on a larger scale than previously possible. Researchers can design qPCR assays for virtually any pathogen for which sequence data is available, including micro-organisms (Kralik and Ricchi 2017), or use the Basic Local Alignment Search Tool (BLAST) tool to match sequences extracted from samples with a database of genetic material (e.g. GenBank; McGinnis and Madden 2004; Soldánová et al. 2017). With today's speed and decreasing cost of gene sequencing and the development of sequence repositories such as GenBank, it is now possible to design studies that use comprehensive molecular techniques to identify suites of pathogens simultaneously.

2.3.2 Quantitative PCR

Quantitative polymerase chain reaction (qPCR) is a commonly used tool in diagnostic laboratories and has arguably become the 'new norm' in diagnostics (Austin and Austin 2016; Kralik and Ricchi 2017). An excellent guide to the design, optimization, and validation of qPCR for diagnostics is provided in detail by Kralik and Ricchi (2017). In brief, primers are designed to match genetic sequences of target organism genes (in this case, infectious agents) and various qPCR methods can quantify the amount of target genetic material within each sample (e.g. probe fluorescence denoting amplification of the target sequence during thermocycling). While not directly comparable to pathogen abundance (except in the case of single copy genes assessed in bacterium or virus), relative abundance of genetic material can be linked with pathogenicity through in situ hybridization and histopathological validation (Miller et al. 2014; de Cicco et al. 2018). Depending on the methodology and equipment employed, qPCR can detect the presence of infectious agents at extremely low infection levels, potentially prior to observable clinical disease (Miller et al. 2017). Such sensitivity also allows for the detection of pathogens that are in low abundance or heterogeneous distribution within the host and thus low probability of being detected by microscopy or histological examination (Cavender et al. 2004).

To determine co-infection rates, individuals must be screened for the presence of multiple infectious agents, a previously time-consuming and expensive task. This can now be achieved quickly and cost-effectively using high-throughput quantitative PCR (ht-qPCR), which runs dozens of assays against dozens of samples simultaneously. For

example, assay panels for dozens of microbial pathogens (viruses, bacteria, protozoans, myxozoans, and fungi) have been created to monitor disease salmon populations off the coast of British Columbia, Canada (Miller et al. 2016). Tissue samples from multiple organs are collected from fish sacrificed as part of the monitoring program, and sample RNA and DNA is extracted for ht-qPCR screening. As many as 80 individual samples can be screened simultaneously for the presence and relative load of 47 infectious agents (Nekouei et al. 2019; Miller et al. 2016). Based on normal working hours, approximately 27,000 PCR reactions can be run in two days when starting from isolated nucleic acids, or about 360 samples screened against 47 duplicate assays (Miller et al. 2016). For a full list of strengths and weaknesses of using a platform such as BioMark see section 7.9 in Miller et al. (2016).

Several studies have successfully applied ht-qPCR to study disease ecology in wild salmon. A survey of 45 infectious agents from adult Chinook salmon *O. tshawytscha* captured during spawning migrations from five stocks detected 20 microparasite species, several of which were new records for the species and/or region, high rates of co-infection, and stock-specific pathogen communities (Bass et al. 2017). Comparison among hatchery and wild stocks screened for 36 infectious agents from juvenile Chinook salmon *O. tshawytscha* found lower pathogen diversity in hatchery reared fish during freshwater phase, however diversity converged with time spent in the marine environment (Thakur et al. 2018). A ten-year study of coho salmon *O. kisutch* of hatchery and wild origin identified 31 microparasites present of the 36 species screened, however there were no consistent differences between hatchery and wild stocks in the marine environment (Nekouei et al. 2019). These studies together not only refute the

hypothesis that hatchery fish are a significant source of pathogens for wild stocks, but perhaps more importantly provide comprehensive baseline data for infectious agent prevalence in the region. These findings facilitate monitoring for novel agents as the risk of potential spread increases with intensified aquaculture and climate change.

The high specificity and sensitivity of qPCR is a both a strength and a potential limitation depending on the context within which it is applied. The often heterogeneous distribution of infectious agents among host tissues creates inherent variation in the amount of genetic material present within a given biopsy (Teffer et al. 2019). While qPCR is superior for detecting the presence of infectious agents, histopathology remains a more reliable way to quantify infection severity and tissue damage (Cavender et al. 2004; Miller et al. 2016) and is useful for ascribing aetiology (e.g. piscine orthoreovirus and heart and skeletal inflammation; de Cicco et al. 2018). High specificity also means it is necessary to have an understanding of what infectious agents are likely to be present and have access to gene sequence information to design effective primers. Consequently, pathogens may be missed if they are not included in the screening panel.

Molecular techniques are also being used on host tissue to determine the physiological response of fish to infectious agents. DNA microarray analysis of viral disease infected individuals has revealed numerous genes induced during infection (Jorgensen et al. 2008; Workenhe et al. 2009; Krasnov et al. 2012). Similarly, qPCR was used to differentiate gene expression profiles between infected and non-infected survivors of viral disease in Pacific salmon smolt (Jeffries et al. 2014). More recently, researchers have been investigating the occurrence of unknown pathogens by using qPCR to characterize the transcriptional response of fish that have been experimentally infected

during challenge studies. (e.g. Viral Disease Development panel based on infection with IHNv; Miller et al. 2017). Samples that demonstrate the same patterns in immune response that do not test positive can then be assessed using NGS to identify previously uncharacterized viruses. This technique has been successfully used to identify three new viral species in Pacific salmon (Mordecai et al. 2019).

While extremely powerful, there are significant up-front costs associated with developing broad scale qPCR screening programs. Knowledge and skill in molecular techniques is a necessity, and operational costs can be significant depending on the number of samples being run. Samples must be collected as sterile as possible, a feat that can be extremely challenging in field settings. For an effective qPCR survey of infectious agents, fish collection and necropsy will also require asceptic methods, something that can be challenging in the field. Finally, it is crucial to recognize that the relative load of infectious agent genetic material present in a tissue sample does not necessarily indicate the level of tissue damage or disease present. While further molecular advances holds promise for applications in diagnostics, at the present time necropsy and histopathology are thus still required to verify disease state.

2.4 Nonlethal sampling for infectious agent screening

2.4.3 Broad-scale screening of the environment: Environmental DNA

Technological advances in gene sequencing and detection have allowed researchers to screen for infectious agents non-lethally. For broad scale detection, environmental DNA, or eDNA, is a molecular approach that has recently been developed and increasingly implemented to identify species presence from abiotic samples (i.e.,

sloughed genetic material in water, soil) (Bohmann et al. 2014). In aquatic environments, sampling regimes are so straight forward that eDNA has been touted as "biodiversity for the lazy" (Hoffmann et al. 2016), and in some cases samples taken by trained citizen scientists are as high quality as those taken by highly experienced genetic researchers (Julian et al. 2019). For biodiversity assessment of free-living vertebrates (amphibians and fish), well designed eDNA study protocol were more accurate for detecting aquatic species than traditional survey-based methods (Valentini et al. 2016). When surveying for pathogens, extracting genetic material from water samples means that there is no need to capture and necropsy target host species and isolate diseased tissues, greatly reducing what can be intensive sampling and permitting regiments. This is particularly important when investigating rare species, either host or pathogen, that require the examination of > 30 individual hosts (Julian et al. 2019). Using DNA for identification can also be highly species-specific depending on assay design, reducing error or uncertainty for species that are extremely difficult to identify based on morphology. Typically, eDNA is most often used to estimate the presence or abundance of rare or invasive species or assess overall diversity of a given habitat (Bohmann et al. 2014). One caveat, however, is that qPCR will not differentiate whether a pathogen is viable. Given that accurate and timely detection of highly pathogenic infectious agents is critical for initiating any mitigation measures, eDNA provides an extremely powerful tool for aquatic wildlife conservation in epizoonotic outbreaks or novel pathogen invasion.

eDNA has been successfully used to detect pathogenic nematodes (*Ribeiroia ondatrae;* Huver et al. 2015), *Ranavirus* (Miaud et al. 2019), and pathogenic bacteria (*Batrachochytrium dendrobatidis*, the causative agent of chytridiomycosis; Kamoroff and

Goldberg 2017) that infect freshwater amphibians. In fish, eDNA has been successfully used to screen for the spread of the mesomycetozoean *Sphaerothecum destruens* in Europe as it is carried to new habitats by an invasive fish (Sana et al. 2018). Screening for *Gyrodactylus salaris*, a monogenean highly pathogenic in Atlantic salmon, from water samples in Norway was also successful for detecting pathogen presence (Rusch et al. 2018). For applications in aquaculture, eDNA screening for pathogens from water samples has undergone preliminary testing in both freshwater (Gomes et al. 2017) and marine (Peters et al. 2017) environments with encouraging results.

In most cases, authors suggest that further development and refinement of eDNA tools is required before eDNA alone can replace current detection methods (e.g. pathogen abundance, Rusch et al. 2018), but believe it will reduce cost, enhance monitoring, and expedite risk-reducing measures in the event of positive detections. This is demonstrated by the screening for *B. dendrobatidis* in lakes in Sequoia King National Park in California, USA. The first detection of the agent was four weeks prior to large-scale die-offs of amphibians from chytridiomycosis in all systems it was detected in (Kamoroff and Goldberg 2017). This buffer between detection and observed mortality may allow for mitigative measures to be launched, such as limiting access to the infected body of water to prevent spread of the pathogen or applying treatment before transmission and/or disease associated mortality reaches critical thresholds. eDNA may also be applied to pathogen screening in aquaculture or host species re-patriation efforts by testing for the presence of infectious agents prior to transfer in to open-net pens or release (Sana et al. 2018). Such efforts may restrict the transfer of infectious agents from cultured to wild

fish, a factor that is currently a major conservation concern for global fisheries (Lafferty et al. 2016).

There remain limitations and considerations for the use of eDNA in infectious agent monitoring. eDNA most reliably confirms only the presence or absence of target species in the environment (Hoffmann et al. 2016). However, DNA persists in the environment for as little as 48 hours (Rupert et al. 2018) to much as 24 days (Goldberg et al. 2016) depending on cellular material and conditions (e.g. UV exposure, temperature, and/or water pH) limiting fine scale spatiotemporal interpretation of species' presence. DNA can also be transported to the area naturally (e.g. predator feces) or artificially by watercraft, waders, etc. (Goldberg et al. 2016). Many infectious agents do not have high host specificity but do demonstrate species-specific pathogenicity (e.g. *G. salaris*; Rusch et al. 2018; *S. destruens*, Sana et al. 2018). In the case where a single host species is the focus of the work, it may then be necessary to also screen for potential reservoir hosts known or suspected to be present in the system (Rusch et al. 2018). In addition, many pathogens have complex life cycles that include multiple hosts and free-living or dormant stages (Marcogliese 2004). Positive detection based on eDNA sample does not provide detail with respect to what species are infected, if infection rates are causing meaningful disease in host species, or even if suitable host species are present in the environment at all (Huver et al. 2015). This also includes trophically transmitted pathogens that may be deposited in the environment (e.g. *Diplostomum* spp. in bird feces; Marcogliese 2004) but unable to establish because of a missing host required for the complete life cycle. Consequently, there are limits to the conclusions that can be made based on the presence of the pathogen alone, especially in the absence of long-term sampling. Refined

methodology may improve the correlation between eDNA copy number and pathogen abundance for implementation in monitoring (Huver et al. 2015; Rusch et al. 2018). Until then, the use of eDNA best complements more traditional methods (Julian et al. 2019) for quantifying species prevalence and abundance, for example by streamlining focus for biological sampling efforts.

2.4.4 Non-lethal biopsies of individual hosts using rt-qPCR

The incorporation of nano-technologies such as microfluidics in rt-qPCR platforms has greatly reduced the amount of tissue that is required for DNA/RNA extraction (e.g. Fluidigm Biomark®; Miller et al. 2016; Teffer et al. 2019). For the BioMark rt-qPCR platform, tissue samples can be as small as 2 mm^3, an amount that can be taken without causing serious harm to the fish (Tavares et al. 2016; Figure 2-2; C). Swabs of the extracellular mucosa can also be taken from external tissues or inserted into the buccal cavity or vent to investigate the presence of pathogens in the alimentary tract (Figure 2-2; D). Indeed many methods currently used to collect and screen tissue lethally could be refined and transitioned to non-lethal (e.g. swabs; Aamelfot et al. 2015), and techniques employed to investigate fish microbiomes are directly transferrable to infectious agent screening (e.g. Hamilton et al. 2019).

While there are considerations associated with using a small tissue sample to represent the pathogen community within the entire host, recent research found a single gill biopsy contains transcripts shed by pathogens infecting internal organs (Teffer et al. 2019). Gill swabs, used in conjunction with rt-qPCR assays, have been used for early detection of the amoeba, *Paramoeba perurans,* responsible for amoebic gill disease

(AGD) in aquaculture settings (Downes et al. 2017). Swabs may also be used to collect mucus from the surface of fish to identify fish pathogens. For example, epithelial mucus swabs to detect Largemouth Bass virus (LMBV) using both conventional and quantitative PCR has been successfully validated (Leis et al. 2018). Consequently, a single sample can provide a snapshot of highly detailed information regarding the infectious agent community on or within the host. This is a major advance for fisheries research by providing the first opportunity for non-lethal screening of individuals for multiple infectious agents. As a result, a myriad of research questions to better investigate broad and fine scale infection dynamics, host responses, and the mechanisms associated with disease outcomes can be addressed for the first time by integrating rt-qPCR screening or NGS in experimental designs and monitoring programs.

2.5 Incorporating pathogen screening in wild fish research

2.5.1 Research Methods

Conventional research methods for investigating the behaviour and ecology of fish in the wild are extremely variable and context-specific. Here I provide a general summary of common research methods that may be useful for investigating infectious agents, and where screening for infectious agents may be informative and easily incorporated into current research programs. Pathogens and subsequent disease can influence host physiology, behaviour, and survival, which can be investigated either independently or in combination (Figure 2-2).

2.5.1.1 In Situ Confinement

In situ confinement or isolation is a cost effective way to investigate temporal shifts in pathogens without the need to transport wild fish to artificial holding tanks. The

ideal scenario for such research is large, isolated areas such as experimental lakes that do not require artificial barriers to separate and confine fish from the environment. Access to such facilities is unique and often not available or applicable to many fish species (e.g. pelagic or migratory species). In such cases, in-system mesocosm or net-pens can be constructed to confine study organisms to a specific area that is monitored or experimentally manipulated in some way. However, artificially constraining fish can have detrimental effects associated with holding stress (see Portz et al. 2006 for a detailed review of holding stress and recommendations for enclosure design) and consequently alter natural infection dynamics. The severity of holding stress can vary depending on the size, behaviour, life-stage, or biology of the study species. For example, smaller species or those with small home-ranges may not be immediately affected by confinement given that fish density does not induce competition-associated stressors (Wedemeyer 1996). Confinement of migratory species in particular can exacerbate holding stress, as individuals unable to execute migratory movements may challenge the integrity of holding materials by jumping or ramming in to containment structures (Cooke et al. 2003; Donaldson et al. 2011). Indeed preventing experimental fish from escaping is often more difficult than anticipated in such studies.

For research investigating pathogen dynamics, holding studies may increase the likelihood of transmission among confined individuals (Teffer et al. 2017). While this can be mitigated by ensuring low host densities and high rates of water exchange, it is not possible to exactly match the natural environment. For example, confinement may prevent natural prophylactic behaviours that reduce transmission under natural conditions (Binning et al. 2017). This is a major consideration when seeking to apply data obtained

in holding studies to wild fishes and the suitability of the study species for confinement and alterations to natural pathogen transmission pathways must be scrutinized to ensure study design addresses the research objectives.

2.5.1.2 Tagging studies

Characterizing ecologically relevant aspects of disease requires that there be as little intervention with normal host behaviour and physiology as possible. Currently, the best method available to study fishes *in situ* is through tagging or telemetry research (Cooke et al. 2013). Tagging allows researchers to individually identify fish with uniquely coded tags that are either passive, i.e. do not emit any signal, or active, such as radio or acoustic tags. Passive tags are typically used in situations where fish will be observed or recaptured, while actively transmitting tags mean the fish can be tracked remotely using receivers (see Cooke et al. 2013 for a review of telemetry applications in fisheries research). There are many potential applications of infectious agent screening in fish tagging studies, and new stabilizing solutions such as RNA Later®, and the small amounts of tissue greatly reduce the time and space required for sample storage. This provides the opportunity for samples to be taken from all tagged fish and examined in detail only if necessary, reducing cost and allowing flexibility based on findings. Below I will cover current knowledge and research opportunities for applying non-lethal infectious agent screening to fish tagging studies.

Passive tags include varieties of external and internal tags that do not emit a signal, requiring that the fish is either recaptured or the tag is directly interrogated by decoding equipment within short range (e.g. Passive Integrated Transponder arrays, Castro-Santos et al. 1996; Gibbons and Andrews 2004). Research using passive tags is

often termed ‘mark-recapture’; a number of fish are tagged and released, and then information on individuals is obtained when tagged individuals are recaptured after time at liberty. Population estimates are determined based on the proportion of tagged individuals captured after a concerted sampling effort. Studies involving large populations or large distributions are generally long-term, taking place over the course of months to years.

To date I am not aware of any published studies on wild fish that have combined mark-recapture data with information on fish pathogens, yet this method is suitable to combine with disease screening in a multitude of systems and research questions. In any case where the presence of infectious agent(s) is expected to be associated with mortality, capture susceptibility, or a measurable physiological outcome expected to change over time (e.g. growth or morphometrics), screening for infectious agents can be extremely informative. Conversely, results from laboratory studies can be validated in the wild. For example, experimental research has demonstrated a positive relationship between the presence of cestodes (specifically *Schistocephalus solidus*) and three-spined stickleback *G. aculeates* growth (Arnott et al. 2000), yet whether this relationship is an artifact of laboratory conditions and access to high-quality feeds is unknown (Barber and Scharsack 2010). A simple mark-recapture study could be designed to record changes in growth and relative load of cestodes by screening using non-lethal anal swabs. Mark-recapture studies are an excellent low-cost method for assessing spatiotemporal changes in infectious agent communities within individuals in study systems where recapture is expected.

Technological advances in hardware, software, and battery engineering has revolutionized aquatic animal tracking, making it one of the most powerful tools in fisheries ecology (Hussey et al. 2015). Tags are now smaller, last longer, emit signals farther, and can include on-board sensors (reviewed in Cooke et al. 2016) that record and transmit depth, tri-axial acceleration, or environmental parameters (e.g. temperature, dissolved oxygen). Consequently, fisheries scientists are able to use remote tracking to investigate an array of research questions at broader and finer scales than ever before, and relate observed movements to environmental factors and/or experimental treatments recorded from the fish's perspective. These advancements have facilitated the transition of numerous research questions out of the laboratory and into the wild, eliminating the potentially confounding effects of holding and observing fish directly, increasing the applicability of results in wildlife ecology.

It is intuitive that aspects of movement can be correlated with individual condition such as infection dynamics; infectious agents can change the physiological state of an animal, potentially manifesting as altered behaviours associated with movement (Dall et al. 2004) or likelihood of survival (Jeffries et al. 2012). Behavioural modifications in fish have been observed in many fish-pathogen interactions and can originate from either the host's response (e.g. prophylactic or therapeutic behaviours) or be the consequence of infection (e.g. adaptive manipulation of the host by the pathogen, reduced energetic capacity; reviewed in Binning et al. 2017). How such behavioural modifications can influence the interpretation of telemetry findings is contextual, depending on the scale at which the movement is recorded and biology of the species being studied.

2.5.2 Factors associated with fish survival

Quantifying natural and/or anthropogenically derived mortality rates in free-ranging fish is a central focus in fisheries conservation and management – such information is rare considering the challenges observing mortalities in the wild. Tagging and tracking fish provides the opportunity to infer mortality based on fish movement (or more specifically, lack thereof), and investigate factors mediating natural mortality rates in the wild, including infectious agents. The level of pathogen-induced mortality that occurs in the wild is unknown. The most direct association with natural mortality occurs when infection causes fatal disease; however, sublethal disease-state may interact with various stressors to influence post-release survival (Jupes et al. 2006). The physiological and physical perturbations associated with factors such as high water temperatures or fisheries capture (e.g. exhaustive exercise, physical abrasion, compression, and lacerations; reviewed in Patterson et al. 2017) may alter fish immunocompetency (Biro and Post 2008; Van Rijn and Reina 2010), exacerbating disease potential. Incorporating screening for infectious agents in telemetry studies investigating fish survival will result in more robust and comprehensive data that capture a critical component of wild fish ecology and evolution. Even a cursory understanding of the infectious agent community of individual tagged fish at the time of release may improve the interpretation of factors influencing post-release survival, particularly in cases where highly pathogenic agents are present. For example, a telemetry study on sockeye salmon *Onchorhynchus nerka* smolts tracked individuals during their ocean-bound migration, revealing high mortality during the first riverine portion of their migration (Clark et al. 2016). Combining further telemetry study with pathogen screening revealed that the presence of Infectious

Haematopoietic Necrosis Virus (IHNv) reduced the probability of an individual smolt surviving that same portion of migration (Jeffries et al. 2014).

Predation of tagged fish is a major consideration in telemetry studies because it results in movement or behaviour data representing that of the predator rather than the intended study animal (Thorstad et al. 2012). While it is possible to identify predated fish based on abrupt changes in movement and behaviour inconsistent with the prey species (Buchanen et al. 2013; Gibson et al. 2015), such analysis relies heavily on numerous assumptions about the consistency of species behaviours (Halfyard et al. 2017). Rather than attempting to infer predation from patterns in data, recent advances in tag technologies have incorporated mortality sensors that are trigged based on exposure to the gastrointestinal tract of the predator (Halfyard et al. 2017). Since they have been made available, predation tags have been used in only a handful of published studies, however major variation in predation rates has already been revealed. For example, over 50% of tagged Atlantic salmon smolts were predated post-release in the Mirimachi River in Eastern Canada (Daniels et al. 2019), while in the Great Lakes, no predation was detected in Atlantic salmon smolts released in Lake Ontario (Larocque et al. 2020).

Individuals that are weakened as a result of infection are believed to be more vulnerable to predation due to reduced swim speed and performance (Bradley and Altizer 2005), yet there is little empirical evidence demonstrating such phenomena. Laboratory research has also provided evidence for parasite-induced predation risk associated with behavioural manipulation of the host (Lafferty and Morris 1996), again something that has not been demonstrated *in situ*. Pairing infectious agent screening with predation tags can be used to investigate the occurrence of such phenomena in the wild. For example,

for species of conservation concern, it may be critical to know whether specific infectious agents, or suites of infectious agents, alter the likelihood of predation. From a theoretical standpoint, concepts of evolutionary stable strategies and optimality in host-pathogen systems (e.g. trophic transmission) could be tested to investigate the adaptive value of pathologies associated with infection.

2.5.3 Factors associated with fish behavior and personality

Better quantification of fine-scale movements using telemetry has revealed high rates of individual variation in behaviours in the wild. Differences in behavioural traits can be consistent among (behavioural traits) and within (behavioural syndromes) individuals (Sih et al. 2004; Dingemanse and Dochtermann 2012; Villegas-Ríos et al. 2017) and are considered an integral pathway for evolutionary processes within species or populations (Wolf and Weissing 2012). To be considered a trait or syndrome, observed variation should be repeatable and a result of both among-individual variance and within-individual consistency in behaviours measured across contexts (e.g. environmental conditions; Killen et al. 2016). Behaviours such as home range size, daily vertical migrations, and overall activity are common metrics used when defining behavioural traits from telemetry data (Taylor and Cooke 2014; Harrison et al. 2014; Villegas-Ríos et al. 2017).

Infection burden is known to influence fish movement and behaviour, thus movement data recorded during tracking and incorporated into behavioural traits or syndromes may reflect some portions of the role infectious agents play within the study population (Poulin et al. 2012; Killen et al. 2016; Binning et al. 2017). Large

ectoparasites can alter swimming behaviour, as observed in the decreased activity and increased metabolic demands in bridled monocle bream *Scolopsis bilineatus* infested with large ectoparasites (Binning et al. 2013). Movement that may be interpreted as "bold" or highly active behaviour is displayed by California killifish *Fundulus parvipinnis* infected with the trematode *Euhaplorchis californiensis* (Lafferty and Morris 1996); individuals with high infection display conspicuous swimming behaviour, making themselves 10-30 times more likely to be consumed by a predator (Shaw et al. 2010). Higher levels of "sociability" based on increased shoal cohesiveness and reduced motor activity has been related to the abundance of the sea lice *Argulus* spp., a horizontally transmitted ectoparasite infesting salmon (Mikheev et al. 2011). Activity and dispersal may also be subject to moderation by pathogens; Chub *Aqualius cephalus* infested with *Anodonta anatine* glochidia had reduced movement and dispersal compared to uninfected individuals (Horky et al. 2014). Considering that the above examples are only considering a single macroparasite in the context of infection, a great deal remains unknown regarding host prophylactic behaviours and/or the synergistic or antagonistic effects that co-infection and microparasites may have on fish movement.

In spite of the clear connection between movement and infectious agents, no telemetry studies specifically investigating fish behavioural traits have incorporated screening for pathogens. The potential effect of disease is often considered only a potential part of the contextual considerations when interpreting telemetry data rather than a meaningful explanatory factor (Killen et al. 2016). While behavioural traits must be consistent and repeatable, the scale at which repeatability is measured may influence how changes in infectious agent dynamics are influencing observed behaviour. Infection

profiles are heterogeneous within a population and likely to change over time, thus individuals that change in infection status during the course of the study (i.e. become infected or clear infection) may exhibit high behavioural plasticity.

2.6 Considerations

Microbial infections are known to have high spatiotemporal variability, and factors driving cycles of zoonoses are often unknown, complex, or unpredictable (Snieszko 1974; Herman 1990). This has presented challenges in research investigating the effects of specific agents on host biology, as repeatability among sampling events may not be possible. Host phenology, behaviour, seasonality, and environmental conditions can drive changes both host immune function (Bowden et al. 2007) and infectious agent dynamics (Altizer et al. 2006) and should be considered during study design when logistically possible. For example, macroparasite loads are generally positively correlated with host size and age, and trophically-transmitted pathogens will only be found in individuals large enough to consume the intermediate host (Marcogliese 2004). Where specific agents are the focus of research, knowledge of the pathogen life-cycle, distribution, and pathogenicity should dictate the timing and locations of sampling. Fish collection methods may introduce bias (e.g. size selectivity) that skew observations, potentially leading to erroneous conclusions. Certain agents may also influence host behaviour to make them more vulnerable to capture, or behavioural types may be more vulnerable to exposure in certain habitats. Such factors should either be controlled for through study design or considered during analysis and data interpretation.

When considering implementing non-lethal sampling to screen for pathogens using genetic techniques, whether or not genetic material from the pathogen is detectible in biopsied tissue should first be validated. For example, previously authenticated identification and/or quantification techniques (e.g. necropsy or histopathology) should at first be paired with planned sampling methods to ensure that 1. Positive detections align with confirmed cases and/or 2. amplification curves can be associated with agent abundance. Because genomic tools are more sensitive than classical identification methods, a higher number of positive cases may be observed compared to traditional parasitological examinations (Kralik and Ricchi 2017); however if molecular methods have lower resolution than classic screening techniques, serious consideration needs to be given to whether or not research objectives can be addressed adequately when false negatives are observed.

Knowledge of general trends and phenomena associated with disease ecology is beneficial when planning to incorporate infectious agent screening in wild fish research. Parasites are often present within host populations without causing any detrimental effects. Indeed, pathogens generally have a negative distribution within host populations; carrying low loads may have little effect on survival and is thus common, and increasing loads are associated with disease and consequent mortality. In contrast, highly pathogenic infectious agents will typically be rare in wild populations because those that are infected quickly succumb to mortality. If investigating specific infectious agents, knowledge of the expected distribution based on previous research and agent pathogenicity is useful to estimate the sample size needed to address research questions. For example, comprehensive screening that seeks to detect a large number of agents, including those

that are highly pathogenic, may require a large sample size (i.e.100s of individuals) to reduce the probability of false negatives compared to investigations focused on highly prevalent pathogens. Collaboration between fish ecologists and epidemiologists or veterinarians should occur during study design, data analysis, and interpretation to ensure all aspects of the research plan are appropriate to address epidemiological and ecological questions and data limitations. Indeed, there is much benefit that can be derived from interdisciplinary research that combines different disciplines and techniques (Dick et al. 2016).

2.7 Conclusions

Parasites and disease of wild fish are a critical component necessary for deciphering the evolutionary drivers underlying fish behaviour and ecology. As access to highly sensitive and specific genetic-based screening techniques increases, it is my hope that wild fish ecologists consider how infectious agent screening and collaboration with disease experts could complement their research programs. While it introduces additional complexity to research methods and analyses, incorporating data on infectious agents provides a holistic view of the study system and may illuminate factors otherwise overlooked. This is particularly important for studies of fish behaviour and survival, where the exclusion of pathogens may lead to erroneous conclusions or fail to identify phenomena important for conservation and management. Research programs could better predict the consequences of climate change, species invasions, control measures, and how factors interact for comprehensive conservation and management recommendations of aquatic ecosystems. Rather than ignoring the potential effects that pathogens may have, I

hope that researchers instead embrace the natural complexity of aquatic ecosystems. Conversely, there are many research opportunities that may be of interest to fish epidemiologists that go beyond the typical funding and monitoring program. For example, investigating transmission pathways within and among populations based on fish movement data and/or network analysis (Hellard et al. 2015). Non-lethal sampling may not provide as comprehensive information as traditional necropsy and histological data, however it facilitates the investigation of complex ecologically relevant theories and phenomena *in situ* for the first time.

The utility of interdisciplinary research programs in wild fish research has been highlighted and advocated for previously (Cooke et al. 2008), however little consideration was given to the influence of pathogens. Collaboration among epidemiologists and fish ecologist may be mutually beneficial and enable all involved to use research approaches not otherwise available. I encourage meaningful and engaging collaboration among multiple disciplines and believe doing so will result in innovative study designs that address the 'real life' complexity of aquatic ecosystems (Hellard et al. 2015; Cooke et al. 2012). Given the effort, cost, and invasive nature of most fisheries sampling, obtaining as much information as possible reduces redundancy in sampling and can mitigates the negative impact of researching wild fishes while strengthening the predictive capacity of models and enhancing the understanding of wild fish ecology.

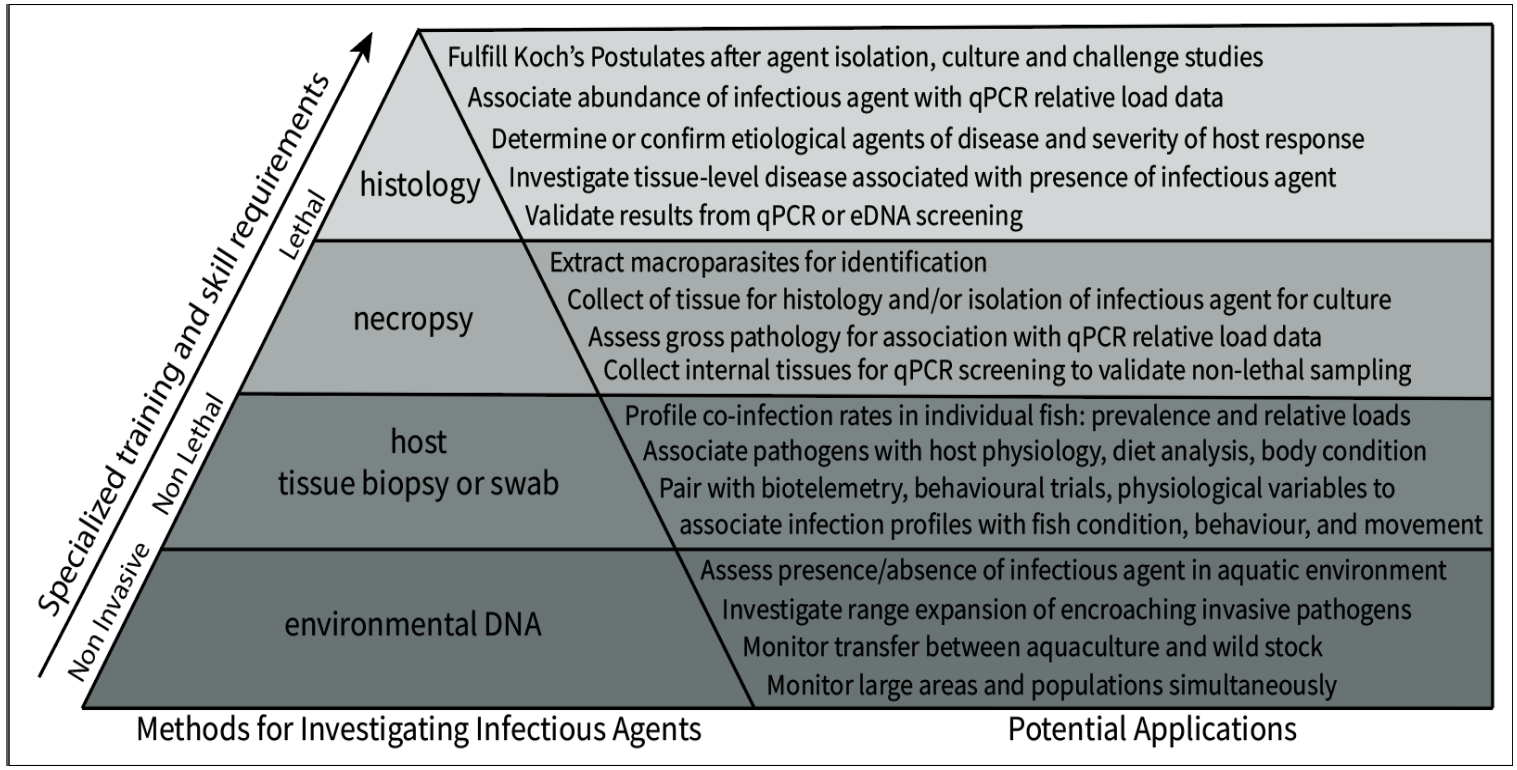

Figure 2 -1: Methods and potential applications for studies investigating wild fish pathogens. Non-lethal methods are genomics-based techniques that can be paired with other methods in fisheries science such as biotelemetry to study the consequences of infection in wild fish. While all methods require specialized training and skill development, the pyramid is reflective of the highly specific tools, training, and applications for each method. For example, eDNA is now used across a number of disciplines while histology remains isolated to microscopy and histopathological work.

Figure 2-1: Investigations of fish pathogens can be conducted in a variety of contexts. A: Necropsy of mortalities collected during a large-scale "fish kill" can provide insight for zoonotic outbreaks, but difficulty preserving host tissue integrity can make identification or quantification of pathogens challenging. B: Experimental studies remove fish from the wild and expose them to experimental conditions in captivity, however stress associated with holding may obscure natural recovery or pathogen transmission. C and D: Nonlethal sampling methods include small gill biopsy or sterile epithelial swab, both of which can be used for molecular genetic based identification techniques.

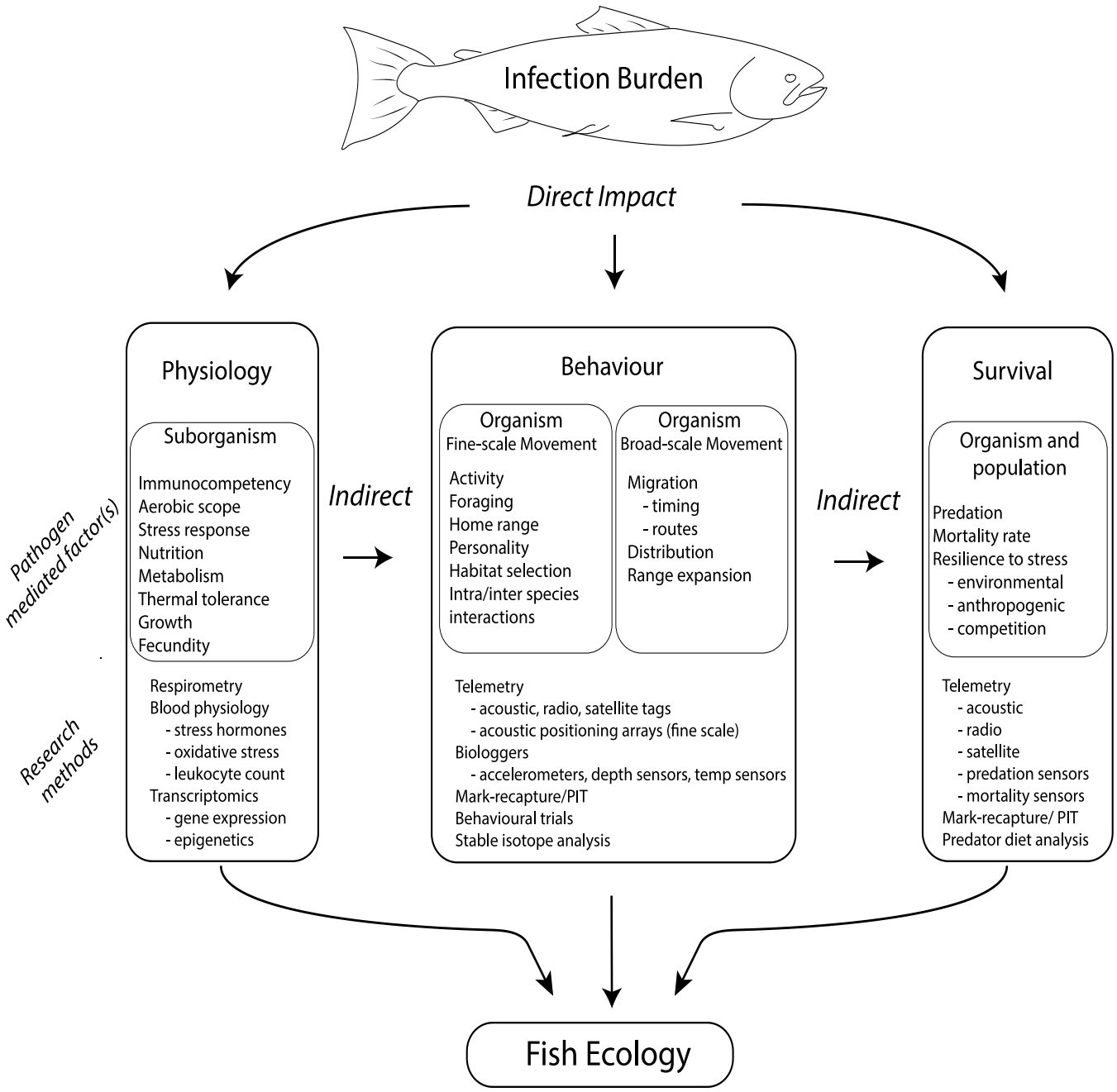

Figure 2-2: Fisheries research areas and factors where individual variation may be associated with pathogen dynamics. Proposed methods can be combined with non-lethal sampling of tissues (e.g. gill biopsy or mucous swab) that is screened using molecular genetic techniques to identify the presence and relative load of pathogens. Comprehensive research can combine multiple scales of individual response to investigate broad-scale evolutionary ecology and provide a holistic understanding of observed phenomena.

Chapter 3: **A survey of microbial infectious agents and characterization of immune response in adult migratory Arctic char (*Salvelinus alpinus*) intercepted during fall migration in three watersheds in the Kitikmeot region of Nunavut, Canada.**

3.1 Abstract

The Arctic is the fastest warming region on Earth, resulting in rapid changes in species phenology and distributions across circumpolar regions. Microbial infectious agents (viruses, bacteria, protozoans and fungi) are a major component of wildlife ecology that are also expected to shift in response to climactic changes and novel species distributions, however limited research has been done on wild Arctic fishes. To investigate microparasite species prevalence and relative load in wild Arctic char I used high-throughput qPCR to rapidly screen 57 adult Arctic char collected from three unique watersheds in the Kitikmeot Region of Nunavut, Canada for the presence of 45 microparasite taxa. Ten species of microparasites were detected, however three (Viral Erythrocytic Necrosis virus, Rikettsia Like Organism, and *Aeromonas salmonicida*) were detected at relative loads below 95% confidence limit of detection for the qPCR platform. New records for this region include three species of bacteria (*Candidatus* Branchiomonas cysticola, *Flavobacterium psychrophilum*, and Piscichlamydia), and four parasite species (Microsporidium *Nucleospora salmonis*; Myxozoan *Parvicapsula pseudobranchicola*; Sarcomastigophora *Spironucleus salmonicida*; and Ciliate *Ichthyophthirius multifiliis*). Biomarkers of cellular immune activity were also quantified and compared with relative infection burden, infectious agent diversity, and *I. multifiliis* to investigate associations of relative load with immune response and demonstrate potential use of biomarker panels to identify novel pathogens, and discuss the implications of microbial infectious agent range

expansion given the increasing observing observation of invasive salmon across the Arctic.

3.2 Introduction

Arctic regions are experiencing unprecedented rates of warming that consistently outpace global averages (Post et al. 2019). Community composition in polar regions are beginning to change as boreal species move Northward in response to warming temperatures and loss of sea ice (Węslawski et al. 2009). Changes in aquatic species demographics have been extensively characterized across Northern latitudes (Cheung et al. 2013; Fossheim et al. 2015), where marine ecosystems are warming at twice the rate of the global average (Hoegh-Guldberg and Bruno, 2010). Marine species are tracking the latitudinal shifts in average ocean temperatures, as thermal regimes in the Arctic become more favourable for species typically bound by their lower threshold of thermal tolerance (Pinsky et al. 2013). The extremely low biodiversity in Arctic ecosystems results in low food web redundancy; consequently, the addition or removal of a single species has the potential to result in regime shifts (Węslawski et al. 2009). Consequently, the impact of rapid and large-scale changes to species composition for endemic Arctic fish species are unknown.

As species colonize new regions, they bring their associated parasite communities. Microparasites – defined here as viruses, bacteria, protozoans, myxozoa, and fungi that have a parasitic life history phase – are of particular interest because they are more likely to cause disease and so have population scale impacts compared to macroparasites. Disease causing agents play a central role in modulating host population sizes, and mediate inter and intra specific competition and ecosystem biodiversity

(Minchella & Scott 1991; Dobson and Hudson 1995; Hudson and Greenman 1998). Microparasites have also been implicated in large scale die-offs of wild fish populations resulting in drastic changes to local ecosystems ((Krkošek et al. 2006). The outcome of invasive host species entering a new environment can be extremely variable; incoming hosts can either act as vectors, bringing new infectious agents in to naïve endemic host populations (Crowl et al. 2008), or "leave their enemies behind" and establish and reproduce rapidly in the new environment due to reduced parasite burden (Torchin et al. 2003; Phillips et al. 2010). In addition, climate change is expected to impact infectious agent diversity and disease trends in wildlife (Altizer et al. 2013). To monitor the potential impacts on microparasite communities, it is crucial to have a baseline understanding of what species are present in endemic populations.

Due to logistical constraints associated with sampling wild fishes, little research has characterized microparasite populations in wild fishes, particularly those in Arctic latitudes. The majority of current knowledge regarding infectious agents of Arctic species is garnered from studies conducted in aquaculture (e.g. Pylkkö et al. 2005, Johansen et al. 2011). For example, while there is no record of being detected in the wild, the bacteria *Candiditus* Piscichlamydia salmonis has been isolated from Arctic char in aquaculture (Draghi et al. 2010). The application of such work to wild fish populations is limited given the high densities, elevated stress, artificial breeding, and veterinary intervention observed in fish rearing facilities (Hoffman 1999). Interest in wild salmonid disease has increased in recent years in response to broad-scale population declines (Cohen 2012), resulting in the creation of a comprehensive monitoring program focusing on microbial pathogen distributions in the Pacific Northwest (e.g. Miller et al. 2016; Teffer et al. 2018;

Bass et al. 2019). This work has revealed broad-scale trends in Pacific salmon microparasite communities, including population-specific variation in agent distributions (Bass et al. 2017). Research regarding microparasite distributions within other fisheries is comparatively lacking.

Across Arctic Canada, Arctic char *Salvelinus alpinus* are culturally important and critical for supporting local food security for Inuit communities (Government of Nunavut 2016). There are also major efforts to initiate small-scale Inuit-run inland commercial char fisheries to support long-term economic development (Government of Nunavut 2016; Schott et al. 2020). Arctic char is an anadromous species that is slow growing and long-lived, maturing at approximately 10 years old at the study latitude and spawning every 3-4 years (Harris et al. 2016). A cold adapted species, Arctic char are widely distributed across the circumpolar North and are the only fish species found in fresh water north of approximately 75 degrees latitude (Power et al. 2008). Adults make their first ocean migration at approximately 4-5 years of age, feeding during the brief ice-free summer in the ocean for approximately 8 weeks prior to returning to freshwater to overwinter in deeper lakes (Dempson and Kristofferson 1987; Scott and Crossman 1998). Unlike most anadromous Pacific salmon, Arctic char are iteroparous and do not appear to have extremely high spawning site fidelity, and thus populations may mix among proximate watersheds (Power et al. 2008; Moore et al. 2017). Little is known about the natural prevalence of microparasite infection in Arctic char populations (for a brief summary of published accounts see Appendix A Table 1). A recent review by Wallace et al. (2017) focused on six microparasites suspected to be transferred to wild fish from aquaculture across Scotland, a small geographic area considering Arctic char's

circumpolar distribution. Currently, no records of microparasites in Arctic Canada have been published since a single study on *Renibacterium salmoninarum* (Souter et al. 1987; Table 5.1-1). At the same time, the range of Pacific salmon has expanded northward rapidly, with over 2400 individual adults collected in Arctic waters across Western Nunavut in 2019 alone, the highest ever recorded (Scott, CBC News, Dec 16 2019). Because Pacific salmon and Arctic char are closely related, the likelihood that microparasites carried by Pacific salmon are also infective to Arctic char is high; indeed, all microbial parasite species that have been reported in Arctic char to date were first identified in other salmonid species. Consequently, the Pacific salmon invasion may lead to changes in microbial pathogen communities as microparasites transfer from Pacific salmon to Arctic char populations. It is necessary to characterize the microparasite communities of wild Arctic char prior to the establishment of Pacific salmon in the region to obtain a baseline of agent prevalence and abundance.

The objective of this study was to provide the first comprehensive microparasite screening for wild Arctic char. For this study I used high-throughput quantitative real-time PCR (HT-qRT PCR) on the Fluidigm Biomark™ platform to screen migratory anadromous adult Arctic char for 45 microparasite taxa known or suspected to cause disease in salmon. Fish were sampled from three unique watersheds, two that have demonstrated little mixing based on previous telemetry research (Moore et al. 2017) and one that is geographically isolated by a large peninsula. Screening was done on gill tissue, which has recently been demonstrated to be the most comprehensive screening tissue for microbial infectious agents in salmonids (Teffer and Miller 2019). Using gill tissue also greatly reduces the sampling time and storage requirements, and facilitates

non-lethal sampling. To characterize how Arctic char respond to parasite burden and investigate transcriptional indication of disease state, I also quantified host immune response to the presence and relative load of pathogens. Previous research has demonstrated the utility of investigating suites of biomarkers to characterize disease using transcriptional profiles in infected salmonids (e.g. Viral Disease Development, Miller et al. 2017). Such transcriptional profiles can not only reveal the agents that may be imposing the strongest physiological impact on fish, but can also aid in identifying when hosts have launched an immune response to an infectious agent that is not present within the microparasite assay panel. Thus, our study objectives were to 1) identify the prevalence and relative load of 45 microparasites known or expected to be present in Pacific salmon populations; 2) investigate site specific differences in microparasite prevalence and host infection burden; 3) characterize the immune response of Arctic char to present infectious agents.

3.3 Methods

3.3.1 Fish collection, tissue sampling, and data recording

This study took place on Victoria Island, Nunavut in three watersheds surrounding the community of Ikaluqtuutiaq (Cambridge Bay; Figure 3-1) from August 16-31st, 2015. Arctic char in this region can be either anadromous or freshwater resident, however I chose to focus on anadromous char as these are more likely to encounter Pacific salmon and are the focus of commercial fisheries in the region. At this latitude, Arctic char migrate to sea in the spring to feed and return 6-8 weeks later to overwinter in lakes deep enough that they do not freeze to the bottom. To collect samples, anadromous

Arctic char were captured shortly after re-entry in to fresh water. At Freshwater Creek, all fish were collected by rod and reel, sampled, and released as part of a separate effort to investigate fishing related mortality. Fish at Halovik and Jayco Rivers were collected as part of Fisheries and Oceans Commercial Fishery data collection program, which is conducted to investigate potential demographic changes associated with harvest pressure in regions where commercial or exploratory fisheries are operating. From each fish, a sample of 2-3 gill filament tips (approximately 0.3 g) was collected using aseptic technique and transferred in to 1.5 mL of RNALater ™ (Life Technologies, Carlsbad, CA, USA) and stored according to manufacturer's instruction. Gill tissue has been demonstrated to harbour RNA from the majority of microparasites known to infect internal organs (Teffer and Miller 2019). For samples taken as part of Fisheries and Oceans data collection at Jayco and Halovik Rivers, fish sex, weight, maturity (scored as spawning prepared, mature but not spawning prepared, or immature), and age based on otolith analysis was obtained as part of the fisheries monitoring sampling program.

3.3.2 Molecular methods

Tissue samples were shipped on dry ice to the Fisheries and Oceans Pacific Biological Station in Nanaimo, BC, Canada and stored in -80°C prior to analysis. Work flow and all methodologies used in laboratory analysis were analytically validated for use in microparasite screening in salmonids as part of the Canadian Science Advisory Secretariat process, and details on the specific sensitivity and specificity, source, and repeatability of each assay can be found in the final report (Miller et al. 2016). Total RNA was extracted from tissue using methods previously described by Miller et al.

(2014, 2016). In brief, tissues were homogenized using a MM301 mixer mill (Restch Inc. Newtown PA) with TRI-reagent™ extraction kits (Ambion Inc, Austin, TX, USA). Extractions were carried out using MagMAX ™ -96 Microarrays Kits on a Biomek FXP automated liquid handler. Extracted RNA concentrations were assessed using a Beckman Coulter DXT 880 Multimode Detector (Brea, CA, USA) and sample RNA concentrations were normalized to 62.5 ng/μL for reverse transcription of cDNA using Superscript ® VILO™ DNA synthesis kits (Life Technologies) and 1 μg of RNA. To allow detection of extremely low transcript copy numbers, specific target amplification was performed using primer pairs for all assays using 1x TaqMan Pre-amp MasterMix (Applied Biosystems, CA, USA) based on manufacturer's instructions. Amplified product was treated with Exo-SAP-IT™ (Affymetrix, Santa Clara, CA, USA) to remove unincorporated primers and 1.25-μL of cDNA from each sample was diluted 1:5 in DNA re-suspension buffer (Teknova, Hollister, CA, USA). Artificial Positive Constructs (APC clones) were created and run in serial dilution to calculate microparasite assay efficiency. APC clones have NED™ probes (Life Technologies) incorporated to facilitate the detection of sample contamination from APC clones. Serial dilution of pooled sample was used to determine biomarker assay efficiency. Non-amplified and no template controls were incorporated at each stage of the process (See Miller et al. 2016 for details).

A 5-μL reaction mix consisting of 2x TaqMan Mastermix (Life Technologies), 20x GE Sample Loading Reagent, nuclease-free water and 2.7-μL of cDNA was added to each assay inlet on the 96.96 gene expression dynamic array (Fluidign Corporation, CA, USA). For each assay, 10-μM primer pairs and 3-μM TaqMan probes were prepared. Probes for microparasites and biomarkers were FAM-MGB, APC clones NED-MGB. 5-

μL of assay and sample were transferred to their respective inlets and loaded into the BioMark chip using the IFC Controller HX (Fluidigm). qPCR was run under the following conditions: 50 °C for 2 mins, 95 °C for 10 mins, then 40 cycles at 95 °C for 15 s and 60 °C for 1 min.

For each assay, cycle thresholds were set using BioMark Real-Time PCR analysis software. Reaction curves for each assay were visually assessed to identify abnormal amplification curves and APC clone contamination based on NED probes. Cycle thresholds were adjusted based on the linear phase of each amplification curve of the APC clones and serial dilutions. Once all assays were assessed, data was exported for analysis in R. Limit of Detection (LOD; based on 95% confidence of true positive determined by Miller et al. 2016) and removal of any case where sample-assay combinations did not both amplify (i.e., test positive) screening was executed for all microparasite assays. Samples below the LOD were removed from all calculations but are presented in graphs in cases where pooled sample also tested positive and curves appear normal. Arctic char biomarkers were normalized to three reference genes, and relative gene expression was assessed using the $2^{-\Delta\Delta Ct}$ to the Ct value of a pooled positive control (Livak and Schmittgen 2001). Assays were failed and excluded from analysis if efficiency was less than 80% (Table 3-1 & 3-2). As Arctic char was a new species for this biomarker panels, many biomarker assays did not perform optimally and were excluded from analysis. A full list of all biomarkers included in the initial panel and associated efficiencies is available in the supplementary material (Appendix A Table 3).

3.3.3 Microbe prevalence, relative load, and relative infection burden

Parasite prevalence was calculated as the number of positive individuals within the sample population, and relative load was transformed such that high values represent larger amounts of transcript by subtracting the Ct from cycle number (40 – Ct). To investigate the effect of high infection burden relative to the sample population and potential virulence of infectious agents detected in low prevalence, an index was calculated (Bass et al. 2019) and summarized as a single "Relative Infection Burden" variable.

$$RIB = \sum_{i \epsilon m}^{m} \frac{L_i}{Lmax_i},$$

Here, L_i is the relative load (40-Ct) of microparasite detected, $Lmax_i$ is the highest relative load of that agent observed in the sample population, and m is the sum of $L_i/Lmax_i$ for all pathogens detected in the sample.

3.3.4 Statistical analysis

Differences in biological characteristics (length, maturity, age-length relationship) of char were compared using analysis of variance (ANOVA), Pearson's chi-squared, and simple linear regression. Differences in microparasite relative loads between sexes and among sampling locations were investigated using Welch's two sample t-test and Wilcox rank sum approximation, data permitting. Microparasite species richness was highly variable among Arctic char, so to test if sampling location was a significant predictor of species richness, differences in the number of microparasite taxa richness per host among

watersheds was tested based on infection categories of low (0-1 species), medium (2-3 species) and high (4 species and over) using Pearson's Chi-square. Site-specific differences in infectious agent communities were investigated using ANOVA and Tukey HSD post-hoc tests, or non-parametric Wilcoxon Rank Sum where appropriate.

To investigate the relationship between host immune response and infectious agent dynamics, biomarkers were summarized using unsupervised PCA analysis. Bartlett's test of sphericity was used to evaluate appropriateness of PCA based on biomarker variance. Scree plots were assessed to determine the number of components to retain (cumulative variance >50%) and generalized linear and mixed effect models with sample location, RIB, and fork length as factors and principal components as the response were constructed to investigate broad-scale trends in gene transcription as they relate to host life history (age, location) and infection dynamics (RIB, species richness). Plots of PCs were assessed for clustering and differentiation to assess the discriminatory performance of the gene panel for Arctic char. Because of the highly pathogenic nature of viruses and potential for spread from invasive Pacific salmon, any viral detection also present in template positive control samples was visually assessed for quality of amplification curve and considered regardless if the Ct was below the LOD.

3.4 Results

A total of 57 fish were sampled; 27 from Freshwater Creek, and 15 each from Halovik River and Jayco River. Fork length was not significantly different among sampling locations (ANOVA $F = 1.939$, $p = 0.257$). Using DFO test fisheries data, age and fork length were correlated (simple linear regression $R^2 = 0.36$, $p = 0.0005$),

allowing fork length to be used as an approximation of age in analyses. Gonadal maturity was not significantly different between Jayco River and Halovik River (Pearson's $X^2 = 1.534$, $p = 0.215$).

Ten species of microparasite were identified based on initial screening, however three were detected in Ct's below the limit of detection (Figure 3-2). Results include the first official record to my knowledge of bacterial species *Candidatus* Branchiomonas cysticola, Gill chlamydia, and *Flavobacterium psychrophilum* in wild Arctic char, and the first record in Canada for *Aeromonas salmonicida.* Parasites detected include the ciliate *Ichthyophthirius multifiliis* and microsporidian *Nucleospora salmonis* and flagellate *Spironucleus salmonicida*, all of which have never been reported in wild Arctic char. Viral erythrocytic necrosis virus was detected in one individual as well as the pool positive control, and amplification curves appeared real but below the LOD. Of the species previously reported in Arctic char, *Myxobolus arcticus,* Salmon alphavirus (SAV), Viral haemorrhagic septicaemia virus (VHSv), and Infectious Pancreatic Necrosis Virus (IPNv) were screened for but not detected in this study.

The proceeding analysis was conducted after LOD screening. Only *C*.B. cysticola was detected with high enough prevalence to investigate site-specific variation in relative load, which did not vary across sampling location (ANOVA $F_{2,44} = 0.397$, $p = 0.67$). Distribution of species prevalence was not equal across sampling locations; for example, *F. psychrophilum* was found only in Freshwater Creek and Halovik River, while *S. salmonicida* was not detected in Freshwater Creek. Microparasite species richness ranged from 0-4 species per individual and was significantly different among sampling locations (Pearson's $X^2 = 29.494$, $df = 6$, $p < 0.0001$); all fish in HR tested positive for at least two

microparasites, while some individuals in Freshwater Creek and Jayco River did not test positive for any species. No fish in Freshwater Creek or Jayco River tested positive for more than three species (Figure 3-3). RIB was also significantly different among the three sites (ANOVA $F_{2,54}$ =17.0, p <0.0001). A post-hoc Tukey test revealed RIB in Halovik River as significantly higher than Freshwater Creek and Jayco River ($p < 0.0001$; Figure 3-4). The relative load of *C.*B. cysticola was also not correlated with RIB (simple linear regression $p = 0.55$), and thus presence of the bacteria did not seem to be associated with infection with other agents. For individuals where sex was known (N=35), sex was not predictive of RIB (Wilcox rank sum, p = 0.414), relative load of *C.*B. cysticola (Welch two sample t-test, t = -1.2209, df = 20.293, p = 0.236), or species richness (Pearson's X^2= 1.6528, df = 4, p = 0.799). Fork length was not associated with RIB or relative loads of *C.*B. cysticola (simple linear regression, p = 0.199 and 0.912, respectively).

PCAs of biomarkers resulted in three retained components for modeling (Table 3-3). Biomarker profiles summarized by PCs were not associated with relative infection burden or fork length, or sampling location based on generalized linear regression models ($p > 0.05$). Because infectious agent prevalence was confounded with sampling location, generalized mixed effect model were constructed using the lme4 and lmerTest packages (Kuznetsova et al. 2019). Infectious agent prevalence was converted to positive or negative, and the occurrence of rare species (prevalence <0.30) combined to facilitate modeling. The final models thus consisted of PCs as the response variable by RIB, *I. multifiliis* infection status (positive or negative binary outcome), and rare species presence as fixed effects, and location as a random effect variable. *C.*B. cysticola was not included in analysis given its ubiquitous distribution and lack of known pathogenic

properties (Gunnarsson et al. 2017). Host transcriptional response as summarized by PCA was not closely associated with tested factors except for PC3, which was significantly correlated with *I. multifliliis* infection (Table 3-4). Major contributors to PC3 included the adaptive immune biomarker MHC-IIB and osmoregulatory genes NKAa1b and CA4. Heat maps of individual genes were constructed to characterize biomarker-specific responses to RIB and relative loads of *I. multifiliis* (Figure 3-4). When investigating significance of correlations, a correction for multiple comparisons was not included, however a conservative $\alpha = 0.01$ was adopted. RIB was significantly correlated with the viral disease associated genes IFI44a, Mx, adaptive immune kinase ZAP70, and inflammatory cytokine IL-11. In positive individuals, *I. multifiliis* relative load was significant for only IL-11 (simple linear regression; $p < 0.01$; Figure 3-4). Finally, visual assessment of PCA plots revealed a single cluster of five individuals reflecting extreme regulation of Viral Disease Development genes (Miller et al. 2017; Figure 3-5).

3.5 Discussion

High throughput qPCR provided the first comprehensive screening of microparasites in wild Arctic char populations. Of the 45 agents included in the screen, I found evidence supporting the presence of eight species, including six that have not been reported in wild Arctic char to my knowledge. These agents included four bacteria, including proteobacteria *Candidatus* Branchiomonas cysticola and Gill chlamydia, *Aeromonas salmonicida,* and *Flavobacterium psychrophilum*, and four microparasites including the ciliate *Ichthyopthurius multifiliis*, flagellate *Spironucleus salmonicida,* the myxozoan *Parvicapsula pseudobranchicola,* and the microsporidian *Nucleospora*

salmonis. Only two agents, *C.*B. cysticola and *I. multifiliis,* were found with prevalence over 10%. This data provides a preliminary baseline for future monitoring of microbial infectious agent prevalence in the region as climactic shifts and species range expansions continue to advance.

3.5.1 Microparasite species richness and infection burden

I found no sex or age-specific variation in microparasite infection burden, and that microparasite prevalence and overall species richness was associated with sampling location. Population-specific trends in infectious agent richness has been demonstrated in salmonids previously (Bass et al. 2017), and in Arctic char, metazoan parasites have been shown to differ between anadromous and non-anadromous individuals and be used as an indicator of anadromy (Bouillon and Dempson 1989). Characterizing a source of variation in exposure to infectious agents is particularly challenging in Arctic char due to their high phenotypic plasticity and complex migratory behaviours. Helminth parasites have also been found to vary within non-anadromous char populations as a function of allopatric polymorphism and associated diet specialization (Curtis et al. 2011). This level of differentiation is not commonly observed in anadromous char morphs (Adams 1999), so is likely not a source of variation in microparasite exposure pathways; However, non-anadromous char populations will inhabit systems with ocean access and may serve as reservoirs of infection to anadromous populations. Individual populations of char are known to overwinter in watersheds adjacent to natal systems with the least energetically demanding migration (Moore et al. 2017). This means that populations may mix among geographically proximate river systems, creating homogenous infectious agent

communities while remaining reproductively isolated. Examining complexes of watersheds may reveal patterns in infectious agent communities associated with migratory behaviour in this complex, long-lived species.

The prevalence and species richness of microbial infectious agents was considerably lower than what has previously been described in adult Pacific salmon species (e.g. Miller et al. 2014; Bass et al. 2017, Teffer et al. 2019) and Atlantic salmon (Teffer et al. 2020). Relatively low infection rates have also been observed in metazoan parasites, which follows global patterns of parasitism; ecosystems such as polar regions with low host species diversity are expected to have low parasitic diversity (Marcogliese 2004). These results suggest that microbial communities of Arctic char were somewhat isolated from Pacific salmon. Since the time of sampling, however, observations of Pacific salmon have increased year over year. As aquatic biodiversity in the Arctic increases, monitoring will be required to observe associated increases in microbial infectious agent communities.

3.5.2 Microparasites

All infectious agents detected exhibit direct transmission except for the myxozoan *P. pseudobranchicola*, which was previously detected in wild Arctic char in Norway (Jørgensen et al. 2011b). Direct transmission pathways do not require consistent presence of intermediate hosts or vectors to complete their life cycle, making them more suited to Arctic waters. Interestingly, *P. pseudobranchicola* has an indirect life-cycle involving an unknown species of annelid worm (Markussen et al. 2015). Given the relatively narrow oceanic range of Arctic char migrations compared to Pacific salmonids, it is reasonable to

assume that the intermediate host inhabits polar regions. The generally low biodiversity in Arctic regions may narrow the number of potential intermediate host species available and facilitate its identification and warrants further investigation. For other dioicious infectious agents known to be carried by *Oncorhynchus* sp., such as the myxozoans *Tetracapsuloides bryosalmonae* and other *Parvicapsula* sp., the lack of suitable intermediate hosts may restrict transmission from invasive salmon to Arctic char and establishment of the infectious agents in the region (Phillips et al. 2010). Arctic char are highly susceptible to *Tetracapsuloides bryosalmonae* and do not appear to launch a comprehensive immune response (Kent et al. 2000), so factors limiting spread to char populations may buffer potential negative effects of Pacific salmon invasion.

Several infectious agents that have been observed in wild Arctic char in other regions were not detected in this study. Previous research has found evidence of *Myxobolus arcticus* infecting the brain tissue of Arctic char in the Nunavik region of Northern Quebec (Desdevises et al. 1998). When investigating the efficacy of various tissues for infectious agent screening, *M. arcticus* was the only species that was positively detected in blood and pooled tissue samples but not in gill tissue (Teffer and Miller 2019). Consequently, results based on gill tissue screening alone is not adequate to rule out the presence of this species. *Renibacterium salmonarum*, the aetiological agent of Bacterial Kidney Disease, has been detected across the circumpolar North, including at Jayco River (Souter et al. 1987, Jónsdóttir et al. 1998, Wallace et al. 2017). During these studies, chronic infection and overt signs of disease were present, suggesting char are susceptible mortality from infection (Souter et al. 1987). Comprehensive future screening

efforts should include lethal samples of brain and kidney tissue to rule any potential false negatives observed here.

The flagellate *Spironucleus salmonicida* has been observed in wild Arctic char collected in Norway (Jørgensen et al. 2011) but has not been reported in Canada to my knowledge. Prevalence in Norway was reported to be 8.3%, similar to the 5% observed here, and there were no reports of disease. Arctic char are suggested as a reservoir for *S. salmonicida.* The microsporidian *Nucleospora salmonis* was detected above the LOD in one individual. This obligate intracellular agent is endemic to the Pacific Northwest, however is also found in Europe, though it is not known if these are endemic populations (Foltz et al. 2009). There is little report of its presence in Arctic char. Further research is required to characterize *N. salmonis* prevalence and virulence in wild Arctic char populations, and determine if it is endemic to the region or evidence of transmission from Pacific salmon species.

3.5.3 Bacteria

While highly prevalent within the sample population, the bacteria *C.*B. cysticola was not associated with any genetic profiles or infection with other infectious agent. Prevalence was high at 82%, however was lower than what has been observed in Chinook salmon *Oncorynchus tshawytscha* (98%; Bass et al. 2017), coho *O. kisutch* (100%; Teffer et al. 2019), and sockeye salmon *O. nerka* (100%; Teffer et al. 2018). Even though the bacteria has been associated with epitheliocysts of the gills (Mitchell et al. 2013), this bacterium it is believed to be low risk or even commensal (Miller et al. 2014; Gunnarsson et al. 2017).

In contrast, *Aeromonas salmonicida* and *Flavobacterium psychrophilum* may pose high risk for mortality in Arctic char based on evidence from aquaculture and challenge studies (Goldschmidt-Clermont et al. 2009; Starliper 2001a; Starliper 2011). *A. salmonicida* is the aetiological agent of furunculosis and/or ulcerative dermatitis, diseases with global distribution in Osteichthyes and with great importance for aquaculture (Hoffmann 1999; Austin and Austin 2016). In aquaculture, a sub-species of *A. salmonicida* subsp. *Smithia* was isolated from Arctic char demonstrating ulcerative and haemorrhagic conditions (Pylkkö et al. 2005; Goldschmidt-Clermont et al. 2009). Considering that *A. salmonicida* is ubiquitous and highly virulent, further investigation should be undertaken to confirm pathology and subspecies type present in wild char populations. *F. psychrophilum* causes Bacterial Coldwater Disease resulting in large areas of tissue necrosis (Austin and Austin 2016). *F. psychrophilum* has been associated with immunocompetency and predictive of mortality in spawning Pacific salmon (Hruska et al. 2010; Dolan et al. 2016), and implicated in mortality rates as large as 90% in rainbow trout *Oncorhynchus mykiss* (Nilsen et al. 2011); however, little evidence is available regarding Arctic char's susceptibility to disease from *F. psychrophilum*.

3.5.4 Viruses

Viruses known to be pathogenic to salmonids and present within Arctic char and Pacific salmon populations were not detected in this study above the limit of detection. Previously detected viral species in wild Arctic char include IPNV, Infectious Salmon Anemia Virus ISAV, and IHNV, however no screening has been conducted this far north. In the Northwest Territories, IPNV was detected in adult char prior to any salmonid

aquaculture in the territory, leading the authors to conclude IPNV is endemic to the region (Souter et al. 1984). Lack of detection of an infectious agent within a small sample population may indicate that the pathogen is not present or is rare, or that it is highly pathogenic and so infected hosts succumb to mortality and are removed from populations quickly. However, based on observations from challenges studies, Arctic char appear to be resistant to the tested strains of virus species, including Infectious Salmon Anemia Virus (Snow et al. 2001), VHSV (Dorson et al. 1991), IPNV (McAllister et al. 2000) and IHNV (McAllister et al. 2000; Alaskan strain; Follett and Burton 2011). However, a high mortality rate of 61% from IPNV infection were reported by Dorson et al. (1991), and the successful viral replication observed by Johansen et al. (2011) suggest strain or host population specific factors may modulate pathogenicity, and that char are reservoir for both IPNV and IHNV. Given that populations are often adapted to the presence of endemic infectious agents and stock- or strain- specific susceptibility is likely to vary across Arctic char distributions in the wild, modeling the impact of the spread of viral agents it is important component of future work on wild stocks of Arctic char.

3.5.5 Transcriptional response

The extremely low prevalence and relative load of infectious agents based on the present assay panel reduces the exploratory power of the disease response biomarker panel. Further, as I did not directly assess individual health (e.g. histopathology), I am not able to directly associate microparasite presence with disease state. However, this biomarker panel was significantly associated with sampling location and the presence of *I. multifiliis* when variation associated with location was controlled. Interestingly, visual

separation of a cluster of individuals based on analyses included a fish with low loads of VEN. In Pacific salmon, such patterns were indicative of viral disease, or in some cases, the presence of *Renibacterium salmoninarum* (Miller et al. 2017), the causative agent of Bacterial Kidney Disease which was not detected in this study. Thus the distinct separation in gene profile by these individuals may indicate the presence of a virus or pathogen that causes similar immune response that was not included in the present assay panel. Further investigation is warranted with a more rigorous sampling regime to determine if VEN is present in the region at higher loads and investigate pathological effects. Additional processing of these samples with high-throughput sequencing may identify novel viruses that should be included in future work.

However, the significant association of *I. multifiliis,* the causative agent of the ubiquitous white spot disease found in fresh waters around the globe, (Hoffmann1999) with PC3, along with the separation of lone individual with potential viral infection supports the potential utility of this approach for identifying disease state for more detailed sequencing. The responsiveness of osmoregulatory associated genes and adaptive immune response may indicate ion imbalance associated with infection. Given the limited research done on wild Arctic char in the wild, it is reasonable to assume that there are microbial pathogens present that were not included in the assay panel. Future surveys should include larger sample sizes to account for the low prevalence of infectious agents and ensure an adequate representation of infection states are present to verify this preliminary molecular signature of disease state.

3.6 Conclusion

This data provides a preliminary baseline for future monitoring of microbial infectious agent prevalence in the region as climactic shifts and species range expansions continue to advance. Current evidence does not support a major shift in microbial pathogen communities associated with the range expansion of Pacific salmon, however more monitoring is required as aquatic diversity continues to increase across the region. If future monitoring is to occur, species such as *Serratia liquefaciens* may be important for wild Arctic char (Starliper 2001b) and should be added to future infectious agent panels. Future research should include a comprehensive, multi-tissue survey to verify the negative detections presented here. Sequencing work guided by genetic profiles may reveal novel infectious agent species as has been seen previously (Miller et al. 2017).

Table 3-1: Abbreviations, names, and groups of infectious agents detected in migratory adult Arctic char *Salvelinus alpinus* collected in the Kitikmeot region of Nunavut system using high-throughput qPCR. P = prevalence (positive detections/screened samples, 57 individuals), E = assay efficiency.

Group	Infectious Agent	Assay ID	Taqman Primer Sequences (5'–3') Probe Sequence (FAM-5'–3'-MGB)	P	E	sequence source
bacteria	*Aeromonas hydrophila*	ae_hyd	F—ACCGCTGCTCATTACTCTGATG / R—CCAACCCAGACGGGAAGAA P—TGATGGTGAGCTGGTTG	0.00	1.00	Lee et al. 2006
bacteria	*Aeromonas salmonicida*	ae_sal	F—TAAAGCACTGTCTGTTACC / R—GCTACTTCACCCTGATTGG / P—ACATCAGCAGGCTTCAGAGTCACTG	**0.02**	0.94	Keeling et al. 2013
bacteria	*Candidatus* Branchiomonas cysticola	c_b_cys	F—AATACATCGGAACGTGTCTAGTG / R—GCCATCAGCCGCTCATGTG / P—CTCGGTCCCAGGCTTTCCTCTCCCA	**0.82**	1.04	Mitchell et al. 2013
bacteria	*Candidatus* Piscichlamydia salmonis	pch_sal	F—TCACCCCCAGGCTGCTT / R—GAATTCCATTTCCCCCTCTTG / P—CAAAACTGCTAGACTAGAGT	0.00	0.93	Nylund et al. 2008
bacteria	*Flavobacterium psychrophilum*	fl_psy	F—GATCCTTATTCTCACAGTACCGTCA/R-TGTAAACTGCTTTTGCACAGGAA P—AAACACTCGGTCGTGACC	**0.07**	0.83	Duesund et al. 2010
bacteria	Gill chlamydia	sch	F—GGGTAGCCCGATATCTTCAAAGT / R—CCCATGAGCCGCTCTCTCT / P—TCCTTCGGGACCTTAC	**0.04**	0.90	Duesund et al. 2010
bacteria	*Piscirickettsia salmonis*	pisck_sal	F—TCTGGGAAGTGTGGCGATAGA / R—TCCCGACCTACTCTTGTTTCATC / P—TGATAGCCCCGTACACGAAACGGCATA	0.00	0.95	Corbeil et al. 2003
bacteria	*Renibacterium salmoninarum*	re_sal	F—CAACAGGGTGGTTATTCTGCTTTC/ R-CTATAAGAGCCACCAGCTGCAA / P—CTCCAGCGCCGCAGGAGGAC	0.00	0.96	Powell et al. 2005
bacteria	Rickettsia-like organism	rlo	F—GGCTCAACCCAAGAACTGCTT / R—GTGCAACAGCGTCAGTGACT / P—CCCAGATAACCGCCTTCGCCTCCG	0.00	1.01	Lloyd et al. 2011
bacteria	*Vibrio anguillarum*	vi_ang	F-CCGTCATGCTATCTAGAGATGTATTTGA/R-CCATACGCAGCCAAAAATCA/ P—TCATTTCGACGAGCGTCTTGTTCAGC	0.00	0.91	Miller et al. 2016
bacteria	*Vibrio salmonicida*	vi_sal	F—GTGTGATGACCGTTCCATATTT / R—GCTATTGTCATCACTCTGTTTCTT / P—TCGCTTCATGTTGTGTAATTAGGAGCGA	0.00	1.06	Miller et al. 2016

platyhelminth	*Gyrodactylus salaris*	gy_sal	F—CGATCGTCACTCGGAATCG / R—GGTGGCGCACCTATTCTACA / P—TCTTATTAACCAGTTCTGC	0.00	1.00	Collins et al. 2010
platyhelminth	*Nanophyetus salmincola*	na_sal	F–CGATCTGCATTTGGTTCTGTAACA/RCCAACGCCACAATGATAGCTATAC / P—TGAGGCGTGTTTTATG	0.00	1.15	Miller et al. 2016
protozoa	*Ceratonova shasta*	ce_sha	F—CCAGCTTGAGATTAGCTCGGTAA / R—CCCCGGAACCCGAAAG / P—CGAGCCAAGTTGGTCTCTCCGTGAAAAC	0.00	1.02	Hallett & Bartholomew 2006
protozoa	*Cryptobia salmositica*	cr_sal	F—TCAGTGCCTTTCAGGACATC / R—GAGGCATCCACTCCAATAGAC / P—AGGAGGACATGGCAGCCTTTGTAT	0.00	1.00	Miller et al. 2016
protozoa	*Dermocystidium salmonis*	de_sal	F—CAGCCAATCCTTTCGCTTCT / R—GACGGACGCACACCACAGT / P—AAGCGGCGTGTGCC	0.00	0.97	Miller et al. 2016
protozoa	*Facilispora margolisi*	fa_mar	F—AGGAAGGAGCACGCAAGAAC / R—CGCGTGCAGCCCAGTAC / P—TCAGTGATGCCCTCAGA	0.00	1.12	Miller et al. 2016
protozoa	*Ichthyophonus hoferi*	ic_hof	F—GTCTGTACTGGTACGGCAGTTTC/ R-TCCCGAACTCAGTAGACACTCAA / P—TAAGAGCACCCACTGCCTTCGAGAAGA	0.00	1.15	White et al. 2013
protozoa	*Ichthyophthirius multifiliis*	ic_mul	F—AAATGGGCATACGTTTGCAAA/R-AACCTGCCTGAAACACTCTAATTTTT / P—ACTCGGCCTTCACTGGTTCGACTTGG	**0.35**	0.98	Miller et al. 2016
protozoa	*Kudoa thyrsites*	ku_thy	F—TGGCGGCCAAATCTAGGTT / R—GACCGCACACAAGAAGTTAATCC / P—TATCGCGAGAGCCGC	0.00	0.85	Funk et al. 2007
protozoa	*Loma salmonae*	lo_sal	F—GGAGTCGCAGCGAAGATAGC/ R-CTTTTCCTCCCTTTACTCATATGCTT / P—TGCCTGAAATCACGAGAGTGAGACTACCC	0.00	1.05	Miller et al. 2016
protozoa	*Myxobolus arcticus*	my_arc	F—TGGTAGATACTGAATATCCGGGTTT / R—AACTGCGCGGTCAAAGTTG / P—CGTTGATTGTGAGGTTGG	0.00	0.92	Miller et al. 2016
protozoa	*Myxobolus cerebralis*	my_cer	F—GCCATTGAATTTGACTTTGGATTA/ R-ACCATTCATGTAAGCCCGAACT / P—TCGAAGCCTTGACCATCTTTTGGCC	0.00	0.97	Kelley et al. 2004
protozoa	*Myxobolus insidiosus*	my_ins	F—CCAATTTGGGAGCGTCAAA / R—CGATCGGCAAAGTTATCTAGATTCA / P—CTCTCAAGGCATTTAT	0.00	1.00	Miller et al. 2016
protozoa	*Neoparamoeba perurans*	ne_per	F—GTTCTTTCGGGAGCTGGGAG / R—GAACTATCGCCGGCACAAAAG / P—CAATGCCATTCTTTTCGGA	0.00	1.07	Fringuelli et al. 2012

protozoa	*Nucleospora salmonis*	nu_sal	F—GCCGCAGATCATTACTAAAAACCT / R—CGATCGCCGCATCTAAACA / P—CCCCGCGCATCCAGAAATACGC	**0.02**	1.06	Foltz et al. 2009
protozoa	*Paranucleospora theridion*	pa_ther	F—CGGACAGGGAGCATGGTATAG / R—GGTCCAGGTTGGGTCTTGAG / P—TTGGCGAAGAATGAAA	0.00	0.99	Nylund et al. 2010
protozoa	*Parvicapsula kabatai*	pa_kab	F—GTCGGATGATAAGTGCATCTGATT / R—ACACCACAACTCTGCCTTCCA / P—TGCGACCATCTGCACGGTACTGC	0.00	0.98	Miller et al. 2016
protozoa	*Parvicapsula minibicornis*	pa_min	F-AATAGTTGTTTGTCGTGCACTCTGT/R-CCGATAGGCTATCCAGTACCTAGT/ P—TGTCCACCTAGTAAGGC	0.00	0.88	Hallett & Bartholomew 2009
protozoa	*Parvicapsula pseudobranchicola*	pa_pse	F—CAGCTCCAGTAGTGTATTTCA / R—TTGAGCACTCTGCTTTATTCAA / P—CGTATTGCTGTCTTTGACATGCAGT	**0.02**	0.87	Jørgensen et al. 2011
protozoa	*Sphaerothecum destruens*	sp_des	F—GCCGCGAGGTGTTTGC / R—CTCGACGCACACTCAATTAAGC / P—CGAGGGTATCCTTCCTCTCGAAATTGGC	0.00	1.00	Miller et al. 2016
protozoa	*Spironucleus salmonicida*	sp_sal	F-AACCGGTTATTCGTGGGAAAG/R-TTAACTGCAGCAACACAATAGAATACT P—TGCCAGCAGCCGCGGTAATTC	**0.05**	0.95	Miller et al. 2016
protozoa	*Tetracapsuloides bryosalmonae*	te_bry	F—GCGAGATTTGTTGCATTTAAAAAG / R-GCACATGCAGTGTCCAATCG / P—CAAAATTGTGGAACCGTCCGACTACGA	0.00	1.04	Bettge et al. 2009
virus	Atlantic salmon paramyxovirus	aspv	F-CCCATATTAGCAAATGAGCTCTATR-CGTTAAGGAACTCATCATTGAGCTT/ P—AGCCCTTTTGTTCTGC	0.00	0.97	Nylund et al. 2008
virus	Infectious haematopoietic necrosis virus	ihnv	F—AGAGCCAAGGCACTGTGCG / R—TTCTTTGCGGCTTGGTTGA / P—TGAGACTGAGCGGGACA	0.00	0.98	Purcell et al. 2013
virus	Pacific salmon parvovirus	pspv	F—CCCTCAGGCTCCGATTTTTAT / R—CGAAGACAACATGGAGGTGACA / P—CAATTGGAGGCAACTGTA	0.00	0.96	Miller et al. 2016
virus	Piscine myocardial virus	pmcv	F—TTCCAAACAATTCGAGAAGCG / R—ACCTGCCATTTTCCCCTCTT / P—CCGGGTAAAGTATTTGCGTC	0.00	1.07	Løvoll et al. 2012
virus	Piscine orthoreovirus	prv	F—TGCTAACACTCCAGGAGTCATTG / R—TGAATCCGCTGCAGATGAGTA / P—CGCCGGTAGCTCT	0.00	0.96	Wiik-Nielsen et al. 2012
virus	Salmonid herpesvirus	omv	F—GCCTGGACCACAATCTCAATG / R—CGAGACAGTGTGGCAAGACAAC / P—CCAACAGGATGGTCATTA	0.00	1.11	Miller et al. 2016

virus	Viral encephalopathy and retinopathy virus	ver	F—TTCCAGCGATACGCTGTTGA / R—CACCGCCCGTGTTTGC / P—AAATTCAGCCAATGTGCCCC	0.00	1.12	Korsnes et al. 2005
virus	Viral erythrocytic necrosis virus	ven	F—CGTAGGGCCCCAATAGTTTCT /R—GGAGGAAATGCAGACAAGATTTG / P—TCTTGCCGTTATTTCCAGCACCCG	0.00	1.12	Purcell et al. 2013
virus	Viral haemorrhagic septicaemia virus	vhsv	F—ATGAGGCAGGTGTCGGAGG / R—TGTAGTAGGACTCTCCCAGCATCC / P—TACGCCATCATGATGAGT	0.00	1.05	Garver et al. 2011

Table 3-2: Names and sequences of biomarker assays. E = assay efficiency.

Assay	E	Gene name	Taqman Primer Sequences (5'–3') Probe Sequence (FAM-5'–3'-MGB)	Source
Adaptive Immune				
IgMs	0.98	Immunoglobulin	F—CTTGGCTTGTTGACGATGAG /R—GGCTAGTGGTGTTGAATTGG /P—TGGAGAGAACGAGCAGTTCAGCA	Raida et al. 2011
MHCI	1.1	Major histocompatibility complex I	F—GCGACAGGTTTCTACCCCAGT /R—TGTCAGGTGGGAGCTTTTCTG /P—TGGTGTCCTGGCAGAAAGACGG	Ingerslev et al. 2009
MHCII-B	0.97	Major histocompatibility complex IIβ	F—TGCCATGCTGATGTGCAG /R—GTCCCTCAGCCAGGTCACT /P—CGCCTATGACTTCTACCCCAAACAAAT	Raida and Buchmann 2008
ZAP70	0.91	Tyrosine-protein kinase (ZAP-70)	F—TCACCTCCGGACCTTTCATT/R—CCATGTGGGAAGCCTTTTCTT/P—TCTTGTATGGTTTTCCTCC	Miller et al. 2016
Innate Immune				
C3	0.88	Complement component 3	F—ATTGGCCTGTCCAAAACACA /R—AGCTTCAGATCAAGGAAGAAGTTC /P—TGGAATCTGTGTGTCTGAACCCC	Raida and Buchmann 2009
C7	0.88	Complement factor	F—ACCTCTGTCCAGCTCTGTGTC /R—GATGCTGACCACATCAAACTGC /P—AACTACCAGACAGTGCTG	Miller et al. 2016
CCL4	0.90	CC chemokine 4	F— TCTCTTCATTGCAACAATCTGCTT/ R—ACAGCAGTCCACGGGTACCT/ P—CTACGCAGCAGCATT	Miller et al. 2016
HERC6	0.85	Probable E3 ubiquitin-protein ligase	F—AGGGACAACTTGGTAGACAGAAGAA/R—TGACGCACACACAGCTACAGAGT/P—CAGTGGTCTCTGTGGCT	Miller et al. 2016
IL-11	0.98	Interleukin 11	F—GCAATCTCTTGCCTCCACTC /R—TTGTCACGTGCTCCAGTTTC /P—TCGCGGAGTGTGAAAGGCAGA	Raida and Buchmann 2008
IL-15	1.00	Interleukin 15	F—TTGGATTTTGCCCTAACTGC /R—CTGCGCTCCAATAAACGAAT /P—CGAACAACGCTGATGACAGGTTTTT	Raida et al. 2011
IL-1B	0.85	Interleukin 1-beta	F—AGGACAAGGACCTGCTCAACT/R—CCGACTCCAACTCCAACACTA/P—TTGCTGGAGAGTGCTGTGGAAGAA	Raida et al. 2011
MMP13	0.96	Matrix Metallopeptidase 13	F—GCCAGCGGAGCAGGAA /R—AGTCACCTGGAGGCCAAAGA /P—TCAGCGAGATGCAAAG	Tadiso et al. 2011
MMP25	1.1	Matrix Metallopeptidase 25	F—TGCAGTCTTTTCCCCTTGGAT/R—TCCACATGTACCCACACCTACAC/P—AGGATTGGCTGGAAGGT	Miller et al. 2016

TF	0.97	Transferrin	F—TTCACTGCTGGAAAATGTGG /R—GCTGCACTGAACTGCATCAT /P—TGGTCCCTGTCATGGTGGAGCA	Raida and Buchmann 2009
TNF	0.80	Tumour necrosis factor	F—CCCACCATACATTGAAGCAGATT/R—GGATTGTATTCACCCTCTAAATGGA/P—CCGGCAATGCAAAA	Ching et al 2010
Stress and Osmoregulation Associated				
CA4	1.01	Carbonic anhydrase 4	F—GGTCATTTTGGTTTTGTACACAGTCT/R—CCTAGATATAGCTATCCACGTACTCACCTA/P—TGATACGTGGTATAGAAAAG	Miller et al. 2016
HIF1A	0.95	Hypoxia-inducible factor 1-alpha	F—TGGCAAATCTGCCTACGAATT/R—GCAGGCTCTTGGTCACATGA/P—ATCATGCCCTGGACTC	Miller et al. 2016
HSC70	0.86	Heat shock cognate 70	F—GGGTCACACAGAAGCCAAAAG /R—GCGCTCTATAGCGTTGATTGGT /P—AGACCAAGCCTAAACTA	Miller et al. 2016
HSP90a	1.05	Heat shock protein 90-alpha	F—AGTACCCTGTTGCACTGAGTTTTAAA/R—GAATGTTTCATTTCCCATTGTTCA/P—ATTGGACTGGTAGATGTGT	Miller et al. 2016
NKAa1-a	1.00	Na/K ATPase α-1a (freshwater)	F—TGGAATCAAGGTTATCATGGTCACT/R—CCCACACCCTTGGCAATG/P—ATCATCCCATCACTGCGA	Miller et al. 2016
NKAa1-b	1.02	Na/K ATPase α-1b (saltwater)	F—GCCTGGTGAAGAATCTTGAAGCT/R—GAGTCAGGGTTCCGGTCTTG/P—CCTCCACCATTTGCTCA	Miller et al. 2016
PDIA4	1.07	protein disulfide isomerase associated 4	F—TGAGGTGCAGGACTTTTTTAAGAA /R—TCGTTGCTCTGTTTCCTGTGA /P—ACATCCTGCCACTGGT	Miller et al. 2016
Tuba1a	0.80	Tubulin alpha-1A chain	F—CTCTGCTGAGAAGGCCTACCAT /R—AGCAGGCGTTGGTGATGTC /P—AGCAGCTGTCTGTTGC	Miller et al. 2016
UBE2Q2	0.96	ubiquitin-conjugating enzyme E2 Q2-like	F—GGCAGGACCACTTGAACGTAA /R— AGGCCTGCACTGAACCAGAT P—TGCTCATTCGGGTGCG	Miller et al. 2016
Viral Disease Associated				
CA054694	0.86	Mitochondrial ribosomal protein (VAR1)	F—CCACCTGAGGTACTGAAGATAAGACA /R—TTAAGTCCTCCTTCCTCATCTGGTA /P—TCTACCAGGCCTTAAAG	Miller et al. 2016
DEXH	0.82	DEXH box helicase, DNA ligase-associated	F—CCATAAGGAGGGTGTCTACAATAAGAT/R—CTCTCCCCCTTCAGCTTCTGT/P—TGGCGCGCTACGTG	Miller et al. 2016
IFI44A	0.9	IFN-induced protein 44-1	F—GCTAGTGCTCTTGAGTATCTCCACAA/R—TCACCAGTAACTCTGTATCATCCTGTCT/P—AGCTGAAAGCACTTGAG	Miller et al. 2016
IFIT5	0.86	Interferon-induced protein tetratricopeptide repeats 5	F—CCGTCAATGAGTCCCTACACATT/R—CACAGGCCAATTTGGTGATG/P—CTGTCTCCAAACTCCCA	Miller et al. 2016

Mx	0.82	Antiviral protein	F—AGATGATGCTGCACCTCAAGTC /R—CTGCAGCTGGGAAGCAAAC /P—ATTCCCATGGTGATCCGCTACCTGG	Eder et al. 2009
VHSV-P10	1.05	VHSV-inducible protein-10	F—GCAAACTGAGAAAACCATCAAGAA/R—CCGTCAGCTCCCTCTGCAT /P—TGTGGAGAAGTTGCAGGC	Miller et al. 2016
Reference				
786d16.1P	1.08	S100 calcium binding protein	F–GTCAAGACTGGAGGCTCAGAG / R–GATCAAGCCCCAGAAGTGTTTG / P–AAGGTGATTCCCTCGCCGTCCGA	Miller et al. 2016
COIL	0.84	Coiled-coil domain-containing protein 84	F—GCTCATTTGAGGAGAAGGAGGATG /R—CTGGCGATGCTGTTCCTGAG /P—TTATCAAGCAGCAAGCC	Miller et al. 2016
MrpL40	0.85	39S ribosomal protein L40, mitochondrial precursor	F—CCCAGTATGAGGCACCTGAAGG /R—GTTAATGCTGCCACCCTCTCAC /P—ACAACAACATCACCA	Miller et al. 2016

Table 3-3: Eigenvalues for principal component analysis of gene expression of biomarkers of immune and stress collected from Arctic char *Salvelinus alpinus*. Maximal contributors to each principal component (>0.30) are highlighted in bold.

Biomarker	PC1	PC2	PC3
C3	0.02	-0.10	0.08
C7	0.01	-0.24	0.13
CA054694	**0.32**	0.14	-0.13
CA4	0.05	-0.17	**0.32**
CCL4	0.21	0.11	-0.04
DEXH	**0.33**	0.03	-0.13
HERC6	**0.31**	0.16	-0.13
HIF1A	0.24	-0.17	0.29
HSC70	0.22	-0.07	0.27
hsp90a	0.12	-0.12	0.02
IFI44a	**0.30**	0.17	-0.08
IFIT5	**0.33**	0.12	-0.12
IgMs	0.00	-0.14	-0.14
IL-11	0.05	**-0.38**	-0.22
IL-15	0.01	-0.11	-0.13
IL-1B	0.05	**-0.36**	-0.23
MHC1	0.03	-0.04	-0.06
MHCII-B	0.01	0.01	**0.31**
MMP13	0.06	**-0.32**	-0.24
MMP25	0.11	-0.25	-0.09
Mx	0.31	0.12	-0.12
NKAa1-a	0.12	-0.18	0.25
NKAa1-b	0.12	-0.12	**0.30**
PDIA4	0.23	-0.09	-0.06
TF	0.02	-0.21	-0.11
TNF	0.05	**-0.31**	-0.25
Tuba1a	0.09	0.06	-0.03
UBE2Q2	0.19	-0.23	**0.32**
VHSV-P10	0.01	-0.09	0.16
ZAP70	0.29	0.09	0.12

Table 3-4: The results of generalized linear mixed effects models of gene expression profiles sampled from Arctic char *Salvelinus alpinus* with sampling location included as a random effect. T-values were calculated using the Satterwaite approximation of degrees of freedom for each covariate.

Fixed effects	Estimate	Std. Error	Df	t value	Pr (>|t|)
PC1 – 23.6% total variation					
RIB	0.5299	0.5057	52.5274	1.048	0.443
I. multifiliis	0.6347	0.8467	16.0762	0.750	0.832
Rare species	-1.4400	0.8937	51.6878	-1.611	0.190
PC2 – 14.3% total variation					
RIB	0.5299	0.5057	51.3625	1.048	0.300
I. multifiliis	-1.80504	0.8467	32.8329	0.750	0.459
Rare species	0.51530	0.8937	50.2560	-1.611	0.113
PC3 – 12.2 % total variation					
RIB	-0.07736	0.46364	15.5103	-0.167	0.8681
I. multifiliis	-1.80504	0.63367	50.1662	-2.849	0.0062**
Rare species	0.51530	0.82709	48.5684	-0.623	0.5359

Figure 3-1: Sampling locations for Arctic char collected during fall migrations in the Kitikmeot region of Nunavut, Canada

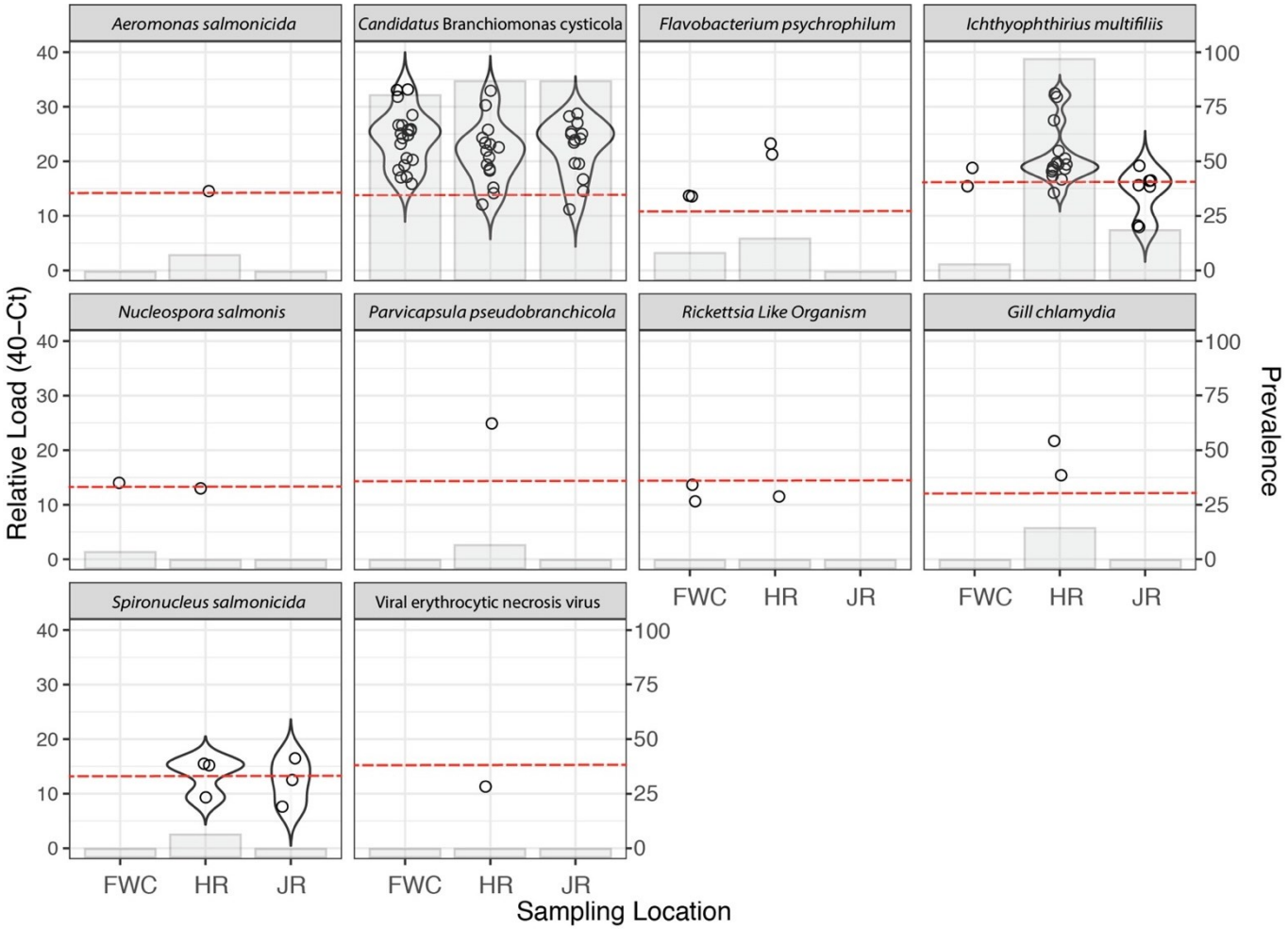

Figure 3-2: Prevalence and relative load of all infectious agents identified by HT qPCR screening of gill tissue in Arctic char collected from three watersheds in the Kitikmeot region of Nunavut, Canada. FWC = Freshwater Creek; HR = Halovik River; JR = Jayco River. Red dashed line indicates the Limit Of Decection for 95% confidence of real amplification of gene target. Bar blots represent infectious agent prevalence within the sample population for each location; FWC n = 27; HR n = 15; JR n = 15.

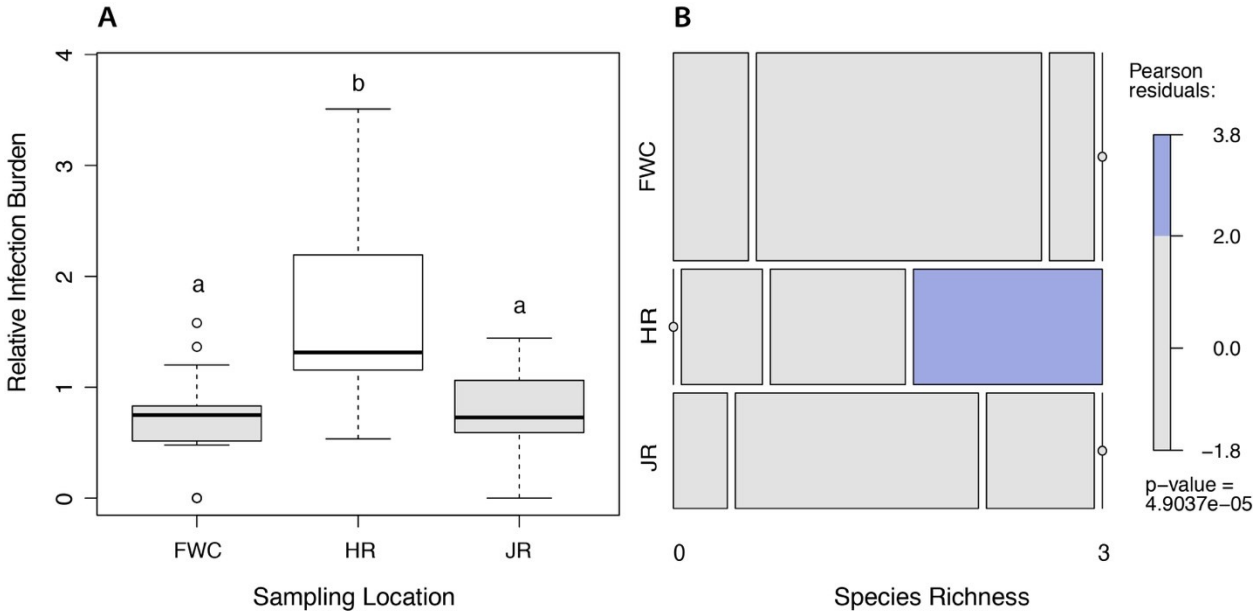

Figure 3-3: Relative Infection Burden, an index summarizing individual pathogen species richness and relative load, and overall species richness per host at each sampling site for microbial infectious agents identified using HT-qPCR on gill tissue from adult anadromous Arctic char.

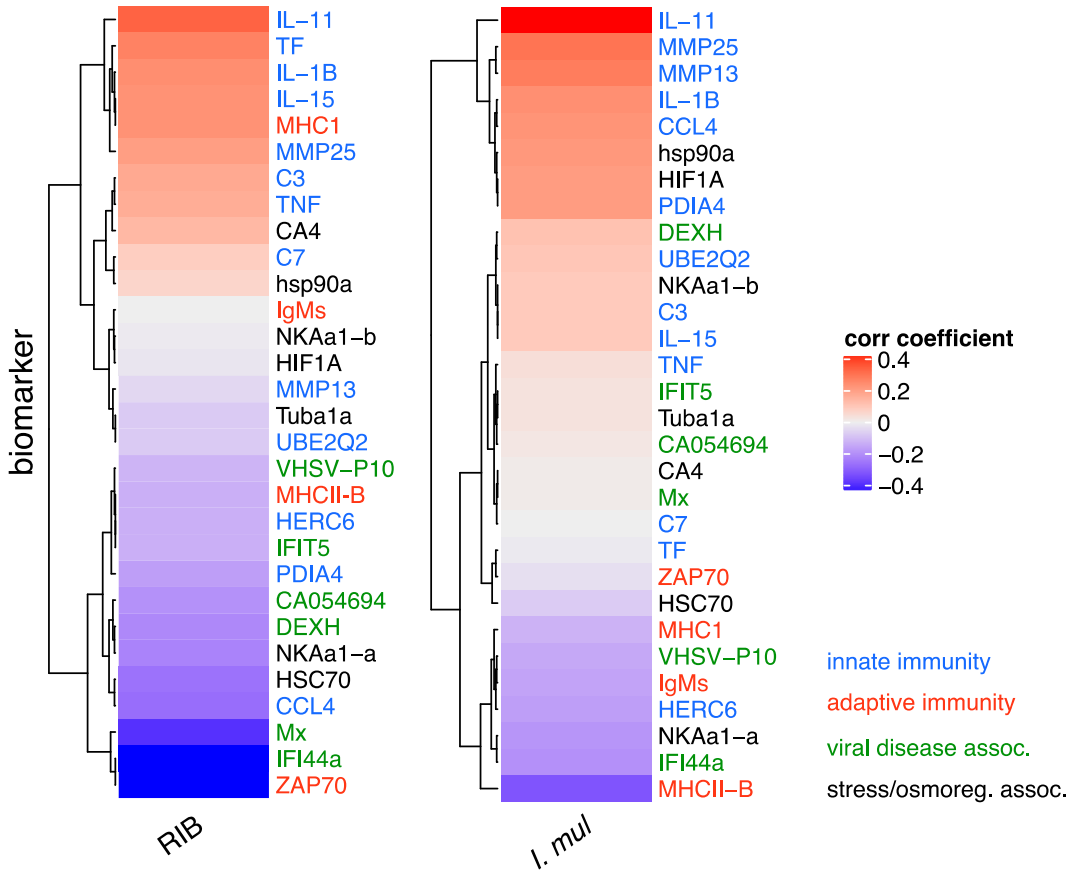

Figure 3-4: Correlation heat map of ddCt gene expression of immune and stress associated biomarkers with relative infection burden (RIB) and relative load of *I. multifiliis* assessed in gill tissue of anadromous adult Arctic char *S. alpinus*.

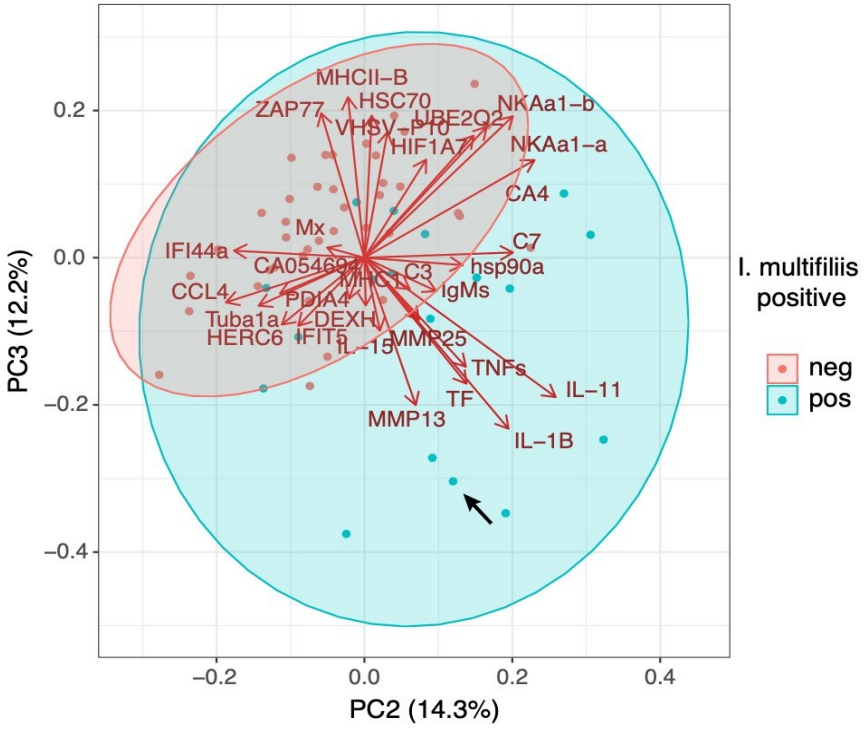

Figure 3-5: Principal component analysis of biomarkers associated with disease development from gill tissue of Arctic char. Ellipse indicate 95% confidence intervals for infection status of *Ichthyopthurius multifiliis*. Arrow indicates individual with viral erythrocytic necrosis virus below the limit of detection.

Chapter 4: **Changes in the condition, infectious agents, and transcription profiles of wild Atlantic salmon experiencing natural temperature increase during fall up-river migrations**

4.1 Abstract

Anadromous Atlantic salmon (*Salmo salar*) populations have declined across their Southern distributions in North America. While river temperature has been identified as a central factor influencing migration behavior and over-winter survival, little research has addressed the prevalence of infectious agents in wild Atlantic salmon populations. Further, current understanding of how temperature may interact with fish condition to influence infection outcomes in the wild is limited. The objective of this research was to investigate changes in an iteroparous anadromous salmon immune, stress, and osmoregulatory transcripts, lipid content, and infectious agent prevalence and relative loads during the initial month of their annual spawning migration. Non-lethal gill samples were collected opportunistically from 27 individual salmon as they entered a fish enumeration fence 150 meters upstream of the estuary. Once sampled, fish were tagged with identification codes and released downstream of the fence and re-sampled each time they entered the counting fence trap for the duration of the study (32 days). Gill samples were screened for a comprehensive panel of 46 known disease-causing agents and 49 biomarkers of adaptive and innate immunity, stress, and viral disease response. Relative infection burden and transcription profiles were modeled with freshwater residency, river temperature, and lipid content. Infectious agents *Tetracapsuloides bryosalmonae, Candidatus* Branchiomonas *cysticola, Flavobacterium psychrophilum, Paranucleospora theridion,* and *Piscichylamidia salmonis* were detected in the population. Relative infection burden and species richness increased over the course of the study. Water

temperature and freshwater residency were related to salmon transcriptional response, but not relative infection burden, highlighting the metabolic cost associated with warming temperatures. The high prevalence and relative load of *T. bryosalmonae* observed in this study may demonstrate increased exposure to transmission pathways as a result of migratory barriers or elevated susceptibility to infection during spawning migrations in anadromous species.

4.2 Introduction

Infectious agents are ubiquitous across ecosystems and species, yet very little is known about their natural distributions and prevalence in wild populations (Walton et al. 2016). This is particularly true for aquatic organisms because outbreaks and disease related mortality is not easily observable. Typically, it is not until major mortality events occur that infectious agents are studied (e.g. whirling disease *Myxobolus cerebralis;* Nehring and Walker 1996). Migratory fish in particular present a major challenge for understanding disease dynamics because of their typically large distributions, complex life-histories, and use of multiple environments. As a result, there is comparatively little known about infectious disease ecology in populations of migratory wild fish (McVicar, 1997; Miller et al. 2014).

Across much of its native distribution, Atlantic salmon (*Salmo salar*) have seen a marked decline throughout the majority of its range over several decades, and conservation efforts limiting harvest have had little impact (Parrish et al. 1998; Jones et al. 2014). In spite of numerous hatchery programs, moratoriums on fishing, and habitat restoration, populations in Atlantic Canada, including those that were previously considered stable, have experienced unprecedented decline (Canadian Science Advisory

Secretariat 2018). Salmon survival in the Atlantic does not appear to be restricted by elevated ocean temperatures experienced during their winter(s) at sea (Russell et al. 2012, Soto et al. 2018), although it is a factor in the Pacific (Siegel and Crozier 2019). Instead, environmental conditions associated with the freshwater phases of Atlantic salmon life-history have been identified as the central factors mediating salmon survival, recruitment, and distribution (Crozier and Kennedy, 2003). For anadromous species already facing demanding physiological changes related to changes in osmoregulation, the metabolic cost imposed by warming water temperatures may induce changes in energy allocation. At the same time, environmental stressors, including increased temperature, have been demonstrated to negatively impact fish immune function and increase infectious disease outbreaks in fishes (Snieszko 1974; Adlard et al. 2015). Increased temperature can also influence the proliferation and development of infectious agents through both direct (e.g. shorter generation times) and indirect (e.g. changes in vector abundance) factors (Adlard et al. 2015), further complicating potential outcomes of increased water temperatures. The effects of infectious agents are now being considered as a potential factor influencing population declines, however the majority of this research addresses transfer of specific infectious agents from aquaculture facilities to wild populations (reviewed in Olivier 2002, Lafferty et al. 2015) rather than investigating infectious agent prevalence and loads in wild populations. As a result, researchers are not able to make comparisons over time or predict how changes to environmental conditions and species distributions may impact infectious agent infection dynamics and consequent wild fish survival.

While experimental studies address fine-scale responses of host/pathogen systems to increasing temperatures (e.g. Strepparava et al. 2018), *in situ* studies are required to

understand how multiple factors interact to influence infectious agent communities and host condition in wild fish. A protocol for assessing the presence and relative load of infectious agents has recently been developed using high-throughput molecular nanofluidic qPCR (Miller et al. 2016). This platform can simultaneously assess host gene expression and infectious agent presence and loading (Miller et al. 2014) and has been used to characterize genetic profiles indicating the early stages of disease development and viral infection in salmonids (Miller et al. 2017). This screening technique requires only a small amount of tissue, facilitating non-lethal and repeated sampling in wild salmon and allowing the investigation of natural changes to infectious agent communities and host response in the face of environmental and anthropogenic pressures (Jeffries et al. 2012; Miller et al. 2014; Bass et al. 2017).

This study sought to characterize the progression of adult Atlantic salmon condition and infectious agent prevalence and relative loads during late-summer up-river migration. Using a Fisheries and Oceans Canada counting fence as a barrier to migration, Atlantic salmon were intercepted and returned to the downstream side of the fence to create a naturalized in-river holding area. Study fish underwent repeated non-lethal sampling to quantify infectious agent prevalence and relative load, host transcriptional profiles, and fat content over the course of the study. My objectives were to 1) to determine infectious agents present in the population; 2) assess changes in infectious agent burden and salmon condition over time spent in fresh water; 3) assess relationships between in-river temperature and infectious agent burden; and 4) characterize the relationship between infectious agent burden and host transcriptional profiles.

4.3 Methods

Twenty-seven anadromous Atlantic salmon were intercepted at a Department of Fisheries and Oceans (DFO) counting fence at the Campbellton River enumeration facility in Campbellton, Newfoundland, Canada during the up-river migration after ocean feeding between 16 June and 20 July 2017. All salmon included in the study were first sea winter fish, also termed grilse, which make up a vast majority of individuals in this population and are easily identifiable by size class (Canadian Science Advisory Secretariate 2018). The fence is located approximately 150 m up-river from the estuary and is non-tidal as a result of the system's relatively high grade in the lower reach. The river below the counting fence is high flow and made up of large boulder riffles and several smaller pools, approximately 1.5 m depth at the deepest areas. Fish were individually dip netted out of the counting trap, transferred to an aerated cooler, and walked approximately 100 m down river to a release site (Figure 4-1). Here, they were quickly sampled for 2x2 mm of gill tissue from the second gill arch on the left side of the fish. Sample was immediately transferred to RNAlater® in 1.5 mL microtubes using sterilized tools. An anterior and posterior muscle lipid measurement was taken with a Distell microwave Fatmeter Sensor (Fish Fatmeter Model-FM 692, Distell, Scotland, UK) as per manufacturer's instructions, and fish were tagged with unique identification codes using highly visible yellow Floy® spaghetti tags for clear identification of tagged fish. The sampling process was repeated each time a tagged fish entered the counting fence trap during the study period (32 days). Microtubes containing gill samples were transferred to 4°C for 24 hrs and then frozen at -20°C prior to transfer to -80°C and

shipped to the DFO Molecular Genetics Laboratory in Nanaimo, BC on dry ice for analysis.

Anterior and posterior Fatmeter measurements were averaged to yield a single fat content estimate at each sampling event. Water temperature was obtained from a monitoring station located at the counting fence approximately 0.5 m below the water surface at the time the fish was sampled. All fish handling was conducted under Animal Care Protocol 101057 issued to Carleton University, and Fisheries and Oceans Canada Scientific Collection Permit NL-3235-16.

4.3.1 RT-qPCR to quantify infectious agent loads and salmon biomarker expression

Gene expression and infectious agent prevalence and relative loads were examined in gill tissue collected from study salmon using high-throughput qPCR on the Fluidigm BioMark Dynamic Array™ Gene Expression platform. All assays were run on isolated RNA after extraction, purification, normalization, and amplification. TaqMan™ assays used in screening were designed to target microbe RNA to allow identification of RNA viruses and microparasites in active states. Consequently, qPCR quantification represents infectious agent productivity rather than absolute quantification, described hereafter as relative load. Details on sensitivity and specificity of each assay, sequence sources, and repeatability of the following RNA extraction, normalization, targeted amplification, and final HT-RT-qPCR using this platform are outlined in the Canadian Science Advisory Secretariat validation of disease screening in salmonids (Miller et al. 2016). To determine what infectious agents were present within the sample population, a subset of amplified product from all samples was pooled and screened for the presence of

46 infectious agents (a full list of screened pathogens is available in supplemental material). Based on this initial screen, all positively detected agents were included in the final assay panel, and selected host biomarkers were added. Infectious agent assays were run in duplicate against each sample, while biomarkers were run as singletons. Assays with efficacies less than 80% were considered failed and removed from analysis. A full list of screened agents, biomarkers, primers, efficiencies, and limits of detection is available in supplemental material. All samples were processed as per methods outlined in Miller et al. (2016) and Teffer et al. (2017).

In brief, RNA extraction was completed using mechanical abrasion in a MM301 mixer mill (Restch Inc. Newtown PA) and Tri-reagent™ followed by addition of 1-bromo-3-chloropropane and purified using Magmax™-96 Microarrays Kits on a Biomek FXP automated liquid handler. Purified RNA quantity and quality was assessed using a Beckman Coulter DTX 880 Multimode Detector (Brea, CA, USA) and sample RNA concentrations were normalized to 62.5 ng/uL. RNA was then converted to cDNA using SuperScript® VILO™ DNA synthesis kit (Life Technologies) following manufacturer's instructions. Specific amplification of target transcripts was performed using primer pairs corresponding to all assays using 1x TaqMan Pre-amp MasterMix as per manufacturer's instructions (Applied Biosystems, CA, USA). Unincorporated primers were removed using Exo-SAP-IT™ (Affymetrix, Santa Clara, CA, USA) and sample was diluted 1:5 with DNA suspension buffer. The effect of this pre-amplification step on final quantification has been investigated thoroughly and was not found to influence the interpretation of results (for extensive detail see Miller et al. 2016). The resulting sample material and assays were loaded directly on to Fluidigm 96.96 Dynamic Array™

integrated fluidic circuit chip for qPCR. 5:1 serial dilution of pooled sample was used to track biomarker efficiency, while combined serial dilutions of artificial construct controls for infectious agent sequences with known copy number were added to the Dynamic Array last and used to track efficiency of each assay on each run. Artificial construct controls contained an extra probe to track potential contamination (see Miller et al. 2016). A series of negative processing controls for RNA extraction, cDNA synthesis, and pre-amplification were also included, as well as a pooled positive control sample of all samples used in the study.

Cycle threshold (Ct) is reported for each biomarker assay using relative expression in the form of $2^{-\Delta\Delta Ct}$ using the averaged expression of three housekeeping genes and the Ct value of a pooled control sample (Livak and Schmittgen 2001). Infective agents are presented as relative load by subtracting the observed Ct value from the total PCR cycles for each qPCR run (i.e. 40-Ct). Infective agents were only considered detected if their Ct was above the assay-specific limit of detection (LOD; 95% level of confidence) as described in Miller et al. (2016), and detected in both duplicate samples. If an infective agent was known to be in the sample population based on detections above the LOD and detected within an individual in subsequent samples, visual assessment of amplification curves was used to confirm positive detections below the LOD.

An index of relative infection burden (RIB) was used to summarize the infective agent load and diversity of agents detected within each individual for each sample. Using this index, both high loads and common and rare pathogens contribute to the burden as follows:

$$RIB = \sum_{i \epsilon m}^{m} \frac{L_i}{Lmax_i},$$

where the relative load of i_{th} infectious agent L_i is divided by the maximum load for that agent observed within the population (i.e., $Lmax_i$), and then summed across all agents found in the sample.

4.3.2 Statistical Analysis

Each fish was sampled at any point it was captured in the counting fence during the 32 day study period. Consequently, samples range from one to six per individual. To visualize changes in infectious agent relative loads and fat content over time, line plots for each individual by study day were constructed. For all subsequent analyses, the study was broken in to three periods to facilitate data reduction techniques without pseudo-replication. Period 1: tagging; period 2: 8-20 days from release; and period 3: 21-32 days from release. In cases where more than one sample from an individual fell within the same period (n = 11), only the last sample within a period was included in analyses. Not all fish were sampled in each study period.

R statistical software version 3.6.1 was used for all data analysis and visualizations. Data were analyzed using statistical models according to each specific research question. Changes in fat content and RIB across study periods was assessed independently by repeated measures ANOVA. Change in infectious agent species richness was tested by chi-squared test of independence. To investigate whether temporal variation and fish condition was related to RIB, a generalized mixed effect model was

constructed with RIB as the response and fat content, temperature (log transformed), study period, and their interaction as fixed effects, and fish id as a random effect. Nonmetric multidimensional scaling with Bray-Curtis dissimilarity distances with 95% confidence ellipse centered on study period was used to visualize patterns in expression of 49 immune, stress, and osmoregulatory associated genes across the three time periods. Significance of separation of transcription profile was investigated using Analysis of Similarity (ANOSIM), and the relationship of RIB, temperature, and fat content was run to visually assess global trends in gene expression using the *Envfit* functions using the *vegan* package (version 2.5-6; Oksanen 2019). To model transcriptional responses to fish condition, infection dynamics, and temperature within each study period, permutational multivariate analysis of variance using based on Bray-Curtis dissimilarity matrices was used (*adonis* function in *vegan*). This function performs a permutational MANOVA to investigate the variation in matrices attributable to specified factors. Ordinations based on biomarker expression for all genes were modeled with temperature, fat content, relative infection burden, and infectious agent species richness, with study period included as a constraining strata for permutations. Heat maps of biomarker expression within each study period were constructed to further investigate gene-specific associations with each factor.

4.4 Results

On average, displaced salmon re-entered the fish counting trap after 11.7 days, with a range of 4:15 hours to 28 days between entries, and all fish survived for the duration of the study. Study fish were observed holding in freshwater areas downstream

of the fence, moving between higher flow areas and deeper pools. It is unknown if any individuals fell back to the marine environment in between sampling events. River temperatures were variable and ranged from 13.1°C – 21°7 C with an average of 17°C (Figure 4-2). A total of 70 samples from 27 grilse were included in analysis: 26 unique samples from individual fish were included in the first study period, 21 in the second, and 17 in the third. All but two salmon screened positive for the presence of infectious agents upon initial sampling, and both of those individuals tested positive in subsequent samples. Infectious agents present in the sample population included the bacteria *Candidatus* Branchiomonas *cysticola* and *Flavobacterium psychrophilum,* and parasites *Paranucleospora theridion*, *Piscichlamydia salmonis*, and *Tetracapsuloides bryosalmonae* (Table 4-1). Prevalence and relative loads of infectious agents increased throughout the duration of the study (Figure 4-3), as did species richness per host (Figure 4-4), which ranged from 0-4 species, and relative infection burden ranged from 0 in uninfected individuals to 3.04 in the most burdened individual (Figure 4-5). *Ca*. B. cysticola was the most prevalent infectious agent, present in 92% of fish at the beginning of the study, followed by *F. psychrophilum*. Co-infection was common among fish sampled; 69% of fish screened positive for two or more infectious agents upon initial sampling and co-infection increased to 100% of the population in study periods 2 and 3 (Figure 4-4). The most drastic change in prevalence and relative load was seen in the myxozoan *T. bryosalmonae,* where the prevalence increased dramatically over the course of the study. In comparison, *Ca*. B. cysticola prevalence and load remained relatively consistent, while *F. psychrophilum* demonstrated a clear increase during study period 2.

Species richness and RIB increased over the course of the study (X^2= 18.037, p = 0.0210; repeated measures ANOVA $F_{2,40.2}$=12.223, p <0.0001), while fat content decreased on average for all individuals ($F_{2,37.9}$=32.563, p <0.0001; Figure 4). Mixed effect models found RIB to be significantly related to fat content but not in-river temperature or study period (Table 4-3).

Gene expression profiles were dissimilar among study periods, with a significant separation observed between initial sampling and subsequent samples in study periods 2 and 3 (ANOSIM R = 0.27; p < 0.001; Figure 4-6). Differences in gene expression within each study period were significantly associated with river temperature and weakly associated with study period and the interaction between temperature and study period , not not RIB (Table 4-4). Genes positively correlated with temperature were not specific to any single functional group, and the temperature effect was insignificant during the first study period. Transcriptional response over time suggests an up-regulation in genes associated with stress and immune function (hsp90a, MMP13, IL-15 and IgMs), and a down-regulation of genes associated with viral disease response (Mx, RSAD, DEXH, IFI44a), oxygen transport (HBA) and transcriptional regulation of protein biosynthesis (EEF2; Figure 4-7A). Transcriptomic response to river temperature was strongest in study period 2, when the average temperature upon sampling was 19.8 C (Figure 4-7B). Strong positive correlations were observed in the thermal stress associated molecular chaperones HSP90a and PDIA4. Innate immune associated inflammatory cytokine IL-15, inflammatory regulators MMP13 and MMP25, and genes associated with adaptive immunity including the immunoglobulin IgT and T-cell stimulating kinase ZAP70 and associated TCRa receptor as well as antigen CD8a were positively correlated with

temperature. Observed relationships shifted in study period 3, where temperature was more consistently associated with stress and osmoregulatory transcripts, however observed correlations were not as strong as in study period 2. Expression of transcription factor EEF2, hemoglobulin component HBA, ribosomal protein RPL6, and sodium potassium ATPase component NKA-a3 were negatively associated with temperature, while molecular chaperones HSP90ab1, HSP90a and PDIA4, structural protein Tuba1a, and transcription factor JUN were positively correlated with temperature. Negative association with temperature were seen in inflammatory cytokines IL-17D and IL-15 and ribosomal protein RPL6. Individual genes were only associated with RIB in study period 3, predominantly represented by weak positive correlations of HBA, MHC1, and osmoregulatory sodium-potassium ATPase isoforms (Figure 4-8).

4.5 Discussion

This study was the first to assess changes in infectious agent dynamics and broad scale transcriptional response in free-ranging migratory adult Atlantic salmon. Increase in relative infection burden, infectious agent richness, and decrease in fat content are consistent with previous work on migratory adult salmon upon freshwater re-entry (Moore 1997; Jonsson & Hansen 1991; Miller et al. 2014). Infectious agents observed were known to be endemic to the region and have been detected in wild salmon in previous research (Hoffman et al. 2009; Sterud et al. 2007; Starliper 2011; Kambestad 2018), however the prevalence of *T. bryosalmonae* observed was extremely high compared to previous surveys conducted by Fisheries and Oceans (M. Robertson,

personal communication) and recent work on Atlantic salmon in North America and Greenland (Teffer et al. In Press).

4.5.3 Fish Condition and Infectious Agent Communities

Decreased fish condition, reflected in fat content, is linked to metabolic shifts associated with energy allocation in adult salmon during spawning migrations (Bombardier et al. 2010). Migratory adult Atlantic salmon cease feeding upon re-entering freshwater. As a result, survival is dependent on available energy stores to be adequate to support up-river migration, gonadal maturation, spawning behavior, over-winter survival, and subsequent out-migration (Moore 1997; Jonsson, Jonsson & Hansen 1991). Considering that investment in defense against infectious agents may reduce fecundity (Van Baalen 1998), and the low probability of survival post spawning, down-regulation of immune associated transcripts is a potential tradeoff that reallocates energetic resources towards reproduction and survival. Significant decreases in body condition and lipid reserves are related to the onset of anorexia and freshwater residency (Kadri 1995; Bombardier et al. 2010), limiting the energy available for cellular maintenance and immune functions. This strategy is similar to that used by semelparous species (e.g. Pacific salmon; Bass et al. 2019) however may leave individuals more vulnerable to opportunistic infection. Increase in infectious agents during exposure to freshwater is typical of migratory salmonids, but the decrease in *F. psychrophilum* observed in some individuals may indicate more immune activity than what is observed in semelparous species, where *F. psychrophilum* typically increases with time in fresh water for Pacific salmon (Teffer et al. 2017; Bass et al. 2018).

The increase in within-host infectious agent species richness observed over the course of the study corresponds to an increase in co-infection over the course of freshwater residency. The order in which infectious agents infect a host can determine the outcome of infectious agent-host interactions due to either synergistic or antagonistic interactions between or among pathogens. Opportunistic co-infection can occur as a consequence of decreased host immunocompetency in response to a present agent allowing for opportunistic infection by additional pathogens (Kotob et al. 2016; Figueroa et al. 2017). In other cases, an antagonistic relationship may exist, where the presence of an infectious agent inhibits the infection or virulence of another (Sofonea 2015). Here, the relative loads of *F. psychrophilum* and *Ca.* B. cysticola decreased alongside a major increase in the prevalence and relative load for *T. bryosalmonae*. Whether this change represents a true antagonistic relationship between *T. bryosalmonae* and *F. psychrophilum* and *Ca.* B. cysticola or is a result of increased exposure to *T. bryosalmonae*, or decreased host immunocompetency requires further investigation.

4.5.4 Transcription Profiles

In the present study, salmon transcription profiles were more closely associated with river temperature than time spent in fresh water or RIB, likely driven by the strong response to elevated river temperatures observed during study period 2. The concurrent up-regulation of genes associated with stress and both adaptive and innate immunity in response to temperature may be linked with temperature stimulated metabolic activity, priming of immune defenses in response to stress, or in response to elevated infection (Sigh, Lindenstôm and Buchmann 2004). Up-regulation of molecular chaperones such as

HSP90 and PDIA4 has previously been observed in adult (Teffer et al. 2018) and juvenile (Houde et al. 2019) Pacific salmon experimentally exposed to thermal stress (also see Akbarzadeh et al. 2018), and Atlantic salmon post-smolts reared in high temperature conditions (Akbarzadeh et al. 2018; Jensen et al. 2015). However, heat shock proteins have also been associated with infection in Atlantic salmon exposed to sea-lice (Provan et al 2013). Higher-temperature conditions were also related with significant up-regulation of the majority of mRNA transcripts compared to low-temperature conditions (Jensen et al. 2015). The reduction of temperature-dependent expression observed in study period 3 may represent chronic temperature stress and metabolic shift, indicated by the observed down-regulation of transcription factors EEF2 and HBA. Reduced expression of transcription factors may indicate a reduction in protein synthesis as fish begin to shift metabolic activity under fasting conditions (Bombardier et al. 2010). The concurrent positive association between HBA with RIB in study period three thus may suggest metabolic shifts in response to infection. Taken with the coinciding positive association of adaptive immune complex MHC1 and biomarkers of genes associated with osmoregulation and ion balance (NKAa1-a, NKA-b1, and NKAa1-b; Shrimpton et al. 2015; Houde et al. 2019), high infection burden may be causing disruption in osmoregulation that could be exacerbated by chronic thermal stress (Akbarzadeh et al. 2018).

Elevated water temperature is known to result in metabolic stress in adult Atlantic salmon (Wilkie et al. 1997), and river temperatures are anticipated to rise across Atlantic salmon distributions in the coming years (IPCC 2019). Here, temperature was more closely associated with transcription profiles than freshwater residency and relative

infection burden, demonstrating the metabolic responsiveness and corresponding energy use imposed on fish under thermal stress. Adult anadromous Atlantic salmon have been shown to behaviourally thermoregulate at temperatures between 17-19°C during holding phases of their spawning migrations (Frechette et al. 2018; Moore et al. 2012). Barriers to migration such as dams and waterfalls can cause migration delay in Atlantic salmon (Thorstad et al. 2003; Twardek et al. 2019), however the high survival rate of fish in this study indicates that the barrier created by the fish fence does not represent a substantial immediate physiological challenge to the population. The fish counting fence restricted salmon to relatively high-flow habitat with extensive hydrological mixing and thus limited access to deep pools where cooler water would be available. Consequently, the observed transcriptional response may not represent the physiological status of fish able to access a larger diversity of habitats, providing insight for future conditions where thermal refugia may not be available. Broad scale behavioural responses to temperature have already been observed in the study region, as timing of Atlantic salmon migrations in Newfoundland has shifted in response to climactic changes (Dempsen et al. 2017). River entry is now 12 days earlier in warm conditions, and later in years where ocean conditions are cooler and there is a higher amount of in-shore sea-ice (Canadian Science Advisory Secretariate 2018). The cumulative effects of reduced time at sea for feeding, potentially leading to reduced condition, and elevated metabolic stress associated with temperature warrant further study to understand how wild fish are able to adjust both physiologically and behaviourally.

4.5.5 *Tetracapsuloides bryosalmonae*

While *T. bryosalmonae* has been observed in the region, the prevalence observed at the end of the study was higher than previous sampling efforts (M. Robertson, personal communication). *T. bryosalmonae* is a myxozoan endoparasite of mainly salmonids that is the causative agent of the potentially fatal proliferative kidney disease (PKD). First attributed to mass mortalities in aquaculture, PKD has been associated with large-scale mortality events in wild juvenile Atlantic salmon (Sterud et al. 2007*)* and population declines of wild brown trout *Salmo trutta* in Europe (Wahli et al. 2002). A freshwater bryozoan is the primary host and thus required to complete the life cycle (Okamura et al. 2011). Bryozoans are benthic, clonal organisms that grow on the substrate. Infection with *T. brysalmonae* occurs when spores released from bryozoans come in to contact with fish gills and/or skin, using amoeboid cells to infiltrate fish tissue (Longshaw et al. 2002). The main site of infection is the kidney, however extrasporogenic stages undergo proliferation in the blood stream and can infect other organs, and spores passed in urine are infective to bryozoan hosts (Hedrick et al. 2004). The sudden increase in *T. bryosamonae* may be the result of study fish being constrained to habitat that held infective bryozoans, artificially increasing exposure risk compared to fish that are able to quickly transit the system.

Clinical pathology of PKD such as kidney hyperplasia and anemia and physiological impairment is positively associated with increased temperatures (Morris et al. 2005, Bruneaux et al. 2017). Consequently, anthropogenic factors associated with the spread and proliferation of *T. bryosalmonae* such as climate warming and barriers to migration have the potential to increase infection rates and pathogen distribution in wild fish (Harris, Machmann and Bakke 2011). All fish sampled remained in high condition

for the duration of the study, however clinical signs of disease are often not observed in adult salmon (Hoffman et al 1998). Inter and intraspecific variation in adaptive immunity to *T. bryosalmonae* has been demonstrated, however survivors appear to develop resistance to subsequent infection and/or disease (Hendrick et al. 1993; Cauwelier et al. 2010). Further, reinfection has the potential to cause decreased immunocompetency and leave individuals susceptible to secondary infections. Adult salmon may also act as vectors, transferring from the bryozoan host to more susceptible juveniles (Sterud et al. 2007). Given the recent declines in population observed in the Campbellton River, the high prevalence of *T. bryosalmonae* observed here indicates this pathogen is present in the population. Whether or not the disease is contributing to population decline warrants further research.

4.6 Conclusion

To my knowledge, data presented here represent the first investigation of temporal changes in infectious agent communities in wild Atlantic salmon. Given the high pathogenic potential of *T. bryosalmonae*, additional monitoring and/or surveys should be executed to determine regional prevalence and potential location of bryozoan vectors. The association between RIB and fat content suggests that fish condition may play a central role in immunocompetency in the wild, however research reducing confounding environmental factors is required. Given the transcript responsiveness to elevated temperatures, future work investigating the potential immunological consequences of thermal shock and chronic thermal stress in wild Atlantic salmon under natural conditions where habitat such as thermal refugia is present is warranted. Field research of this kind should be preferred over experimental holding studies, where

confinement stress and artificial environments reduce the applicability of data to natural environments (Portz et al. 2006).

Table 4.1: Abbreviations, names, and groups of infectious agents detected in migratory adult Atlantic salmon collected in the Campbellton River system using high-throughput qPCR. P = prevalence, E = assay efficiency.

Group	Infectious Agent	Assay ID	Taqman Primer Sequences (5'–3') Probe Sequence (FAM-5'–3'-MGB)	P	E	sequence source
bacteria	*Aeromonas hydrophila*	ae_hyd	F—ACCGCTGCTCATTACTCTGATG / R—CCAACCCAGACGGGAAGAA P—TGATGGTGAGCTGGTTG	0.00		Lee et al. 2006
	Aeromonas salmonicida	ae_sal	F—TAAAGCACTGTCTGTTACC / R—GCTACTTCACCCTGATTGG / P—ACATCAGCAGGCTTCAGAGTCACTG	0.00		Keeling et al. 2013
	Candidatus Branchiomonas cysticola	c_b_cys	F—AATACATCGGAACGTGTCTAGTG / R—GCCATCAGCCGCTCATGTG / P—CTCGGTCCCAGGCTTTCCTCTCCCA	**0.96**	0.95	Mitchell et al. 2013
	Candidatus Piscichlamydia salmonis	pch_sal	F—TCACCCCCAGGCTGCTT / R—GAATTCCATTTCCCCCTCTTG / P—CAAAACTGCTAGACTAGAGT	**0.07**	0.97	Nylund et al. 2008
	Flavobacterium psychrophilum	fl_psy	F—GATCCTTATTCTCACAGTACCGTCA/R-TGTAAACTGCTTTTGCACAGGAA P—AAACACTCGGTCGTGACC	**0.74**	0.92	Duesund et al. 2010
	Gill chlamydia	sch	F—GGGTAGCCCGATATCTTCAAAGT / R—CCCATGAGCCGCTCTCTCT / P—TCCTTCGGGACCTTAC	0.00	.97	Duesund et al. 2010
	Piscirickettsia salmonis	pisck_sal	F—TCTGGGAAGTGTGGCGATAGA / R—TCCCGACCTACTCTTGTTTCATC / P—TGATAGCCCCGTACACGAAACGGCATA	0.00		Corbeil et al. 2003
	Renibacterium salmoninarum	re_sal	F—CAACAGGGTGGTTATTCTGCTTTC/ R-CTATAAGAGCCACCAGCTGCAA / P—CTCCAGCGCCGCAGGAGGAC	0.00		Powell et al. 2005
	Rickettsia-like organism	rlo	F—GGCTCAACCCAAGAACTGCTT / R—GTGCAACAGCGTCAGTGACT / P—CCCAGATAACCGCCTTCGCCTCCG	0.00		Lloyd et al. 2011
	Vibrio anguillarum	vi_ang	F-CCGTCATGCTATCTAGAGATGTATTTGA/R-CCATACGCAGCCAAAAATCA/ P—TCATTTCGACGAGCGTCTTGTTCAGC	0.00		In house
	Vibrio salmonicida	vi_sal	F—GTGTGATGACCGTTCCATATTT / R—GCTATTGTCATCACTCTGTTTCTT / P—TCGCTTCATGTTGTGTAATTAGGAGCGA	0.00		In house

platyhelminth	*Gyrodactylus salaris*	gy_sal	F—CGATCGTCACTCGGAATCG / R—GGTGGCGCACCTATTCTACA / P—TCTTATTAACCAGTTCTGC	0.00	0.98	Collins et al. 2010
	Nanophyetus salmincola	na_sal	F–CGATCTGCATTTGGTTCTGTAACA/RCCAACGCCACAATGATAGCTATAC / P—TGAGGCGTGTTTTATG	0.00	1.15	In house
protozoa	*Ceratonova shasta*	ce_sha	F—CCAGCTTGAGATTAGCTCGGTAA / R—CCCCGGAACCCGAAAG / P—CGAGCCAAGTTGGTCTCTCCGTGAAAAC	0.00	1.02	Hallett & Bartholomew 2006
	Cryptobia salmositica	cr_sal	F—TCAGTGCCTTTCAGGACATC / R—GAGGCATCCACTCCAATAGAC / P—AGGAGGACATGGCAGCCTTTGTAT	0.00	1.00	In house
	Dermocystidium salmonis	de_sal	F—CAGCCAATCCTTTCGCTTCT / R—GACGGACGCACACCACAGT / P—AAGCGGCGTGTGCC	0.00	0.97	In house
	Facilispora margolisi	fa_mar	F—AGGAAGGAGCACGCAAGAAC / R—CGCGTGCAGCCCAGTAC / P—TCAGTGATGCCCTCAGA	0.00	1.12	In house
	Ichthyophonus hoferi	ic_hof	F—GTCTGTACTGGTACGGCAGTTTC/ R-TCCCGAACTCAGTAGACACTCAA / P—TAAGAGCACCCACTGCCTTCGAGAAGA	0.00	0.98	White et al. 2013
	Ichthyophthirius multifiliis	ic_mul	F—AAATGGGCATACGTTTGCAAA/R-AACCTGCCTGAAACACTCTAATTTTT / P—ACTCGGCCTTCACTGGTTCGACTTGG	0.00	0.97	In house
	Kudoa thyrsites	ku_thy	F—TGGCGGCCAAATCTAGGTT / R—GACCGCACACAAGAAGTTAATCC / P—TATCGCGAGAGCCGC	0.00	0.88	Funk et al. 2007
	Loma salmonae	lo_sal	F—GGAGTCGCAGCGAAGATAGC/ R-CTTTTCCTCCCTTTACTCATATGCTT / P—TGCCTGAAATCACGAGAGTGAGACTACCC	0.00	1.05	In house
	Myxobolus arcticus	my_arc	F—TGGTAGATACTGAATATCCGGGTTT / R—AACTGCGCGGTCAAAGTTG / P—CGTTGATTGTGAGGTTGG	0.00	0.92	In house
	Myxobolus cerebralis	my_cer	F—GCCATTGAATTTGACTTTGGATTA/ R-ACCATTCATGTAAGCCCGAACT / P—TCGAAGCCTTGACCATCTTTTGGCC	0.00	0.97	Kelley et al. 2004
	Myxobolus insidiosus	my_ins	F—CCAATTTGGGAGCGTCAAA / R—CGATCGGCAAAGTTATCTAGATTCA / P—CTCTCAAGGCATTTAT	0.00	1.00	In house

	Neoparamoeba perurans	ne_per	F—GTTCTTTCGGGAGCTGGGAG / R—GAACTATCGCCGGCACAAAAG / P—CAATGCCATTCTTTTCGGA	0.00	1.07	Fringuelli et al. 2012
	Nucleospora salmonis	nu_sal	F—GCCGCAGATCATTACTAAAAACCT / R—CGATCGCCGCATCTAAACA / P—CCCCGCGCATCCAGAAATACGC	0.00	1.06	Foltz et al. 2009
	Paranucleospora theridion	pa_ther	F—CGGACAGGGAGCATGGTATAG / R—GGTCCAGGTTGGGTCTTGAG / P—TTGGCGAAGAATGAAA	**0.13**	1.16	Nylund et al. 2010
	Parvicapsula kabatai	pa_kab	F—GTCGGATGATAAGTGCATCTGATT / R—ACACCACAACTCTGCCTTCCA / P—TGCGACCATCTGCACGGTACTGC	0.00	0.98	In house
	Parvicapsula minibicornis	pa_min	F-AATAGTTGTTTGTCGTGCACTCTGT/R-CCGATAGGCTATCCAGTACCTAGT/ P—TGTCCACCTAGTAAGGC	0.00	0.92	Hallett & Bartholomew 2009
	Parvicapsula pseudobranchicola	pa_pse	F—CAGCTCCAGTAGTGTATTTCA / R—TTGAGCACTCTGCTTTATTCAA / P—CGTATTGCTGTCTTTGACATGCAGT	0.00	0.84	Jørgensen et al. 2011
	Sphaerothecum destruens	sp_des	F—GCCGCGAGGTGTTTGC / R—CTCGACGCACACTCAATTAAGC / P—CGAGGGTATCCTTCCTCTCGAAATTGGC	0.00	1.00	In house
	Spironucleus salmonicida	sp_sal	F-AACCGGTTATTCGTGGGAAAG/R-TTAACTGCAGCAACACAATAGAATACT P—TGCCAGCAGCCGCGGTAATTC	0.00	0.95	In house
	Tetracapsuloides bryosalmonae	te_bry	F—GCGAGATTTGTTGCATTTAAAAAG / R-GCACATGCAGTGTCCAATCG / P—CAAAATTGTGGAACCGTCCGACTACGA	**0.55**	0.96	Bettge et al. 2009
virus	Atlantic salmon paramyxovirus	aspv	F-CCCATATTAGCAAATGAGCTCTATR-CGTTAAGGAACTCATCATTGAGCTT/ P—AGCCCTTTTGTTCTGC	0.00	0.97	Nylund et al. 2008
	Infectious haematopoietic necrosis virus	ihnv	F—AGAGCCAAGGCACTGTGCG / R—TTCTTTGCGGCTTGGTTGA / P—TGAGACTGAGCGGGACA	0.00	0.98	Purcell et al. 2013
	Pacific salmon parvovirus	pspv	F—CCCTCAGGCTCCGATTTTTAT / R—CGAAGACAACATGGAGGTGACA / P—CAATTGGAGGCAACTGTA	0.00	0.96	In house
	Piscine myocardial virus	pmcv	F—TTCCAAACAATTCGAGAAGCG / R—ACCTGCCATTTTCCCCTCTT / P—CCGGGTAAAGTATTTGCGTC	0.00	.96	Løvoll et al. 2010

Piscine Orthoreovirus	prv	F—TGCTAACACTCCAGGAGTCATTG / R—TGAATCCGCTGCAGATGAGTA / P—CGCCGGTAGCTCT	0.00	0.92	Wiik-Nielsen et al. 2012
Salmonid herpesvirus	omv	F—GCCTGGACCACAATCTCAATG / R—CGAGACAGTGTGGCAAGACAAC / P—CCAACAGGATGGTCATTA	0.00	1.11	In house
Viral encephalopathy and retinopathy virus	ver	F—TTCCAGCGATACGCTGTTGA / R—CACCGCCCGTGTTTGC / P—AAATTCAGCCAATGTGCCCC	0.00	1.12	Korsnes et al. 2005
Viral erythrocytic necrosis virus	ven	F—CGTAGGGCCCCAATAGTTTCT /R—GGAGGAAATGCAGACAAGATTTG / P—TCTTGCCGTTATTTCCAGCACCCG	0.00	1.10	James Winton, pers. comm.
Viral haemorrhagic septicaemia virus	vhsv	F—ATGAGGCAGGTGTCGGAGG / R—TGTAGTAGGACTCTCCCAGCATCC / P—TACGCCATCATGATGAGT	0.00	1.05	Garver et al. 2011

Table 4-2: Assay name and sequences for biomarkers of immune function and stress. E = assay efficiency.

Functional group	Assay name	Gene name	Primer and probe sequences	Source	E
adaptive immunity	B2M	Beta 2-microglobulin	F—TTTACAGCGCGGTGGAGTC /R—TGCCAGGGTTACGGCTGTAC P—AAAGAATCTCCCCCCAAGGTGCAGG	Miller et al. 2016	1.12
	CD8a	T-cell surface glycoprotein CD8 alpha chain	F—ACACCAATGACCACAACCATAGAG/R-GGGTCCACCTTTCCCACTTT P—ACCAGCTCTACAACTGCCAAGTCGTGC	Raida et al. 2011	1.03
	IgMs	Immunoglobulin	F—CTTGGCTTGTTGACGATGAG /R—GGCTAGTGGTGTTGAATTGG P—TGGAGAGAACGAGCAGTTCAGCA	Raida et al. 2011	1.05
	IgT	Immunoglobulin tau	F-CAACACTGACTGGAACAACAAGGT/R-CGTCAGCGGTTCTGTTTTGGA P—AGTACAGCTGTGTGGTGCA	Tadiso et al. 2011	1.07
	MHCI	Major histocompatibility complex I	F—GCGACAGGTTTCTACCCCAGT /R—TGTCAGGTGGGAGCTTTTCTG P—TGGTGTCCTGGCAGAAAGACGG	Ingerslev et al. 2009	1.06
	MHCII-B	Major histocompatibility complex IIβ	F—TGCCATGCTGATGTGCAG /R—GTCCCTCAGCCAGGTCACT P—CGCCTATGACTTCTACCCCAAACAAAT	Raida & Buchmann 2008	1.00
	RSAD	Radical S-adenosyl methionine Domain-containing protein 2	F—GGGAAATTAGTCCAATACTGCAAAC/R–GCCATTGCTGACAATACT GACACT / P—CGACCTCCAGCTCC	Miller et al. 2016	0.95
	TCRa	T cell receptor alpha chain	F—ACAGCTTGCCTGGCTACAGA/R—TGTCCCCTTTCACTCTGGTG P—CAGCGCACACAAGGCTAATTCG	Miller et al. 2016	1.02
	ZAP70	Tyrosine-protein kinase (ZAP-70)	F—TCACCTCCGGACCTTTCATT/R—CCATGTGGGAAGCCTTTTCTT P—TCTTGTATGGTTTTCCTCC	Miller et al. 2016	0.91
innate immunity	C1Qc	complement C1q subcomponent subunit C	F—CGCCGGTGAGTGGAATCTA/R—CTTCTCCATCATGTGGTGTGCTA P—ACCTCCAAACATAGAAGAG	Miller et al. 2016	0.94
	C3	Complement component 3	F—ATTGGCCTGTCCAAAACACA/R-AGCTTCAGATCAAGGAAGAAGTTC P—TGGAATCTGTGTGTCTGAACCCC	Raida & Buchmann 2009	1.05
	C7	Complement factor	F—ACCTCTGTCCAGCTCTGTGTC /R—GATGCTGACCACATCAAACTGC P—AACTACCAGACAGTGCTG	Miller et al. 2016	0.90
	CCL4	CC chemokine 4	F— TCTCTTCATTGCAACAATCTGCTT/R-ACAGCAGTCCACGGGTACCT P—CTACGCAGCAGCATT	Miller et al. 2016	0.93

	GAL3	Galectin-3-binding protein precursor	F—TTGTAGCGCCTGTTGTAATCATAT/R-TACACTGCTGAGGCCATGGA P—CTTGGCGTGGTGGC	Miller et al. 2016	1.12
	HERC6	Probable E3 ubiquitin-protein ligase	F-AGGGACAACTTGGTAGACAGAAGAA/R–TGACGCACACACAGCTA CAG / P—CAGTGGTCTCTGTGGCT	Miller et al. 2016	0.96
	IL-15	Interleukin 15	F—TTGGATTTTGCCCTAACTGC /R-CTGCGCTCCAATAAACGAAT P—CGAACAACGCTGATGACAGGTTTTT	Raida et al. 2011	1.14
	IL-17D	interleukin 17-delta	F— CAACAGAAGTGCGAACGATG /R—GATGCCACATCGCATAACAG P—TGGTCGAGTATCTTTCGTGTGTTTGC		1.04
	IL-1B	Interleukin 1-beta	F—AGGACAAGGACCTGCTCAACT/R-CCGACTCCAACTCCAACACTA P—TTGCTGGAGAGTGCTGTGGAAGAA	Raida et al. 2011	0.93
	IL-8	Interleukin 8	F—GAGCGGTCAGGAGATTTGTC/R-TTGGCCAGCATCTTCTCAAT P—ATGTCAGCGCTCCGTGGGT	Ingerslev et al. 2009	1.04
	MMP13	Matrix Metallopeptidase 13	F—GCCAGCGGAGCAGGAA /R—AGTCACCTGGAGGCCAAAGA P—TCAGCGAGATGCAAAG	Tadiso et al. 2011	1.02
	MMP25	Matrix Metallopeptidase 25	F—TGCAGTCTTTTCCCCTTGGAT/R-TCCACATGTACCCACACCTACAC P—AGGATTGGCTGGAAGGT	Miller et al. 2016	0.93
	TNF	Tumour necrosis factor	F—CCCACCATACATTGAAGCAGATT/R—GGATTGTATTCACCCTCTAAA TGGA / P—CCGGCAATGCAAAA	Ching et al. 2010	0.82
	UBE2Q2	ubiquitin-conjugating enzyme E2 Q2-like	F—GGCAGGACCACTTGAACGTAA/R—AGGCCTGCACTGAACCAGAT P—TGCTCATTCGGGTGCG	Miller et al. 2016	0.87
stress/osmoregulation	DEXH	DEXH box helicase, DNA ligase-associated	F—CCATAAGGAGGGTGTCTACAATAAGAT/R—CTCTCCCCCTTCAGCT TCTGT / P—TGGCGCGCTACGTG	Miller et al. 2016	0.99
	EF-2	Elongation factor 2	F—AGGTCACAGCCGCCCTTAG/R—ACACAGTCTCTGTCTGCACACACA P—CGACTGCGTCTCAGGT	Miller et al. 2016	0.87
	HBA	Hemoglobin subunit alpha	F—GCCCTGGCTGACAAATACAGA/R—GAGCAGGAACTGGAGTCCAAT P—ACCATCATGAAAGTCC	Miller et al. 2016	0.98
	HIF1A	Hypoxia-inducible factor 1-alpha	F—TGGCAAATCTGCCTACGAATT/R—GCAGGCTCTTGGTCACATGA P—ATCATGCCCTGGACTC	Miller et al. 2016	1.00
	HSP90a	Heat shock protein 90-alpha	F—AGTACCCTGTTGCACTGAGTTTTAAA/R—GAATGTTTCATTTCCCA TTGTTCA / P—ATTGGACTGGTAGATGTGT	Miller et al. 2016	1.05
	HSP90ab1	heat shock protein 90-beta	F—GACACGGTGTTGGGTTGGTT /R—TTGCAGTCAACTCTCCATGCA P—TCATGTGCAACATAACAT	Miller et al. 2016	1.14

	JUN	AP-1 Transcription Factor Subunit	F—TTGTTGCTGGTGAGAAAACTCAGT/R—CCTGTTGCCCTATGAATTG TCTAGT/P—AGACTTGGGCTATTTAC	Miller et al. 2016	0.88
	LDHB	lactate dehydrogenase B	F—GTCACTGCTCCCATTTTACACTCTAG/R—CCCAAACTCCCTCCCAG ATAAC/P—CTGTTCTTAGCTTCCC	Miller et al. 2016	0.93
	NKA_a3	Na/K ATPase α-3a (freshwater)	F—GGAGACCAGCAGAGGAACAG /R—CCCTACCAGCCCTCTGAGT P—AAGACCCAGCCTGAAATG	Stefansson et al. 2007	1.15
	NKAa1-a	Na/K ATPase α-1a (freshwater)	F—TGGAATCAAGGTTATCATGGTCACT/R—CCCACACCCTTGGCAATG P—ATCATCCCATCACTGCGA	Miller et al. 2016	1.09
	NKAa1-b	Na/K ATPase α-1b (saltwater)	F—GCCTGGTGAAGAATCTTGAAGCT/R—GAGTCAGGGTTCCGGTCTT P—CCTCCACCATTTGCTCA	Miller et al. 2016	1.01
	PDIA4	protein disulfide isomerase associated 4	F—TGAGGTGCAGGACTTTTTTAAGAA /R—TCGTTGCTCTGTTTCCTGT P—ACATCCTGCCACTGGT	Miller et al. 2016	0.92
	SHOP21	Salmon hyperosmotic protein 21	F—GCGGTAGTGGAGTCAGTTGGA /R—GCTGCTGACGTCTCACATCAC P—CCTGTTGATGCTCAAGG	Miller et al. 2016	1.00
	Tuba1a	Tubulin alpha-1A chain	F—CTCTGCTGAGAAGGCCTACCAT /R—AGCAGGCGTTGGTGATGTC P—AGCAGCTGTCTGTTGC	Miller et al. 2016	0.85
viral disease associated	CA054694	Mitochondrial ribosomal protein (VAR1)	F—CCACCTGAGGTACTGAAGATAAGACA/R—TTAAGTCCTCCTTCCTC ATCTGGTA / P—TCTACCAGGCCTTAAAG	Miller et al. 2016	0.87
	CD9	CD9 antigen	F—CGCCACCACAACCAAGGT /R—TCCTCAGCCTCTTCTTCTTGAAG P—AGATCCCCAAGACTCTGTCAGACGCCT	Miller et al. 2016	0.96
	IFI44A	IFN-induced protein 44-1	F—GCTAGTGCTCTTGAGTATCTCCACAA/R— TCACCAGTAACTCTGTATCATCCTGTCT/P—AGCTGAAAGCACTTGAG	Miller et al. 2016	0.86
	IFIT5	Interferon-induced protein w tetratricopeptide repeats 5	F—CCGTCAATGAGTCCCTACACATT/R—CACAGGCCAATTTGGTGATG P—CTGTCTCCAAACTCCCA	Miller et al. 2016	0.91
	IRF1	Interferon regulatory factor 1	F—CAAACCGCAAGAGTTCCTCATT/R-AGTTTGGTTGTGTTTTTGCATGT AG / P—CTGGCGCAGCAGATA	Miller et al. 2016	1.12
	Mx	Antiviral protein	F—AGATGATGCTGCACCTCAAGTC /R—CTGCAGCTGGGAAGCAAAC P—ATTCCCATGGTGATCCGCTACCTGG	Eder et al. 2009	0.86
	NFX	Zinc finger NFX1-type	F—CCACTTGCCAGAGCATGGT/R—CGTAACTGCCCAGAGTGCAAT P—TGCTCCACCGATCG	Miller et al. 2016	0.91

	RPL6	Neoplasm-related protein C140	F—CGCCACCACAACCAAGGT /R—TCCTCAGCCTCTTCTTCTTGAAG P—AGATCCCCAAGACTCTGTCAGACGCCT	Miller et al. 2016	1.07
	SRK2	Tyrosine-protein kinase FRK	F—CCAACGAGAAGTTCACCATCAA/R—TCATGATCTCATACAGCAAGA TTCC / P—TGTGACGTGTGGTCCT	Miller et al. 2016	0.92
	VHSV-P10	VHSV-inducible protein-10	F—GCAAACTGAGAAAACCATCAAGAA/R—CCGTCAGCTCCCTCTGCAT P—TGTGGAGAAGTTGCAGGC	Miller et al. 2016	0.96
	VHSVI-P4	VHSV-inducible protein-4	F—TGGCTTCCCACATTGCAA /R—CCTCCTCCCCCCTGCAT P—AGATGGAGACAGGAATG	Miller et al. 2016	0.95
reference	COIL	Coiled-coil domain-containing protein 84	F—GCTCATTTGAGGAGAAGGAGGATG/R—CTGGCGATGCTGTTCCT GAG / P—TTATCAAGCAGCAAGCC	Miller et al. 2016	0.94
	MRPL40	39S ribosomal protein L40, mitochondrial precursor	F—CCCAGTATGAGGCACCTGAAGG/R—GTTAATGCTGCCACCCTCTCA P—ACAACAACATCACCA	Miller et al. 2016	0.92

Table 4-3: Generalized Linear mixed effect model estimates of relative infection burden (RIB) with fat content, temperature and study period and their interaction as fixed effects and fish id as a random effect. Significance is given based on Satterhwaite's method.

Model Term	Mean Sq	df	ddf	*F* value	*P* value
fat content	2.12	1	53	9.22	0.00371*
log(temperature)	0.0655	1	53	0.278	0.600
study period	0.216	2	53	2.86	0.0660
Log(temp)*study period	0.642	2	53	2.79	0.0706

Table 4-4: Results from Permutational Multivariate Analysis of Variance model with study period as strata for biomarker expression based on Bray-Curtis distances

Model Term	*df*	*SS*	R^2	*F* value	*P* value
log(temperature)	1	0.558	0.130	9.19	0.001 *
Fat content	1	0.0659	0.015	1.08	0.441
RIB	1	0.0398	0.009	0.656	0.726
Residuals	60	3.64	0.846		
Total	63	4.30	1.00		

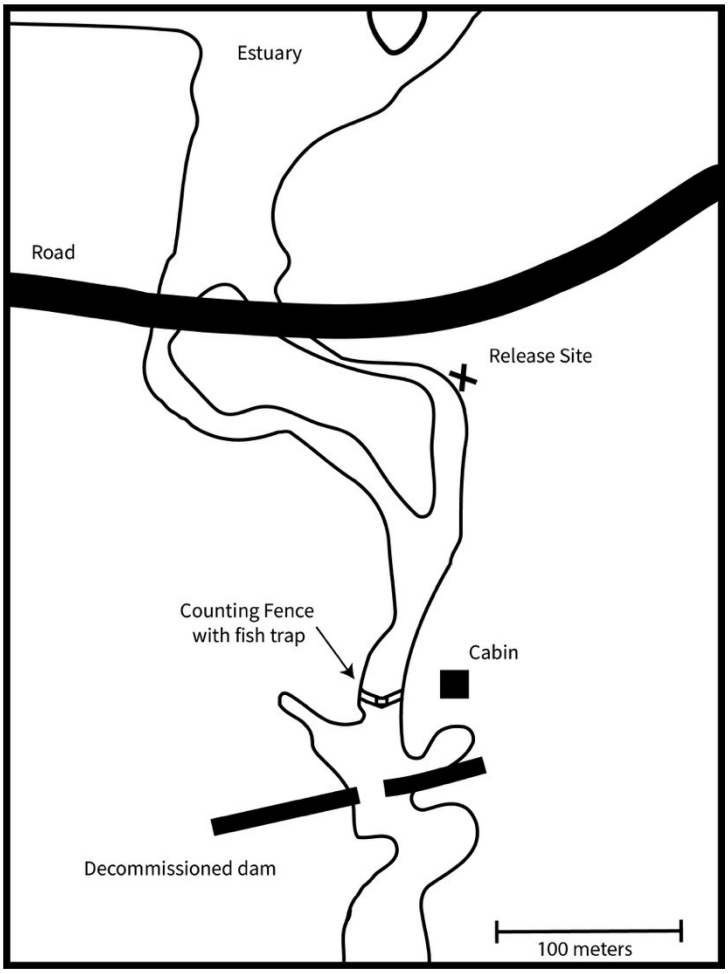

Figure 4-1 Schematic of study site at the Campbellton River, Newfoundland

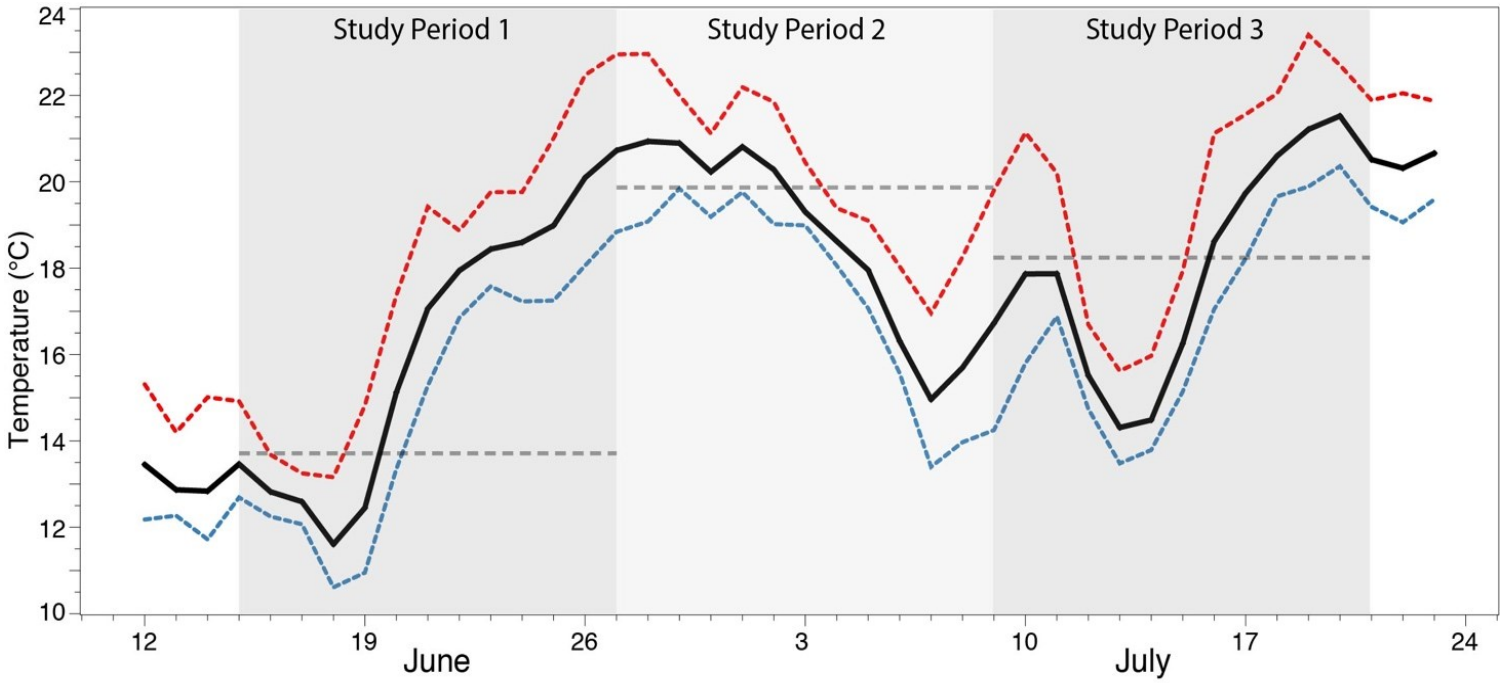

Figure 4-2: Water temperature of the Campbellton River over the course of the study represented as daily average (black), maximum (red), and minimum (blue). Average temperature for samples taken from Atlantic salmon for gene expression and infectious agent screening during each study period are indicated by vertical dashed lines

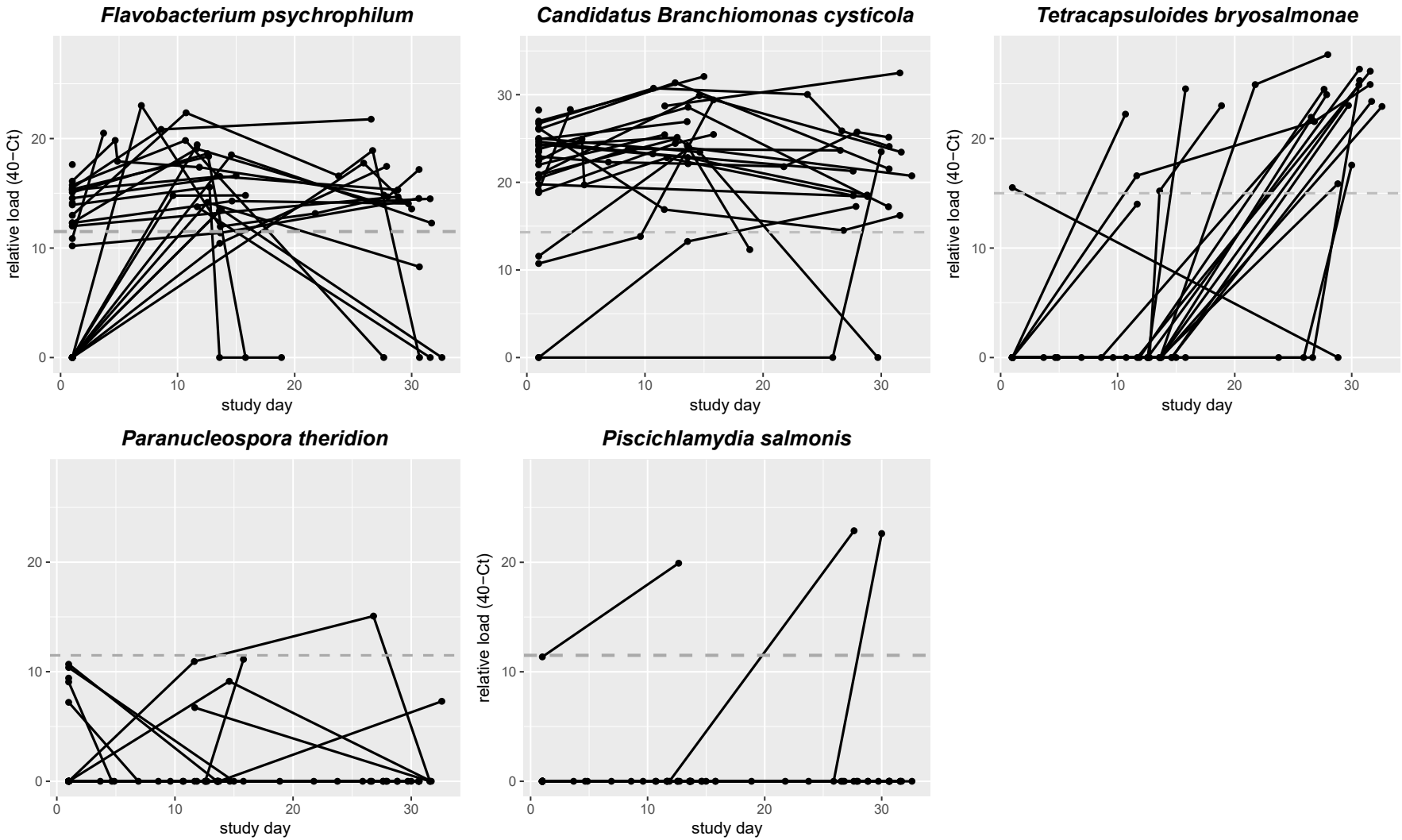

Figure 4-3: Line plots of infectious agent relative loads from individual adult Atlantic salmon collected from the Campbellton River in Newfoundland, Canada. Each fish was externally tagged with an identification code and released downstream of a counting fence after each sample. Samples were opportunistically taken over the course of 32 days study period when fish re-entered a passive trap. Each line represents an individual fish (n=27), with each point as a sample (1-6 per individual).

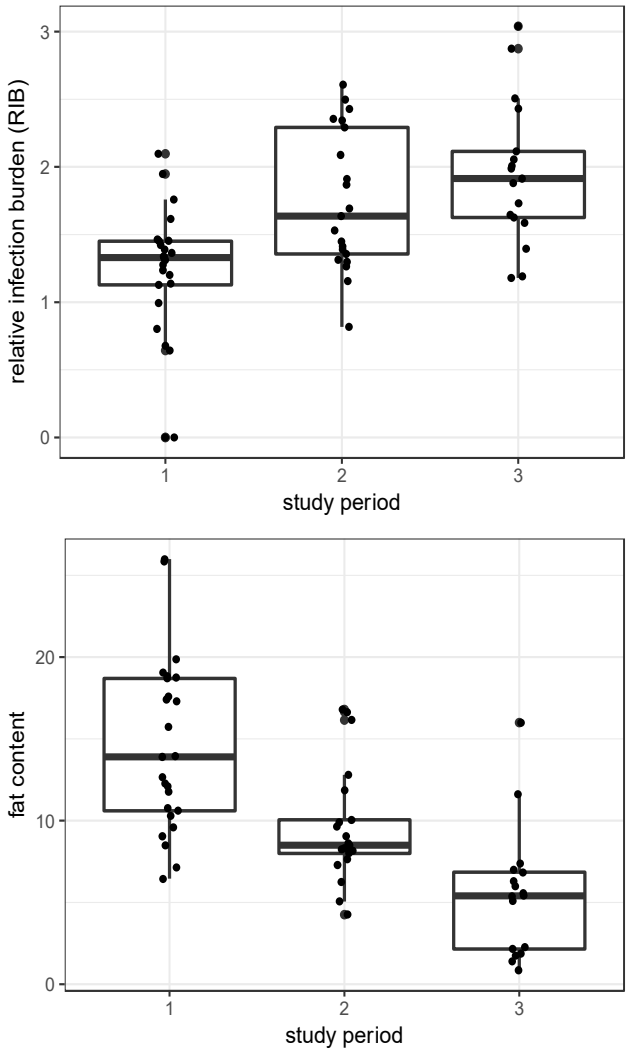

Figure 4-4: Change in relative infection burden (RIB) and fat content collected from individual Atlantic salmon sampled in three time periods over the course of 32 days in the Campbellton River, Newfoundland.

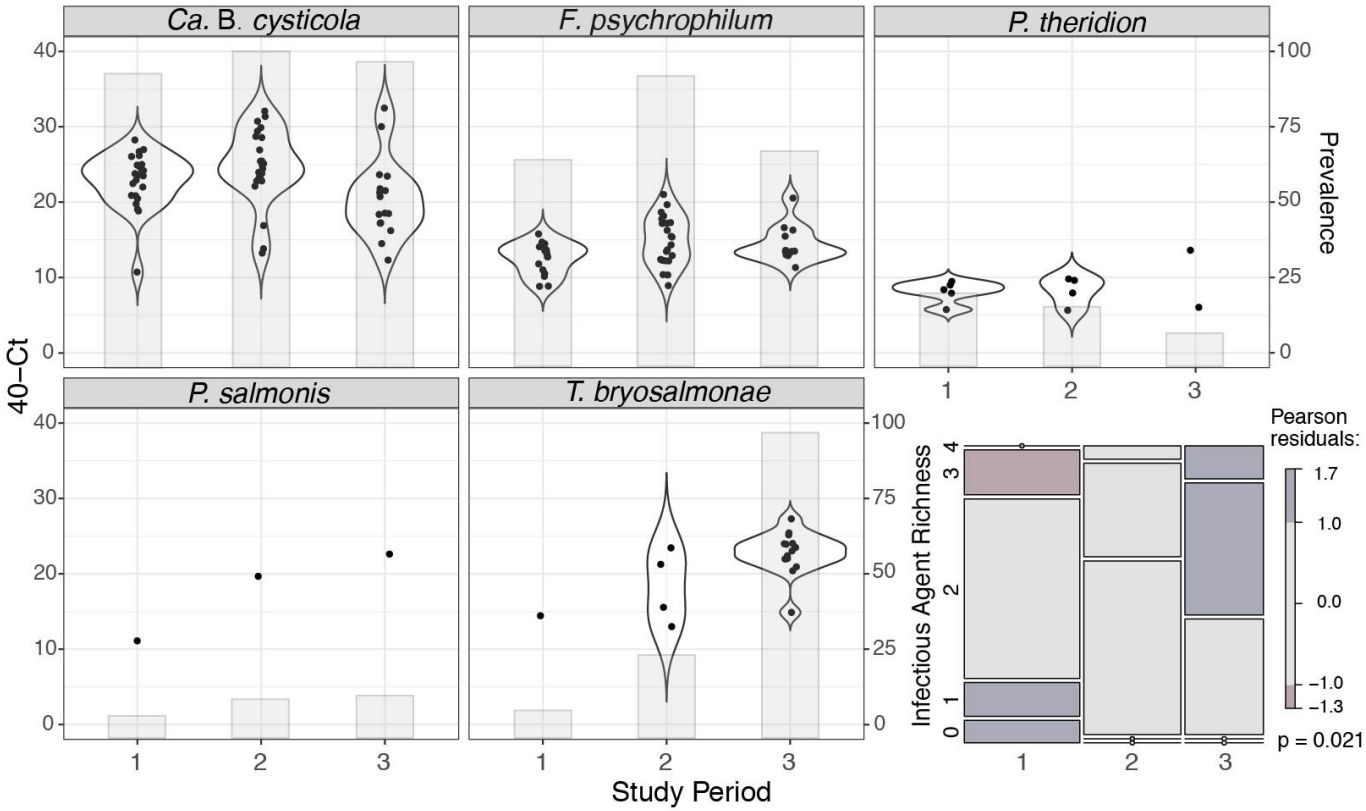

Figure 4-5: Infectious agent relative load, prevalence, and overall species richness from Atlantic salmon non-lethal gill tissue samples taken from spawning migrants in the Campbellton River, Newfoundland.

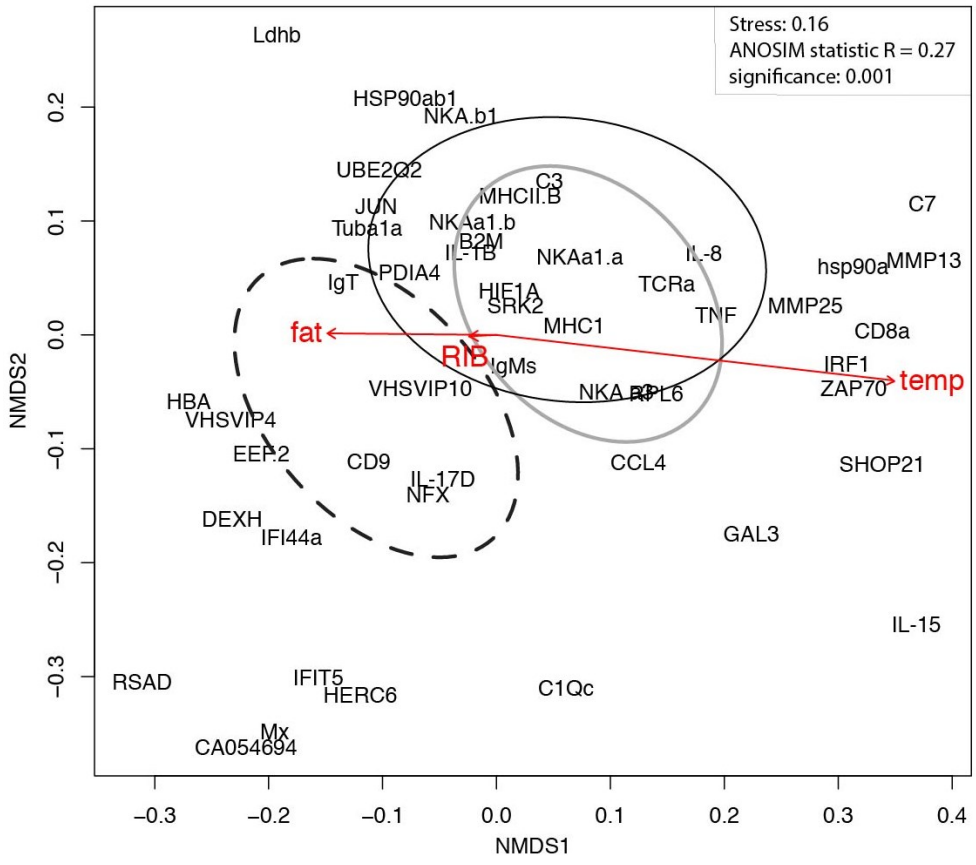

Figure 4-6 NMDS ordination using Bray-Curtis dissimilarity distances of expression of immune and stress associated transcripts non-lethally sampled from adult migratory Atlantic salmon. Ellipses represent each study period as follows: study period one, black dashed line; study period 2, solid black line; study period 3, solid gray line.

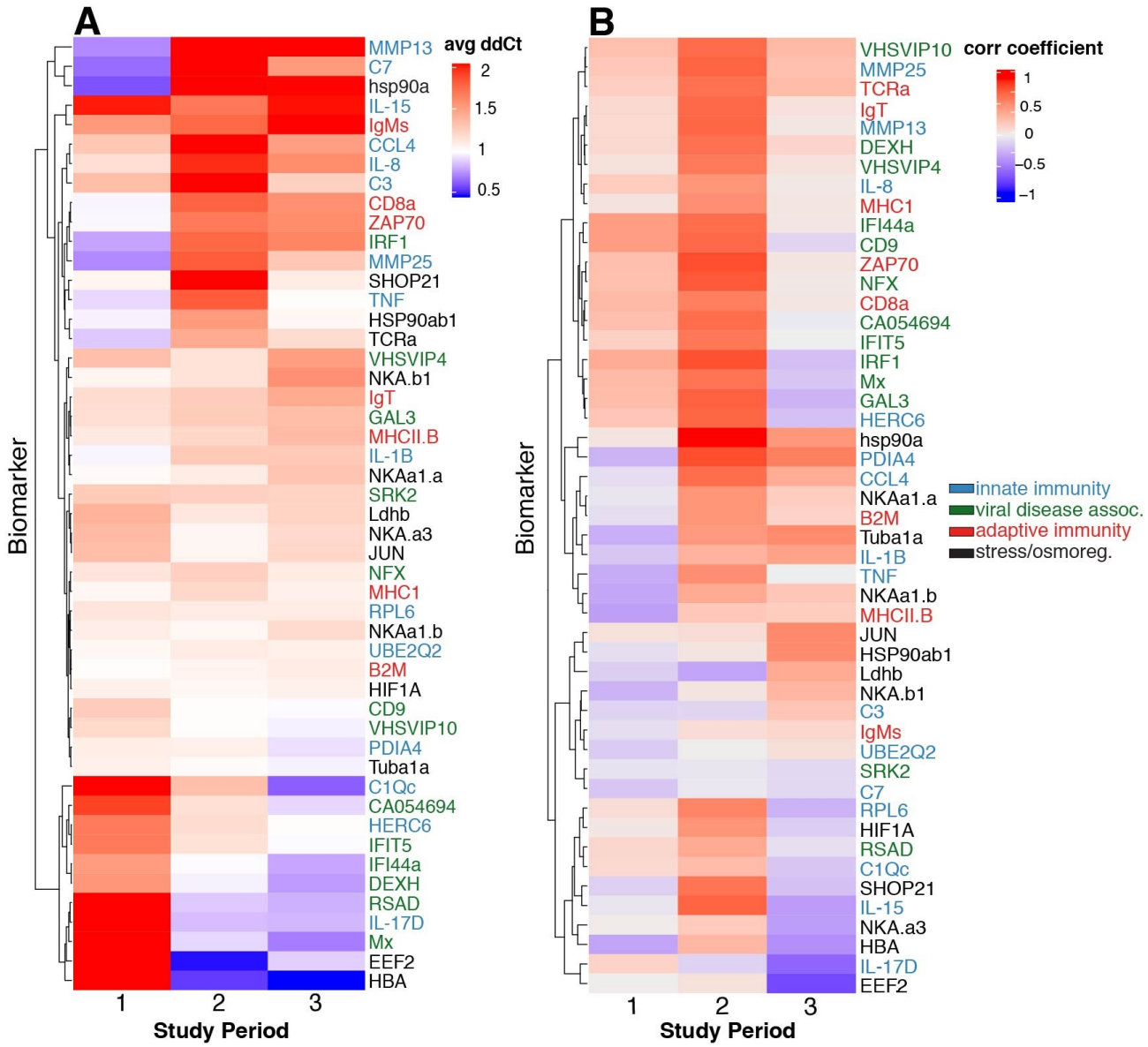

Figure 4-7: Heat maps of gene expression for transcripts associated with immune function and stress in adult Atlantic salmon. A: Average ddCt expression observed in each study period, approximately representing time spent in fresh water; B: correlation of river temperature with gene expression for each transcript.

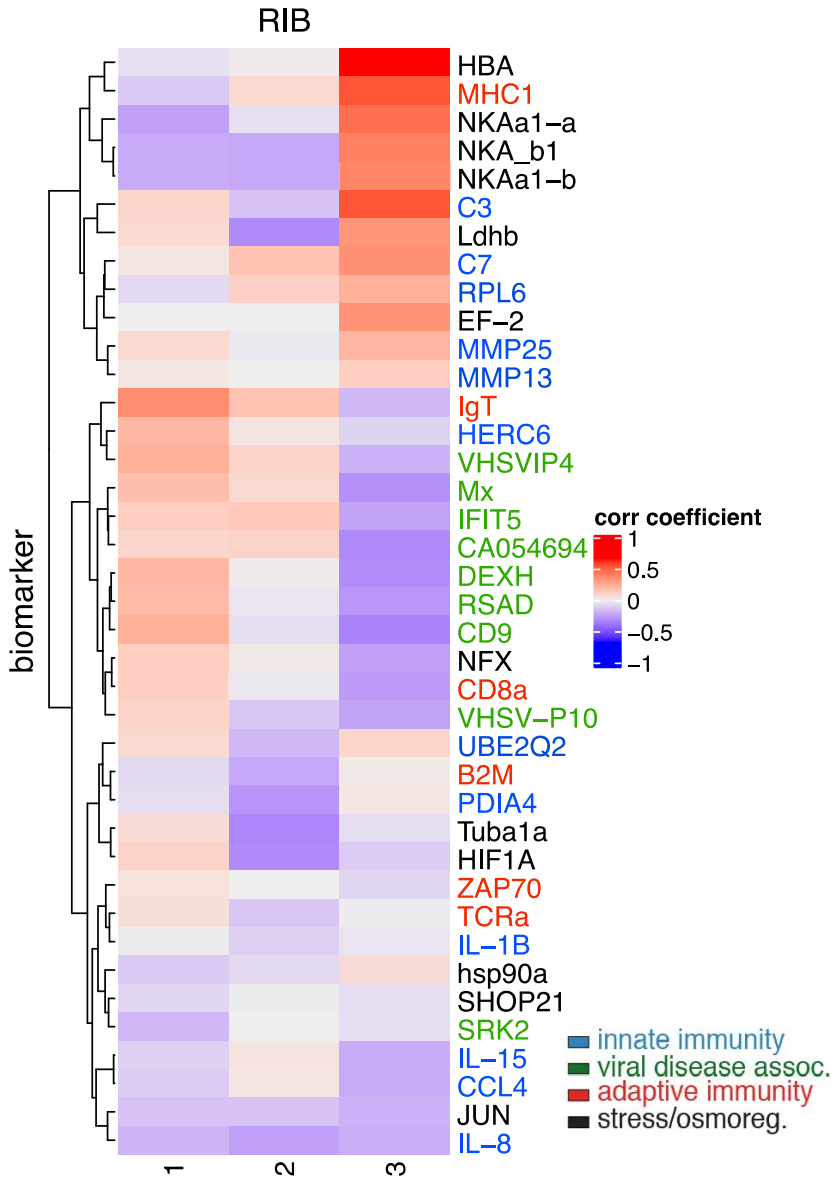

Figure 4-8 Heat map of correlation between gene expression and relative infection burden (RIB) from transcripts associated with immune function and stress in adult Atlantic salmon.

Chapter 5: **Handling, infectious agents, and physiological condition influence survival and post-release behaviour in migratory adult coho salmon after experimental displacement**

5.1 Abstract

For Pacific salmon captured and released by fisheries, post-release behaviour and survival may be influenced by their health and condition at time of capture. I sought to characterize the interactions between infectious agent burden, fish immune and stress physiology, and fisheries stressors to investigate the potential for capture-mediated-pathogen-induced mortality in adult coho salmon *Oncorhynchus kisutch.* I used radio-telemetry paired with high-throughput qPCR of non-lethal gill biopsies for infectious agents and host biomarkers from 200 tagged fish experimentally displaced and exposed to various experimental fisheries treatments (gill net entanglement, recreational angling, and recreational angling with air exposure vs. non-sampled control). I characterized relationships among post-release behaviour and survival, infectious agent presence and loads, physiological parameters, and transcription profiles of stress and immune genes. All infectious agents detected were endemic and in loads consistent with previous adult Pacific salmon monitoring. Individuals exposed to fisheries treatments were less likely to reach spawning habitat compared to controls, and handling duration independent of fisheries gear had a negative effect on survival. High infectious agent burden was associated with accelerated migration initiation post-release, revealing behavioural plasticity in response to deteriorating condition in this semelparous species. Prevalence and load of infectious agents increased post-migration, and transcription signatures reflected changes in immune and stress profiles consistent with senescence. Results from this study further the understanding of factors associated with fisheries that increase risk

of post-release mortality and characterize some physiological mechanisms that underpin migratory behaviour.

5.2 Introduction

Associations among pathogens and their hosts in wildlife populations are relevant to conservation efforts but remain relatively under-studied due to logistical constraints (Grenfell and Dobson 1995). For cryptic aquatic species, the majority of information available on host-pathogen relationships is derived from monitoring of disease that occurs in domesticated aquaculture strains in captive facilities (Kurath and Winton 2011; Atlas of Fish Disease 2009). Pathogen-related disease in wild aquatic animals is generally only considered a conservation issue when observable mortality or large-scale population declines occur during cases of epizootic outbreaks (e.g. Viral Hemorrhagic Septicemia in the Laurentian Great Lakes). As a result, surveys of infective agents in aquatic wildlife populations generally investigate relatively rare events or anomalies and do not assess infectious agents in the context of general ecology and conservation (Miller et al. 2014). Research has demonstrated that microbial infectious agents and their effect on fish is a complex relationship that depends on multiple factors including but not limited to environmental conditions, host life-history stage, immunocompetency, and co-infection dynamics (Tort 2011). As aquatic ecosystems continue to experience change as a result of human activities (Dudgeon et al. 2006; Reid et al. 2019), it is increasingly necessary to characterize pathogen dynamics outside of aquaculture and episodic disease events to focus instead within the context of 'natural' pathogen dynamics and impacts of anthropogenic stressors. As infectious disease is often believed to be the ultimate cause

of mortality in stressful environments (Joseph et al. 2013; Miller et al. 2014) and stressors rarely occur in isolation outside of laboratory settings, it is necessary to investigate synergistic effects of multiple stressors when considering stress in wild organisms (Crain et al. 2008).

While many anthropogenic stressors are broad-scale with obvious negative connotations, such as climate change or habitat destruction, fish can also experience acute stress as a result of conservation actions such as release from fisheries. Fisheries interactions represent a multifactorial stressor that can impose exhaustive exercise, physical injury, and hypoxia upon captured fish (Davis 2002; Patterson et al. 2017). Stressors by definition induce stress responses at primary, secondary, and tertiary organismal scales (Barton 2002), which can trigger behavioural changes and a cascade of alterations in physiological processes. These can include gene expression and protein synthesis, metabolism and energetics, and immune and endocrine function (Tort 2011). The link between stress and fish immunocompetency has been demonstrated to be particularly complex; depending on the type of stressor and recipient's condition at time of induction, a cellular stress response can both activate (Aluru and Vijayan 2009; Kassahn et al. 2009) and suppress (Dhabhar 2002; Biro and Post 2008; Van Rijn and Reina 2010) immune associated genes. As individual genes are generally involved in multiple physiological pathways, and may respond to multiple stressor types, detection of a specific stressor based on shifts in expression of single gene transcripts will likely yield erroneous conclusions (Houde et al. 2019). Alternately, assessing patterns of transcription for a broad range of immune and stress related genes may help characterize observed outcomes after exposure to acute stressors. I have demonstrated that specific

stressor and disease states can be accurately discriminated based on curated biomarker (gene) panels that are specifically co-expressed under specific stressor conditions (Miller et al. 2017; Akbarzadeh et al. 2018, Houde et al. 2019), and these panels can be effective even in multi-stressor scenarios (Houde et al. 2019). Moreover, tracking and holding studies have revealed transcriptional signatures associated with shifts in migratory behaviour, survival, age, and/or environmental exposure that have been demonstrated across multiple studies (e.g. "mortality-related signature" or MRS described in Miller *et. al* 2011, Drenner *et. al* 2018, Stevenson et al. 2019; viral disease response or "VDD" in Jeffries et al. 2013; Miller et al. 2017; Twardek et al. 2019; Stevenson *et. al* 2019).

Although the goal of many fisheries is to capture and harvest fish, many fish are also released (i.e., capture-and-release) to comply with regulations or voluntarily due to conservation ethic (e.g., recreational voluntary catch-and-release) or lack of market in the case of commercial fisheries. Fisheries stressors include exhaustive exercise, air exposure, and physical injury from contact with fishing gear (hooks, nets) and handling (reviewed in Raby et al. 2015). These interactions can leave released fish physiologically compromised (reviewed in Davis 2002; Wilson et al. 2014) and potentially vulnerable to pathogen infection and disease progression (Patterson et al. 2017). How a capture-and-release interaction may influence disease-related mortality is termed "capture-mediated-pathogen-induced mortality", and while estimates of post-release mortality in general have been determined for many capture-and-release fisheries, the level of pathogen-induced mortality that occurs in the wild is unknown.

Biotelemetry allows researchers to track the migration behaviour and post-release survival and reproductive success of fish released from fisheries (Donaldson et al. 2008),

such as Pacific salmon *Oncorynchus* spp. (Donaldson et al. 2012; Raby et al. 2012). By combining physiological sampling with telemetry, detailed questions regarding host condition at time of release can be addressed (reviewed in Cooke et al. 2013; Patterson et al. 2017). This approach is particularly powerful when combined with advanced pathogen screening, allowing the empirical characterization of infection and physiological state at time of tagging to be directly related to mortality outcomes (Miller et al. 2014; Bass et al. 2017). Further, simultaneously assessing host transcription profiles of immune and stress-related genes creates a comprehensive picture of not only the microparasite community present within the host at the time of tagging, but also whether the host exhibits physiological or transcriptional responses associated with infections (e.g. Teffer et al. 2017, 2019). This methodology allows for the investigation of fundamental research questions regarding host-pathogen interactions and factors that influence these relationships, while also addressing population scale effects potentially important for the management and conservation of host species. For Pacific salmon, this technique has been used to identify new and endemic pathogens in British Columbia's adult (Bass et al. 2017) and juvenile salmon (Tucker et al. 2018) and to identify pathogenic microbes that influence migratory survival of juvenile salmon (Jeffries et al. 2013), and demonstrate links between host transcriptional responses and survival in both smolts and migratory adults (Miller et al. 2011, 2014, 2017; Jeffries et al. 2013; Teffer et al. 2018).

To understand how infectious agent dynamics and fish physiology and immune responses influence post-release impairment and migration success, I measured infectious agent loads and host physiological and transcriptional status in adult coho *O. kisutch* exposed to standardized fishery treatments. Adult coho salmon that have entered fresh

water are vulnerable to recreational and commercial in-river fisheries that use a variety of gear types, including hook and line and gillnets. coho salmon populations are generally regarded as being in decline in the Fraser River basin with one population categorized as Threatened by the Committee on the Status of Endangered Wildlife in Canada (Interior Fraser River coho salmon; COSEWIC 2016). Releasing coho salmon following capture is a tactic that is used in Fraser River commercial fisheries (when coho salmon are captured as by catch) and recreational fisheries (depending on region and stock origin e.g. hatchery versus wild) to ensure that fisheries activities do not further exacerbate declines in adult coho escapement. Yet there haves been very few studies on the effects of releasing coho salmon from capture on their subsequent migration survival (e.g. Raby et al. 2012, 2015) and none that have examined the effects of individual condition and infectious agent burdens on migration success. Recent experimental holding studies on adult coho salmon demonstrated links between fisheries stressors and transcriptional profiles, infectious agent communities, and survival (Teffer et al. 2019a), but such links have not been identified the wild. To investigate these factors, I exposed migrating adult coho salmon to experimental fisheries simulations, measured infection burdens and host transcriptional responses in gill, quantified stress indices in blood, and then used radio telemetry to relate post-release migratory behaviour and survival to upstream spawning habitat to physiological and disease-associated metrics. To determine changes in infectious agent burdens and host condition, fish were opportunistically recaptured post-migration and experimentally displaced 75 km down river for experimental treatments, biopsy, and tagging. I tested the hypothesis that the severity of fisheries treatment, infection burden and physiological status at release influence migration rate and survival,

and that infection burden would increase in fish recaptured post-migration (as observed in other Pacific salmon examined during spawning migrations (e.g. Bass et al. 2019)). I predicted that fisheries treatments and high infection burden would be related to migratory impairment and decreased survival, and that infectious agent diversity and relative load increased during migration.

5.3 Methods

5.3.6 Capture and transport

Adult coho salmon were collected at the Fisheries and Oceans Canada Chilliwack River Hatchery in Chilliwack, British Columbia (20-24, October 2014; Figure 5-1). Individuals present at the hatchery at the time of sampling had already migrated from the ocean to the hatchery; however, work began early in the migration period of this population to ensure coho salmon, which tend to enter rivers early and stage near spawning grounds for weeks prior to spawning (McPhail and Lindsey 1970), were in good condition with several weeks remaining before spawning commenced. I chose to use the hatchery as a collection site rather than attempting to intercept fish during migration to avoid stress and injury associated with in-river capture methods, to condense the number of days across which fish were released, and to ensure robust sample sizes. At time of capture, all fish were silver in colour with no secondary sexual characteristics indicating gonadal maturation is not complete and relatively recent river entry (Davidson, 1935). Two hundred adult coho salmon were dip netted from hatchery collection channels and immediately transferred to an aerated truck-mounted transport tank filled with sand-filtered, UV-treated water sourced from within the same watershed (10-12°C).

Selection of fish done haphazardly. Water was oxygenated using compression canisters and temperature and dissolved oxygen were monitored continuously during transport. Fish were then transported 75 river kilometers downstream (approx. 1 h transit time) and transferred using dip nets to in-river holding pens with front to back flow-through. All fish survived capture and transportation. Fish were monitored during holding and allowed to recover for 1 h prior to experimental treatments. All research complied with Canadian Council for Animal Care Protocol #102022 and was conducted under Movement of Live Fish in British Columbia permit # 13533 and Scientific License XR 362 2014.

5.3.7 Experimental treatments

Experimental treatments aimed to replicate stress and exertion associated with in-river fisheries; myself and the supporting research team have worked alongside fishers in the study region to design treatments that simulate real-world aspects of fisheries interactions commonly experienced by migrating salmon. Treatments were randomly assigned and included two recreational angling simulations representing best practices (no air exposure, biopsied) and poor practices (1 min air exposure, biopsied), gill-net by-catch simulation (1 min air exposure, biopsied), and a non-treated control (no biopsy). Air exposure treatments were included for angling and gill net treatments to represent the typical experience for these fisheries (Cook et al. 2019), and a no air exposure angling treatment was included to assess if current 'best handling practices' may potential mitigate the deleterious effects of air exposure (Brownscombe et al. 2017). Experimental treatments were conducted in a large (500 L) tank supplied with continually pumped river water. For recreational angling simulations, fish were transferred to the measuring trough

and manually hooked through the maxilla using a no. 2 circle hook rigged on 4.5 kg test monofilament fishing line and released into the experimental arena for 2 minutes. Tension was placed on the line by the experimental angler to encourage the fish to fight as it would in a typical angling event. Fish were then either immediately submerged in a tagging trough with a continual flow of fresh river water over the gills and body (simulating best angling practices via fast return to the water) or exposed to 1 min of air exposure prior to entering the sampling trough (simulating poor handling practices). Hook removal occurred either during the air exposure treatment, or immediately after transfer to the tagging trough while submerged.

Gillnet simulations consisted of a 3 min experimental entanglement in 140 mm mesh commercial regulation gillnet. Netting was held in an aluminum frame at low tension such that mesh was open but not taught to simulate suspended netting potentially encountered in the river by migrating fish. Each fish was individually dip netted from the in-river holding pen, transferred to a corner of the experimental arena and released to swim into the gillnet. After 3 min of entanglement, fish were removed and treated with 1 min of air exposure as described above, and then transferred to the tagging trough for biopsy and tagging. In all cases, total handling time was recorded for each fish from the time it was dip-netted out of the holding tank to when it was released.

5.3.8 Biopsy and Tagging Procedure

The tagging trough was foam-lined with flow-through water pumped continually from the river. Fish were measured (fork length), biopsied for gill tissue (2 filament tips, ~2 mm in length) and blood (2 mL from the caudal vasculature; 21-gauge needle and

heparinized 3 mL Vacutainer, Becton-Dickson, NJ; as per Miller et al. 2014; Bass et al. 2017). Gill tissue was immediately transferred into 2 mL RNAlater® for RNA stabilization and preservation for transcript analysis. Blood samples were kept in an ice slurry for up to 15 min and then centrifuged for 6 min at 7000 *g* to isolate plasma, which was transferred (~1.5 mL) to microtubes and flash frozen in liquid nitrogen for subsequent analysis of metabolites and hormones. No biopsy was taken from fish in the control group (no treatment and no biopsy; n=50) prior to tagging. All fish were internally tagged by gastric insertion with Sigma-eight Inc. (Markham, ON) coded radio tags programmed for a 4 sec signal transmission interval and externally tagged with individual ID spaghetti tags (Floy Manufacturing, Seattle, WA), then released. Signal transmission and ID decoding were confirmed immediately prior to deployment using a Lotek Wireless (Newmarket, ON) SRX-600 radio telemetry receiver.

Nine fixed Sigma-8 Orion radio receivers were installed upstream of the release site; seven along the predicted migration route, and three up-stream of the Chilliwack-Vedder confluence with the Fraser River (Figure 1). Manual tracking of the system occurred every 48 h along roadways adjacent to the river system where possible, and by foot at river access points. Migration progress was estimated based on sequential detection at receiver stations up-river of the release site and by manual tracking (Lotek SRX 600). Migration "success" was defined as detection of fish at or beyond river km 65, where habitat suitable for spawning begins and spawning has been observed (Chilliwack River Hatchery staff, personal comm.). Fish that were detected upstream of the release site but not detected at or beyond river km 65 were considered en-route mortalities, while fish that were not detected after release are considered immediate post-release mortalities.

Lethal sampling was conducted at the collection location (Chilliwack River Hatchery) to provide baseline condition, gene expression, blood physiology, and microbial infection data at the beginning and end of the study. Any tagged fish that was recaptured upon return to the Chilliwack Hatchery was also lethally sampled. On the first and last day of the study, N=10 adult coho salmon were taken directly from the hatchery channel and immediately euthanized by cerebral percussion (referred to as baseline initial and baseline final respectively). Length, weight, blood and tissue samples, and reproductive status were collected for each fish. For comparative purposes, this study uses biomarker and pathogen data isolated from gill tissue only. Gill biopsies from sacrificed fish were taken from a similar location on the gill and in a similar manner and size to nonlethal biopsies.

5.3.9 Blood assays and molecular techniques

Plasma samples were processed at the Department of Fisheries and Oceans Canada (DFO) West Vancouver Laboratory, West Vancouver, BC. Plasma sodium, potassium, and chloride were measured to investigate potential osmoregulatory disturbance (Barton et al. 2002) with a Cole Parmer single-channel flame photometer (model 2655-00), and glucose and lactate were measured to assess the metabolic response to stress with a YSI STAT Plus glucose/lactate analyzer (model 2300). All analyses were run in duplicate and averaged following procedures outlined in Farrell et al. (2001). Plasma testosterone, estradiol, and cortisol concentrations were processed and run in duplicate, and measured as per manufactures instructions using ELISA kits (Neogen Corp., Lansing MI, USA). Testosterone and estradiol levels for blood-sampled tagged

fish were compared to those from baseline destructives samples (hatchery) where sex was known (n = 40) to inform sex assignment of tagged fish. Consequently, sex is unknown for control fish as no blood was taken.

Gene expression of host genes and infectious agent prevalence and relative loads were examined in gill tissue using high-throughput qPCR on the Fluidigm BioMark Dynamic Array™ Gene Expression platform at the DFO Molecular Genetics Laboratory in the Pacific Biological Station, Nanaimo, BC (Tables 5-1 & 5-2). Biomarkers believed or known to be linked with infection and mortality were selected. To determine the presence of infectious agents within samples, a pool of all samples was screened for 46 infectious agents expected or known to cause disease in salmon globally (a full list of screened pathogens is available in supplemental material). Only those that were positive in the pooled sample were run on individual samples. TaqMan™ assays used in screening were designed to target microbe RNA to allow identification of RNA viruses and microparasites in active states. Consequently, qPCR quantification represents microbial productivity rather than absolute quantification, described hereafter as relative load. Details on sensitivity and specificity of each microbe assay, sequence sources, and repeatability of the following RNA extraction, normalization, targeted amplification, and final HT-RT-qPCR using this platform are outlined in the Canadian Science Advisory Secretariat validation of the BioMark for infectious agent monitoring in salmonids (Miller et al. 2016).

5.3.10 RNA extraction, normalization, and cDNA PCR

All samples were processed as per methods outlined in Miller et al. (2016) and Teffer et al. (2017). In brief, RNA extraction was completed using mechanical abrasion in a MM301 mixer mill (Restch Inc. Newtown PA) and Tri-reagent™ followed by addition of 1-bromo-3-chloropropane, and purified using Magmax™-96 Microarrays Kits on a Biomek FXP automated liquid handler. Purified RNA quantity and quality was assessed using a Beckman Coulter DTX 880 Multimode Detector (Brea, CA, USA) and sample RNA concentrations were normalized to 62.5 ng/uL. RNA was then converted to cDNA using SuperScript® VILO™ DNA synthesis kit (Life Technologies) following manufacturer's instructions. Specific targeted amplification of target transcripts was performed using primer pairs corresponding to all assays using 1x TaqMan Pre-amp MasterMix as per manufacturer's instructions (Applied Biosystems, CA, USA). Unincorporated primers were removed using Exo-SAP-IT™ (Affymetrix, Santa Clara, CA, USA) and sample was diluted 1:5 with DNA suspension buffer. The effect of this pre-amplification step on final quantification has been investigated thoroughly and was not found to negatively influence the reliability of results (for extensive detail see Miller et al. 2016). The resulting sample material and assays were loaded directly on to Fluidigm 96.96 Dynamic Array ™ integrated fluidic circuit chip for qPCR. Combined serial dilutions of artificial construct controls with known copy number were added to the Dynamic Array last and used to track efficiency of each assay on each run. These controls contained an extra probe to track potential contamination (see Miller et al. 2016). A series of negative processing controls for RNA extraction, cDNA synthesis, and pre-amplification were also included, as well as a pooled positive control sample of all samples used in the study.

For host biomarkers, cycle threshold (Ct) is reported for the average of each duplicate biomarker assay using relative expression in the form of $2^{-\Delta\Delta Ct}$ using the averaged expression of two housekeeping genes and the Ct value of a pooled positive control (Livak and Schmittgen 2001). Infective agents were only considered detected if their Ct was below the assay-specific limit of detection (95% level) described in Miller et al. 2016) and detected in both duplicate samples. The mean Ct of detected infective agents was calculated and is presented herein as relative load by subtracting the observed Ct value from the total PCR cycles for each qPCR run (e.i. 40-Ct).

5.3.11 Statistical analysis

All data filtering and statistical tests were conducted using R Statistical Software (R Core Team, 2018). Downloaded telemetry data was filtered and false detections were removed from fixed receiver stations for any detection that did not i.) correspond with deployed tag's unique frequency and code designations, or ii.) follow 4 sec signal transmission intervals. A positive detection was determined by at least two detections within a 20-sec period that were multiples of 4 sec. All fish taken from the system by anglers were removed from the dataset at the reported time of capture. Post-release behaviour was determined by assessing delay in migration initiation, designated by passage of the first up-river receiver, and swimming ground speed (km/hr) between stationary receivers located in the Chilliwack River. Pearson's chi-squared test was used to assess the relationships between fisheries treatment and migratory fate for all tagged fish (i.e. non-biopsied control and treatment groups; n=190).

5.3.11.1 Microbe prevalence, relative load, and relative infection burden

Prevalence for each infectious agent detected was calculated for the tracked fish and again for all fish sampled. Pathogens with high prevalence (>70%) within the sample population are analyzed independently using non-parametric Wilcoxon Rank Sum approximation to assess sex-specific and temporal differences in relative load. To assess the potential effects of high loads within the sample population, infection with rare pathogens, and potential cumulative effects of multiple infections, an index accounting for both load and diversity of agents detected within each individual was used to include potential effects of pathogens found in low prevalence. Infectious agents found in each host were summarized as a single variable representing the "Relative Infection Burden" (Bass et al. 2019), whereby high loads and both common and rare pathogens contribute to the burden index as follows:

$$RIB = \sum_{i \epsilon m}^{m} \frac{L_i}{Lmax_i},$$

where L_i is the pathogen relative load (40-Ct) for that sample, $Lmax_i$ is the highest relative load of that agent observed in the sample population, and m is the sum of $L_i/Lmax_i$ for all pathogens detected in the sample.

The relationship between RIB, sex, and treatment on blood physiology was tested using multivariate analysis of variance (MANOVA) with type I sum of squares to account for sex specific variation in physiological variables. Ordinal Logistic Regression models (*polr* function in MASS r package), as well as GLMs (family = binomial, link = logit) with binary response (survival to spawning habitat) were used to investigate the influence of RIB, treatment, and sex on migratory fate and survival. RIB, blood ions, and

metabolites were $\log_{10}$ transformed to address non-normal distributions for regression models, otherwise non-parametric tests were used in cases where data structure was in violation of parametric test assumptions.

5.3.11.2 Transcription profiles and infectious agent communities

Principal Component Analysis was used to reduce dimensionality of gene expression data. Scree plots were used to visually estimate the number of components to retain (cumulative variance >50%) and Bartlett's test of sphericity was used to ensure dataset structure was suitable for ordination. Factors influencing gene expression principle components (PCs) were investigated using generalized linear models with PCs as the dependent variable. Model covariates included RIB, blood ions (i.e., potassium, sodium), metabolites (i.e., glucose, lactate), cortisol, sex, and survival.

Infectious agent community composition was summarized using Non-metric Multi-Dimensional Scaling (NMDS) ordination with a Bray-Curtis dissimilarity metric, with each host as an individual replicate. NMDS is well suited for community data because NMDS is not restricted by linear ordination structures as it uses non-euclidean distance measures in *n* dimensional space, and can thus accommodate non-parametric and count data. I used the *metaMDS*, *envfit* and *anosim* functions from the R package *vegan* (version 2.9.2) to create ordinations and investigate the relationship between pathogen community structure and transcription profiles, fate, and sampling time. The optimal number of dimensions to include in each ordination was visually assessed using scree plots. In NMDS, the calculated stress statistic represents the level of dissimilarity within the original data captured by the produced ordination, where values < 0.2 are generally

accepted as indicating an interpretable representation of data dissimilarity for ecological systems (Kruskal and Wish 1978; Jaworska and Chupetlovska-Anastasova 2009).

5.4 Results

5.4.1 Fisheries treatments and post-release mortality

Of the 200 tagged and released fish, 42.5% successfully migrated to spawning habitat, 27.5% were immediate post-release mortalities, and the remaining 30% were en-route mortalities. A high degree of variation in migratory behaviour was observed, with migrations times ranging between 2.9 to 37 d (mean = 14.1 d) to traverse the 65 km from the release site to the hatchery. Six percent (n = 12) were reported as captured in fisheries, two of which were captured near spawning habitat and included in analysis. A total of 147 samples were screened for infectious agents and gene expression characterized: 112 gill samples at tagging release, 20 from fish recaptured post migration, and 16 baseline samples.

Migratory fate (immediate mortality, en-route mortality, or successful migration) was significantly related to treatment group (χ^2=13.03, df=6, p=0.04; Figure 5-2); treatment fish experienced somewhat greater immediate post-release mortality compared to non-handled controls (χ^2=6.45, df=3, p=0.07), and control fish were more likely to reach spawning habitat compared to all treatment groups (χ^2=12.6, df=3, p=0.005). Among treatment groups, there was no difference in migratory fate (χ^2 = 1.33, df=4, p=0.856). Plasma cortisol was significantly higher in females than in males (t=10.641, df=106.57, p<0.001), and was not related to experimental fisheries treatments ($F_{2,89}$=0.5623, p>0.05), and there was no significant interaction between sex and

treatment ($F_{2,89}$=0.1461, p>0.05; Table 5-3). Post-release behaviour characterized by migratory delay, total duration of migration, and swim rate through low and high velocity flow portions of the river was not significantly predicted by treatment (two-way MANOVA; $F_{12,213}$=0.89, *p*=0.557), handling time ($F_{4,69}$=1.61, p=0.182), or their interaction ($F_{12,213}$=0.3, df=9, p=0.975). There were no significant differences in sex (χ^2=3.32, df=3, p=0.344) or fork length (t=0.88, df=128, p=0.380) among treatment groups.

Handling time was significantly lower for control fish compared to all treatment groups (Kruskal-Wallis χ^2 p<0.001) with no significant variation among other treatments (Kruskal-Wallis χ^2 p>0.05; Figure 5-2). The difference in handling time between control and fisheries treatments was 24.1 seconds and represents the time required to take gill biopsies and blood samples. Handling time significantly predicted migratory fate for controls (Kruskal-Wallis χ^2 p=0.006; Bonferroni corrected for multiple comparisons α = 0.0125) but not for fish exposed to fisheries treatments.

5.4.2 Factors associated with infection burden

Fourteen infectious agents were detected in the screened population, including four bacteria (*Aeromonas salmonicida*, *Flavobacterium psychrophilum*, '*Candidatus* Branchiomonas cysticola', *Rickettsia*-like organism [RLO]), nine microparasites (myxozoans: *Ceratonova shasta*, *Parvicapsula pseudobranchicola*, *Parvicapsula minibicornis*; protists: *Dermocystidium salmonis, Sphaerothecum destruens*; flagellate: *Cryptobia salmositica*; ciliate: *Ichthyophthirius multifiliis*; microsporidea: *Paranucleospora theridion, Loma salmonae*) and one virus (Viral Erythrocytic Necrosis [VEN]; Table 1; Figure 4). All screened gill samples contained at least two infectious

agents. Relative loads and prevalence did not vary by sex except for increased prevalence of *L. salmonae* and high relative loads of *C. shasta* in females compared to males (Wilcoxon χ^2 approximation p=0.017). RIB of tagged fish at the time of capture was not significantly different among treatment groups (F=1.87, p=0.160) or between sexes (F=1.7, p=0.195). RIB, treatment, sex, and their interaction terms were not predictive of migratory fate (ordinal logistic regression: $p > 0.05$). Post-release migratory delay was weakly related to RIB; fish with higher infection rates initiated migration sooner compared to less infected individuals independent of sex, treatment, and blood cortisol values (R^2=0.12, p=0.003; Figure 5-4). Beyond 24 rkm, however, there was no effect of RIB on migration rate for any river segment or total remaining migration. ANOVA tests on individual blood ions and hormones (predictor variables: sex, RIB, treatment, RIB-sex interaction, RIB-treatment interaction) revealed significant effects of sex and RIB on blood cortisol only, where higher RIB values are associated with lower circulating blood cortisol levels (Table 5-4).

Increases in RIB due to higher prevalence and loads of several pathogens were observed in recaptured fish sampled post-migration, supporting pathogen accruement during migration (Figure 5-3). Comparing initial and final samples, *A. salmonicida, C. salmositica,* and RLO were not detected at release but were detected upon recapture, and significant increases in the relative loads of Ca. B. *cysticola* ($p = 0.044$), *C. shasta* ($p = 0.0274$), *F. psychrophilum* ($p < 0.001$), and *I. multifilliis* ($p = 0.004$) were observed. Relative loads of detected infectious agents were similar in recaptured and final baseline groups except for higher loads of *F. psychrophilum* in fish that completed the migration twice (Wilcoxon ranked sum $p = 0.001$).

5.4.3 Transcription Profiles

Three retained PCs summarized 57.2% of the ordination variation (Figure 5-5). No single genes or functional gene groups contributed maximally to PC1. Stress associated genes HSP90 and SHOP-21 and innate immune gene C7 loaded negatively on PC2, and adaptive immune genes MHCI and MHCII-B and cytokine IL-15 loaded positively on PC2. Genes associated with stress ATP5G3, TF, and SHOP 21, as well as broad scale immunity and inflammation MMP13, were maximal contributors to PC3. Fish that were recaptured showed distinct separation in PC structures, particularly in PC2 (Figure 5-6). Generalized linear models to investigate factors influencing transcriptional profiles of tracked coho salmon identified effects of blood ions and metabolites, but no significant contribution of sex, treatment, RIB, or migratory fate (Figure 5-7). Blood lactate and glucose are significant covariates explaining PC1, ($p = 0.03$, $p = 0.04$ respectively), while blood sodium levels alone were highly predictive of gene expression summarized in PC2, summarizing osmoregulatory, immune, and stress genes associated with maturation ($p < 0.001$). PC3, representing stress and broad scale immune genes, was strongly associated with blood glucose ($p = 0.002$) and weakly associated with blood cortisol values and migratory success ($p = 0.09$).

No patterns in gene expression associated with migratory fate were observed. Transcription of several genes differed when comparing individuals sampled upon recapture (Figure 7); significant up-regulation in ATP5G3-C, C7, SHOP 21, TF, and NKA a1b was observed in fish recaptured post migration (Kruskal-Wallis ranked sum $p< 0.007$), and notable down regulation in HSC70 and MHCII-B ($p < 0.01$). Small sample

size of initial and final baseline samples and high variance in samples taken upon tagging prohibits interpretation of tagging effects alone on expression of individual genes.

5.4.4 Gene Expression, Infectious Agent Community, and Fish Condition

NMDS ordination of infectious agent community yielded a stress value of 15.8, indicating a good level of dissimilarity represented by the ordination. ANOSIM analysis of sample type (initial and final baseline, tagging, and recapture) revealed changes in infectious agent community structure over time ($p < 0.001$, ANOSIM statistic $R = 0.2$; Figure 8A). Gene expression summarized by PC2 was highly associated with infectious agent community structure based on *envfit* analysis ($r2 = 0.17$, $p < 0.0001$). NMDS of only samples taken at tagging produced a similar stress value of 16.0. ANOSIM analysis revealed no significant relationship between migratory fate and infectious agent community ($p = 0.532$; Figure 8B). *Envfit* analysis of NMDS ordinations with transcription profile PCs was significant but with small effect size for PC3 ($r2 = 0.03$, $p = 0.03$). More detailed analysis of the infectious agent community ordination using individual biomarkers and physiological variables revealed no distinct signal when considering only tagged fish, however a strong osmoregulatory response is associated with species that increase in prevalence and relative load in fish sampled at the end of the study (i.e., final baselines and recaptured fish).

5.5 Discussion

This study sought to characterize factors associated with coho salmon health and condition to investigate the incidence and mechanisms of potential capture-mediated-

pathogen-induced mortality in wild salmon following fisheries interactions. All infectious agents detected are considered endemic and within prevalence ranges observed in previous surveys in the southern BC region (Miller et al. 2014; Bass et al. 2017, Teffer et al. 2018; Table 1). Fisheries treatments were associated with decreased survival compared to controls, and handling time alone was related to mortality of control fish, with longer handling times associated with increased post-release mortality regardless of treatment. As there was no effect of fisheries treatment on measured physiological variables, variation in individual condition regardless of fisheries treatment was investigated. Physiological parameters were significantly associated with gene expression and sex but not with migratory behaviour and survival. I identified a negative correlation between cortisol and infectious agent burden that was sex-specific but not predictive of survival. Finally, infectious agent community composition was predictive of immediate post-release migratory behaviour but not migratory fate, and changes in infectious agent community and salmon physiology were observed over the course of the study and were associated with biomarkers related to osmoregulation, metabolism, and stress.

5.5.1 Fisheries Stress and Post Release Survival

The combined post-release mortality of 57.5% (27.5% immediate, 30% delayed) observed in this study is high compared to previous estimates for adult coho (Raby et al. 2012, 2015) and likely a result of cumulative effects of the repeated migration and extending post-release monitoring duration. Although non-biopsied controls survived better than treated fish, impairment related to the severity of fisheries treatment was not observed; post-release migratory behavior and success were similar among fisheries

treatments. My results are contrary to previous research on Pacific salmon, where experimental gill-net entanglement was related to elevated indicators of stress physiology and migratory delay post-release compared to control groups (Nguyen et al. 2014, Keefer et al. 2017; Teffer et al. 2017; Bass et al. 2018). Previous work has demonstrated high resilience to fisheries stressors as salmon approach spawning grounds (Raby et al. 2013; Bass et al. 2018). coho salmon experimentally treated with fisheries stressors at temperatures similar to those in this study (10.7-12.5°C) were resilient to capture stress, while fish exposed to high temperature (15°C) experienced significantly higher mortality (Teffer et al. 2019). Consequently, coho included in this study may have been resilient to the variation in severity of fisheries treatment, yet vulnerable to the energetic and physiological demands associated with transport and repetition of 75 km of migration experienced during this study.

The amount of time a fish was handled, regardless of treatment group, was found to influence immediate mortality post-release. Raby et al. (2012) found no relationship between fish handling time and migratory fate in wild coho salmon released from beach seine fisheries, contrary to marginally non-significant results of the present study. Harmful factors unique to handling include loss of mucous coat and externally applied pressure (reviewed in Brownscombe et al. 2016, Cook et al. 2018), which are influenced by the duration of handling. Research assessing the impacts of various tagging methods on physiological indicators of sockeye salmon found capture, handling, and holding effects were more pronounced than tagging alone (Dick et al. 2018). In this study, all fish handlers had extensive experience working with adult salmon and the biopsy and tagging methods employed have been widely applied with high survival rates (Donaldson et al.

2012; Nguyen et al. 2014; Raby et al. 2013; Cook et al. 2017; Bass et al. 2018; Bass et al. 2019). Variation in handling times was attributed to a diversity of factors and was not defined by gear type but rather practical constraints; for example, handlers often need to pause or use additional restraint while a fish is struggling to prevent its escape. This is particularly true for control fish that were not exposed to exhaustive exercise prior to handling, as they are more vigorous during tagging. While not explicitly recorded as part of the experimental design, long handling times of control fish are likely attributed to such cases despite best efforts to minimize handling stress.

The significant negative effect of handling alone on fish survival independent of fishery treatment is a critical consideration for best practices in catch-and-release fisheries. Many best-practice recommendations prioritize reducing air exposure over handling time (e.g. Brownscombe et al. 2017). Yet, these results indicate the duration of handling time alone can have a greater impact on post-release survival in some fisheries. In sectors where catch-and-release is used as a conservation strategy by managers, best-practice recommendations should stress the importance of reducing the duration of all interactions with captured fish alongside gear and air exposure recommendations.

5.5.2 Relative infection burden and sex-related stress response

Consistent with previous research on migratory Pacific salmon in the region (e.g. Bass et al. 2017; Teffer et al. 2019), RIB increased in fish resampled post-migration. Condition of fish upon recapture was markedly different from condition at tagging; recaptured fish demonstrated advanced sexual dimorphism, spawning preparedness, scale reabsorption, and development of visible fungal infections. Natural recruitment of

infectious agents occurs during spawning migrations, particularly at spawning grounds when fish are in high densities and immunocompromised (Miller et al. 2014; Teffer et al. 2019). The significant increase in prevalence and relative loads of *C. salmositica* in recaptured fish suggests that fish taken from the hatchery and transported downstream had not been in fresh water for an extended period of time; previous work at the same hatchery noted high prevalence and relative loads of *C. salmositica* with extended residency in hatchery channels (Bass et al. 2018). The load and prevalence increase of freshwater bacteria (*F. psychrophilum*) and microparasites (*I. multifiliis*) also support temporal increases that occur during migration (Teffer et al. 2017; Bass et al. 2018). Higher prevalence and loading of *F. psychrophilum* in recaptured fish compared to those sampled at the hatchery at the end of the study (final baseline samples) may indicate the effects of experimental relocation, treatment, and handling on resistance to *F. psychrophilum* infection. Considering *F. psychrophilum* has been identified as an infectious agent that poses moderate risk to Pacific salmon (Miller et al. 2014), potential increase in vulnerability to infection as a result of fisheries stress and handling warrants further investigation.

There was no observed interaction between fisheries treatments and RIB and neither of these factors predicted migratory fate. The fact that high RIB was associated with shorter migratory delay suggests that high relative loads of multiple infectious agents are related to intensified up-river migration behaviour. Infection rates have been demonstrated to increase as Pacific salmon get closer to spawning (Teffer et al. 2015; Bass et al. 2018; Teffer et al. 2019); available energy is depleted, and immune response is significantly diminished (Dolan et al. 2016). The semelparous life history strategy seen in

Pacific salmon has selected for reallocation of energy away from physiological processes not directly associated with gamete production and spawning behaviour such as immune function (Teffer et al. 2019); consequently, individuals with higher RIB may be further along their senescence trajectory compared to those with lower RIB (Dolan et al. 2016; Teffer *et a.* 2019). In contrast, previous work found that pathogens such as *C. salmositica* is associated with decreased migration rates (Bass et al. 2018). Here, the prevalence of *C. salmositica* in the tagged sample population was too low to detect an effect. Accelerated migration rates associated with fish condition similar to those observed here have been observed in compromised sockeye salmon (Drenner et al. 2018; Miller et al. 2011).

Interestingly, RIB was negatively related to circulating cortisol levels in this study, and this relationship was more pronounced for males than for females. Previous research on adult coho salmon has identified sex-specific differences in response to stressors, including prolonged physiological disturbance after gill net entanglement in females compared to males (Teffer et al. 2019). Female Pacific salmon have been consistently shown to have higher circulating blood cortisol levels compared to males (Kubokawa et al. 2001; Cook et al. 2011; Raby et al. 2011) independent of infection burden (Teffer et al. 2019). Additionally, glucose metabolism is higher in females during spawning migrations, indicating sex-specific energy demands and metabolism (Teffer et al. 2017, 2019). The lack of observed correlation between RIB and cortisol in females may be attributed to such sex-specific energy allocation and stress responsiveness. The negative association of RIB with circulating cortisol in males may indicate an impaired or suppressed stress response associated with high infectious agent burden or a more

advanced stage of senescence. This result differs from previous work on Chinook salmon released after mild gill-net entanglement, where no relationship between RIB and circulating cortisol for males or females was detected (Bass et al. 2019). However, migratory sockeye salmon *O. nerka* with visual pathologies associated with fungal infections (likely *Saprolegnia ssp.*) had elevated cortisol levels compared to those without visible infection both in-river and at spawning grounds (Baker et al. 2013). These results indicate that further research is necessary regarding potential impaired stress response in Pacific salmon associated with infective agent burden.

5.5.3 Transcription profiles

The lack of detectable relationship between transcription profiles, RIB and migratory fate highlights the challenges associated with characterizing individual health and post-release survival outcomes in wild fish. Gene transcription relating to stressors can be highly transient and variable. Alternately, chronic stressors responses are more stable and consistent; in fact, recent research has shown that fish experiencing chronic thermal or osmotic stress can be specifically identified based on the co-activation of panels of 8-10 genes (Houde et al. 2019). Unfortunately, I did not have such panels at the time of this study, and rather employed a broad range of genes known to be activated when fish are experiencing stress or responding to infection. While these can provide clues as to the range of physiological processes being activated, especially when combining with other measured variables such as blood chemistry and infectious agents, they are not as informative of more curated panels. Given the high variability in exposure of free-ranging salmon to stress, it may be necessary to employ larger sample

sizes and attempts to reduce confounding effects (e.g. transport stress) to detect transcriptional signals that have been observed in more controlled experimental laboratory holding studies (e.g. Teffer et al. 2017, 2019).

In the present study study, gene transcription was, however, related to aspects of individual physiology; plasma ions and metabolites were significantly related to transcription profiles summarized by PC1 and PC2 at release, yet there was no detected secondary stress response to experimental fisheries treatments. Donaldson et al. (2012) found that changes in transcription of stress associated genes peaked 2-4 hours post induction of an acute stressor, while lactate and cortisol took approximately 24 h to return to baseline levels. Here, sampling occurred 2-5 hours after initial capture. Transcriptomic response to transport and holding stress is not easily observed in this study given the small sample sizes for initial baseline and high variance in individual gene transcription. However, given that lactate and glucose are known biomarkers of exhaustive exercise (Barton 2002), and sodium and glucose together have been associated with stress response in salmon (Wagner and Congleton 2004) the gene expression profiles represented by PC1 and PC2 may describe broad scale transcriptional response associated with the exercise and stress experienced during initial capture and transport.

Several biomarkers of a stress and immune response that have previously been associated with mortality in salmonids (SHOP21, ATP5G3, TF, MMP13) and several immune genes (CD83, MHCII-B, MMP13) were major contributors to PC3, which was related to plasma glucose and marginally associated with cortisol and migration survival in tracked fish. When considering changes over time, all stress related genes that contributed maximally to PC3 demonstrated a significant fold increase in transcription

post-migration, while MHCII-B expression decreased significantly. These patterns are consistent with senescence in Pacific salmon, including adult coho (Teffer et al. 2019) and suggest that transcriptional profiles represented by PC3 may be associated with senescence and/or spawning preparedness. Stress associated genes were also linked with the infectious agent community composition of tagged coho at release and recapture; however, the low variation explained by PC3 and small effect size of the relationship indicates a highly variable physiological (Cooke et al. 2006) and/or transcriptional (Hammill et al. 2012; Teffer et al. 2019) response to stress. Indeed, although blood glucose is typically positively related to cortisol levels, semelparous salmonids sampled during spawning migrations have demonstrated variable and muted glucose responses relative to cortisol levels (McConnachie et al. 2012; Dick et al. 2018).

The relationship between infectious agent communities and gene expression when considering changes over time highlights stress and immune genes that have previously been associated with both increased infectious agent loads and senescence (PC2 SHOP21, C7, and MHCIIB; Teffer et al. 2017). Previous experimental work on sockeye salmon observed similar trends: individuals with high loads of *F. psychrophilum* and *C. shasta* demonstrated similar expression profiles of stress and immune genes including SHOP21 and C7, while MHCIIB was associated with *P. minibicornis* and *Ca.* B. cysticola (Teffer et al. 2017). As semelparous fish undergo senescence and infection burdens increases, osmotic stress also increases. Damage to the integument can be caused by agents such as *F. psychrophilum* and *Ca*. B. cysticola, which have also been associated with osmotic impairment and subsequent mortality (Miller et al. 2011; Jeffries et al. 2012). The observed up-regulation of SHOP21 and C7 post-migration

corresponding with down-regulation of MHCII-B may be in response to disease associated osmotic stress resulting from increased infection burden and decreased immuno-competency. Together, these results indicate the synergistic trajectory of natural senescence and disease ecology in Pacific salmon and highlight the challenges associated with teasing apart causal relationships among gene expression, stress physiology, and the numerous infectious agents carried by wild salmon in the final stages of freshwater migration. However, as these methods are applied across multiple species, populations, and freshwater ecosystems, the similarities in connections and relationships observed will inform associations that may indeed be causal.

The experimental manipulation of fish that includes downstream transport is not entirely realistic (Cooke et al. 2013), however such approaches facilitate experimental comparisons and enable the application of mechanistic and comparative approaches to understanding the relative effects of different fisheries stressors to inform evidence-based conservation (Cooke et al. 2017). Investigating fisheries stress responses independent of capture or confinement effects is a logistical challenge for stress physiology research in wild animals (Patterson et al. 2017). Previous work on Pacific salmon has shown even temporarily holding actively migrating fish is itself stressful with potential to result in near total mortality upon releasing fish (Donaldson et al. 2011). Therefore, experimental effects cannot be easily addressed with changes in blood sampling time. Further, collecting fish that had already reached the hatchery biased the sample population towards surviving fish, which may carry lower infectious agent loads than those that died en-route. To further understand the context-specific responses of fish to fisheries capture, intercepting fish during migration enhances the likelihood of detecting highly pathogenic

infectious agents or fish in poor condition. Due to naturally occurring co-infections (every fish sampled was positive for at least two infectious agents), co-infection dynamics should also be considered by future studies. This method would further benefit the investigation of co-infection dynamics and sex-specific responses to infection and fisheries stress in the wild, however large sample sizes are required to account for pathogens occurring in low prevalence.

Several factors may have influenced my ability to detect disease-related transcriptional response and mortality in this study. While the panel of infectious agents used here includes most pathogens likely to be present on the Pacific coast, there is potential for unknown agents to exist within the host sample population. Pathogens not detected by these methods may decrease my ability to characterize transcriptional profiles of infected versus uninfected fish because presumed healthy fish may indeed be inducing associated immune responses (Miller et al. 2017). Any disease-induced mortality associated with undetected pathogens remains unknown. Finally, non-lethal sampling necessitates non-invasive collection of gill tissue and while this method has been demonstrated to have high concordance with pooled multi-tissue sample (Teffer et al. 2019), there is potential for over-representation of agents infecting the gill (*Ca*.B. cysticola and *L. salmonae*) and under-representation of pathogenic agents found commonly in other tissues (e.g. *P. minibicornis, T. bryosalmonae*).

5.6 Conclusions

Characterizing the factors associated with individual variation in the susceptibility of Pacific salmon to post-release mortality is a complex challenge that requires the

consideration of both external and internal environments at time of capture. Recent evidence suggests a role of infectious agents in mediating post-release survival (Bass et al. 2017, Patterson et al. 2017, Teffer et al. 2017) and these results support an influence of cumulative infection profiles on migratory delay following capture and release. I identified a sex-specific link between RIB and the stress response, with stronger relationships among males. A broad scale response in transcription of stress and immune genes may have been reflective of capture, transport, and confinement that was reflected in physiological variables measured from the blood.

The degree to which individual infectious agents influence the fate of salmon released from fisheries deserves additional and long-term investigation due to the challenges associated with the low prevalence of highly pathogenic species, co-infection dynamics, and individual and temporal variation in host immunocompetence. Further, as variability in river temperatures and flow increases with climate change, having a solid foundation of knowledge is critical to adequately monitor the effects dynamic environmental conditions and potential introduction of new infectious agents to Pacific waters (Fenkes et al. 2016). These findings increase the understanding of naturally occurring infections in wild Pacific salmonids and how these infectious agents increase over the course of migration alongside changes in immune function and stress physiology, information that is critical for ongoing monitoring efforts and research as environmental conditions change. Additional insight regarding context-specific response to fisheries interactions is necessary (Raby et al. 2015). Further research on wild salmon is required to identify specific infectious agents and transcription profiles that are predictive of negative outcomes in migrating coho salmon in the wild.

Table 5-1: Abbreviations, names, and groups of infectious agents detected in migratory adult coho salmon collected in the Chilliwack River system using hight-throughput qPCR. LOD = Limit of Detection at 95% confidence.

Assay ID	Infectious Agent	Group	LOD (40-Ct)	Mean Relative Load ± SD	Taqman Primer Sequences (5'–3') Probe Sequence (FAM-5'–3'-MGB)	Source sequence
ae_sal	*Aeromonas salmonicida*	Bacterium	14.4	12.2	F—TAAAGCACTGTCTGTTACC R—GCTACTTCACCCTGATTGG P—ACATCAGCAGGCTTCAGAGTCACTG	Keeling et al. 2013
c_b_cys	*Candidatus* Branchiomonas *cysticola*	Bacterium	14.3	27.4 ± 3.1	F—AATACATCGGAACGTGTCTAGTG R—GCCATCAGCCGCTCATGTG P—CTCGGTCCCAGGCTTTCCTCTCCCA	Mitchell et al. 2013
fl_psy	*Flavobacterium psychrophilum*	Bacterium	10.5	17.8 ± 2.8	F—GATCCTTATTCTCACAGTACCGTCAA R—TGTAAACTGCTTTTGCACAGGAA P—AAACACTCGGTCGTGACC	Duesund et al. 2010
rlo	*Rickettsia-like organism*	Bacterium	14.8	22.5	F—GGCTCAACCCAAGAACTGCTT R—GTGCAACAGCGTCAGTGACT P—CCCAGATAACCGCCTTCGCCTCCG	Lloyd et al. 2011
env	Erythrocytic necrosis virus	Virus	15.1	16.4	F—CGTAGGGCCCCAATAGTTTCT R—GGAGGAAATGCAGACAAGATTTG P—TCTTGCCGTTATTTCCAGCACCCG	Purcell et al. 2013
cr_sal	*Cryptobia salmositica*	Parasite	15.7	13.6 ± 1.1	F—TCAGTGCCTTTCAGGACATC R—GAGGCATCCACTCCAATAGAC P—AGGAGGACATGGCAGCCTTTGTAT	Miller et al. 2016
ce_sha	*Ceratonova shasta* (formerly *Ceratomyxa shasta*)	Myxozoan	11.5	18.1 ± 6.7	F—CCAGCTTGAGATTAGCTCGGTAA R—CCCCGGAACCCGAAAG P—CGAGCCAAGTTGGTCTCTCCGTGAAAAC	Hallett and Bartholomew 2006
de_sal	*Dermocystidium salmonis*	Parasite	14.5	18.4 ± 1.9	F—CAGCCAATCCTTTCGCTTCT R—GACGGACGCACACCACAGT P—AAGCGGCGTGTGCC	Miller et al. 2016
ic_mul	*Ichthyophthirius multifiliis*	Parasite	16.3	16.2 ± 6.4	F—AAATGGGCATACGTTTGCAAA R—AACCTGCCTGAAACACTCTAATTTTT P—ACTCGGCCTTCACTGGTTCGACTTGG	Miller et al. 2016
lo_sal	*Loma salmonae*	Parasite	14.6	22.8 ± 8.2	F—GGAGTCGCAGCGAAGATAGC R—CTTTTCCTCCCTTTACTCATATGCTT	Miller et al. 2016

pa_ther	*Paranucleospora theridion*	Parasite	11.8	17.8 ± 3.7	P—TGCCTGAAATCACGAGAGTGAGACTACCC F—CGGACAGGGAGCATGGTATAG R—GGTCCAGGTTGGGTCTTGAG P—TTGGCGAAGAATGAAA	Nylund et al. 2010
pa_pse	*Parvicapsula pseudobranchicola*	Myxozoan	14.8	13.6 ± 7.5	F—CAGCTCCAGTAGTGTATTTCA R—TTGAGCACTCTGCTTTATTCAA	Jørgensen et al. 2011
pa_min	*Parvicapsula minibicornis*	Myxozoan	10.4	18.5 ± 5.2	P—CGTATTGCTGTCTTTGACATGCAGT F—AATAGTTGTTTGTCGTGCACTCTGT R—CCGATAGGCTATCCAGTACCTAGTAAG	Hallett and Bartholomew 2009
sp_des	*Sphaerothecum destruens*	Parasite	13.5	12.5 ± 6.1	P—TGTCCACCTAGTAAGGC F—GCCGCGAGGTGTTTGC R—CTCGACGCACACTCAATTAAGC P—CGAGGGTATCCTTCCTCTCGAAATTGGC	Miller et al. 2016

Table 5-2 : Abbreviations, gene names, functional groups, and primer/probe sequences for biomarkers associated with stress and immune function and reference genes assessed in migratory adult coho salmon using high-throughput qPCR.

Assay name	Gene name	Functional group	Primer and probe sequences	Efficiency	Source
ATP5G3-C	ATP synthase lipid-binding protein	Stress	F - GGAACGCCACCATGAGACA R - CGCCATCCTGGGCTTTG P - AGCCCCATTGCCTC	0.975	Workenhe et al. 2009
CD83_onmy	Complement factor CD83	Adaptive Immunity	F - GATGCACCCCTTGAGAAGAA R - GAACCCTGTCTCGACCAGTT P - AATGTTGATTTACACTCTGGGGCCA	1	Raida et al. 2011
IL-1R_onmy1	Interleukin 1 receptor	Innate Immunity	F - ATCATCCTGTCAGCCCAGAG R - TCTGGTGCAGTGGTAACTGG P - TGCATCCCCTCTACACCCCAAA	0.975	Raida et al. 2011
JUN	Transcription factor	Osmoregulation/Stress	F—TTGTTGCTGGTGAGAAAACTCAGT R—CCTGTTGCCCTATGAATTGTCTAGT P—AGACTTGGGCTATTTAC	0.905	In house
CXCR4	C-X-C motif chemokine receptor 4	Immune	F - GGAGATCACATTGAGCAACATCA R - GCTGCTGGCTGCCATACTG P - TCCACGAAGATCCCCA	0.93	In house
FKBP	FK binding protein	Immune	F - GCACGCCGGACTTTGC R - GAGTTGGGTGGGATGATACCA P - TATGGCAGCAAAGGG	1.1	In house
HSP90a	Heat shock protein 90-alpha (alike)	Stress	F—TTGGATGACCCTCAGACACACT R—CGTCAATACCCAGGCCTAGCT P—CCGAATCTACCGGATGAT	0.97	In house
IgMs_onmy	Immunoglobulin	Adaptive Immunity	F—CTTGGCTTGTTGACGATGAG R—GGCTAGTGGTGTTGAATTGG P—TGGAGAGAACGAGCAGTTCAGCA	1.09	Raida et al. 2011
IL-11	Interleukin 11	Innate Immunity	F—GCAATCTCTTGCCTCCACTC R—TTGTCACGTGCTCCAGTTTC	1.01	Raida and Buchmann 2008

IL-15_onmy	Interleukin 15	Innate Immunity	P—TCGCGGAGTGTGAAAGGCAGA F—TTGGATTTTGCCCTAACTGC R—CTGCGCTCCAATAAACGAAT	1.09	Raida et al. 2011
IL-8	Interleukin 8	Innate Immunity	P—CGAACAACGCTGATGACAGGTTTTT F—GAGCGGTCAGGAGATTTGTC R—TTGGCCAGCATCTTCTCAAT	1.04	Ingerslev et al. 2009
MHCI_sasa1	Major histocompatibility complex I	Adaptive Immunity	P—TGTCAGCGCTCCGTGGGT F—GCGACAGGTTTCTACCCCAGT R—TGTCAGGTGGGAGCTTTTCTG	1.16	Ingerslev et al. 2009
MHCII-B_onmy	Major histocompatibility complex IIβ	Adaptive Immunity	P—TGGTGTCCTGGCAGAAAGACGG F—TGCCATGCTGATGTGCAG R—GTCCCTCAGCCAGGTCACT	1.01	Raida and Buchmann 2008
MMP13_SASA	Matrix Metallopeptidase 13	Multi-function Immunity	P—CGCCTATGACTTCTACCCCAAACAAAT F—GCCAGCGGAGCAGGAA R—AGTCACCTGGAGGCCAAAGA	1	Tadiso et al. 2011
NKA_a1b	Na/K ATPase α-1b (saltwater)	Osmoregulation	P—TCAGCGAGATGCAAAG F—GCCTGGTGAAGAATCTTGAAGCT R—GAGTCAGGGTTCCGGTCTTG	0.99	Richards et al. 2003
SHOP21	Salmon hyperosmotic protein 21	Osmoregulation	P—CCTCCACCATTTGCTCA F—GCGGTAGTGGAGTCAGTTGGA R—GCTGCTGACGTCTCACATCAC	0.92	In house
TF_onmy	Transferrin	Stress	P—CCTGTTGATGCTCAAGG F—TTCACTGCTGGAAAATGTGG R—GCTGCACTGAACTGCATCAT	0.94	Raida and Buchmann 2009
C7	Complement factor	Innate Immunity	P—TGGTCCCTGTCATGGTGGAGCA F—ACCTCTGTCCAGCTCTGTGTC R—GATGCTGACCACATCAAACTGC	1.02	In house
HSC70	Heat shock cognate 70	Stress	P—AACTACCAGACAGTGCTG F—GGGTCACACAGAAGCCAAAAG R—GCGCTCTATAGCGTTGATTGGT	0.96	In house

COIL-P84-2	Coiled-coil domain-containing protein 84	Reference	P—AGACCAAGCCTAAACTA F—GCTCATTTGAGGAGAAGGAGGATG R—CTGGCGATGCTGTTCCTGAG	1.09	In house
r78d16.1	S100 calcium-blinding protein	Reference	P—TTATCAAGCAGCAAGCC F - GTCAAGACTGGAGGCTCAGAG R - GATCAAGCCCCAGAAGTGTTTG P - AAGGTGATTCCCTCGCCGTCCGA	0.98	In house

Table 5-3: ANOVA effect tests for cortisol, blood ions, and blood metabolites for sex, experimental fisheries treatments, relative infection burden (RIB), and their interaction with RIB from adult migratory coho salmon *O. kisutch*. * = significance at p<0.001, ** significance at p<0.01.**

Factor	Predictor	df	SS	F-ratio	p-value
Cortisol	sex	1	27.7239	171.7269	<0.001***
	RIB	1	1.6279	10.0832	0.00199**
	treatment	2	0.0579	0.1795	0.836
	Treatment*RIB	2	0.6469	2.0035	0.140
	Sex*RIB	1	0.2763	1.7117	0.194
Glucose	sex	1	0.0520	0.4743	0.493
	RIB	1	0.1007	0.9194	0.340
	treatment	2	0.2763	1.2614	0.288
	Treatment*RIB	2	0.4726	2.1577	0.121
	Sex*RIB	1	0.0002	0.0019	0.965
Lactate	sex	1	.0514	0.9983	0.320
	RIB	1	.0479	0.9309	0.337
	treatment	2	.0616	0.5991	0.551
	Treatment*RIB	2	.0773	0.7507	0.477
	Sex*RIB	1	.0097	0.1879	0.667
Chloride	sex	1	0.08360	2.7273	0.102
	RIB	1	0.00116	0.0380	0.846
	treatment	2	0.03076	0.5017	0.607
	Treatment*RIB	2	0.00714	0.1164	0.890
	Sex*RIB	1	0.00044	0.0143	0.905
Sodium	sex	1	0.01591	2.4395	0.121
	RIB	1	0.00036	0.0550	0.815
	treatment	2	0.00699	0.5358	0.589
	Treatment*RIB	2	0.00541	0.4145	0.662
	Sex*RIB	1	0.02200	3.3732	0.0692
Potassium	sex	1	0.1587	0.7118	0.401
	RIB	1	0.0596	0.2671	0.606
	treatment	2	0.9399	2.1078	0.127
	Treatment*RIB	2	0.6032	1.3527	0.263
	Sex*RIB	1	0.3509	1.5738	0.212

Table 5-4: Characteristics for each experimental group of tagged coho salmon. For treatment groups, C = control, GN = gill net, AA = angled with air exposure, and A = angled with no air exposure. For migratory fate, H = harvested during migration by anglers, IM = immediate mortality, ERM = enroute mortality, SUC = successful migrant. Plasma ions and sex steroids were quantified from blood samples taken after each experimental fisheries treatment and not taken in the control group.

Trmt	N	Migratory Fate				sex		FL ± SD	Cortisol (ng/mL)± SD		Lactate	Glucose	Sodium
		H	IM	EM	S	f	m	cm	f	m	mmol/L ± SD		
C	50	3	7	9	31	-	-	58.2 ± 3.10	-	-	-	-	-
GN	52	0	19	16	31	25	23	59.1 ± 4.13	387 ± 149	138 ± 52.7	7.18 ± 1.79	8.02 ± 2.97	158 ± 12.1
AA	48	5	13	11	19	25	16	59.2 ± 3.75	408 ± 162	138 ± 48.5	7.52 ± 1.45	6.94 ± 1.98	160 ± 15.3
A	50	2	16	14	18	20	23	59.1 ± 4.54	408 ± 84.4	193 ± 146	7.38 ± 1.71	7.85 ± 3.95	158 ± 12.1

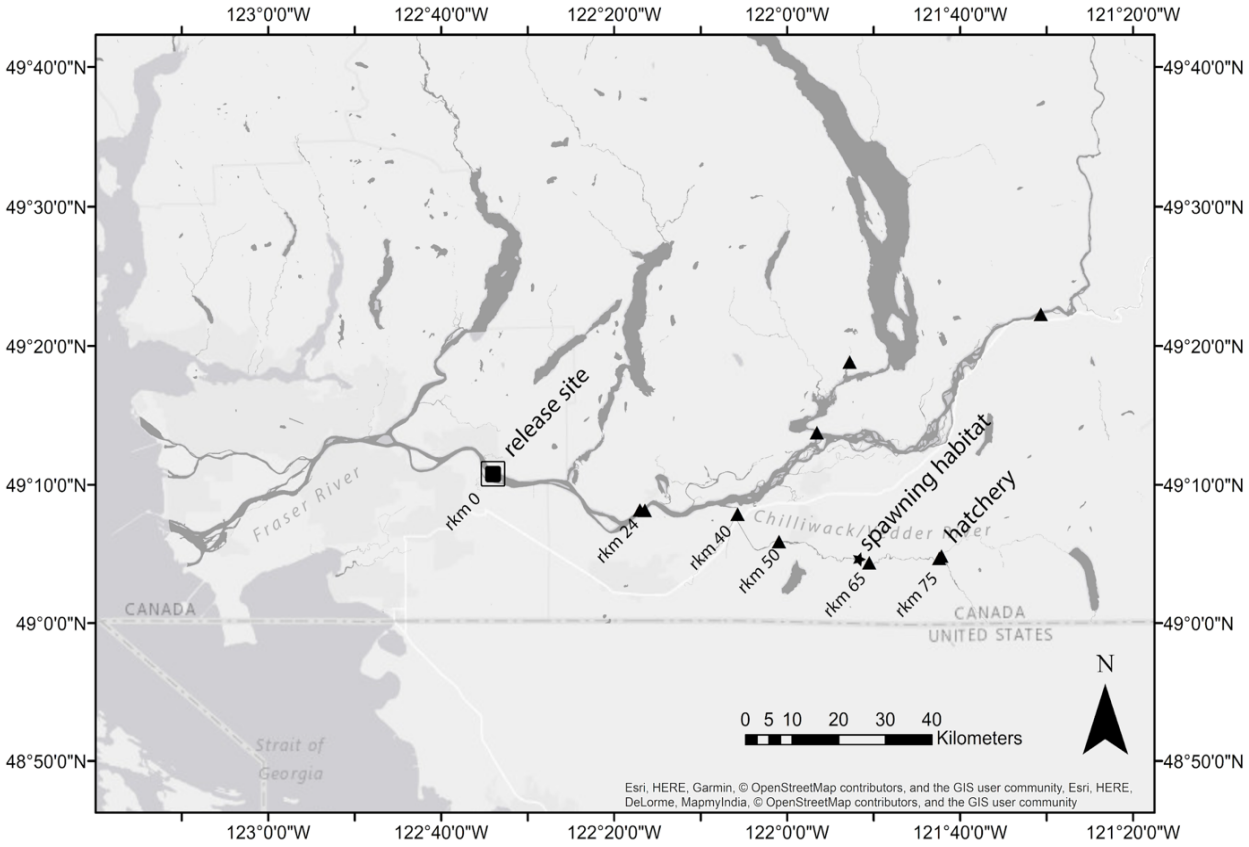

Figure 5-1: Map of the study area, including release site, receiver locations (triangles), and the beginning of spawning habitat for coho salmon experimentally displaced from the Chilliwack River Hatchery and exposed to experimental fisheries treatment prior to tagging, sampling, and release.

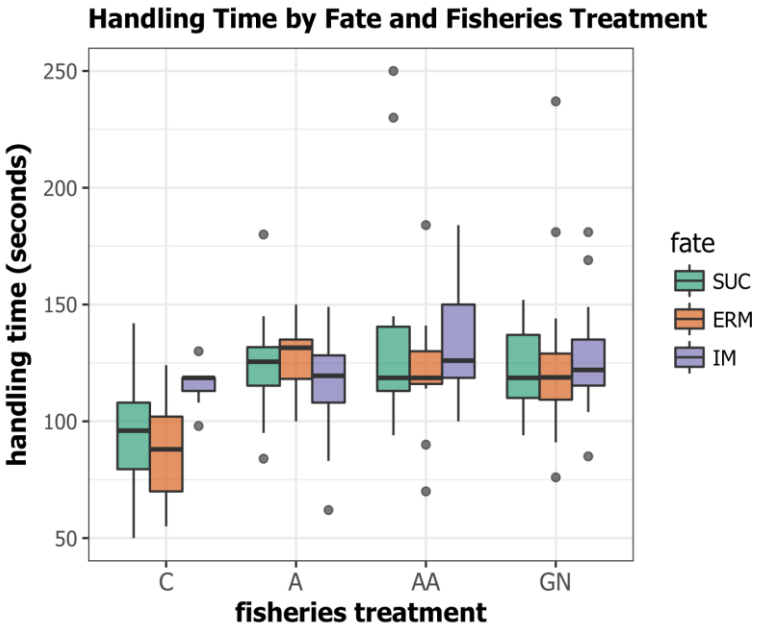

Figure 5-2: Standardized handling time and migratory fate for coho salmon exposed to experimental fisheries treatments (C: control with no fisheries treatment; A: fish angled for two minutes with no air exposure; AA: fish angled for two minutes and air exposed for one minute; GN: fish entangled in 140 mm gill net for three minutes and air exposed for one minute). Handling time excludes experimental treatment duration and air exposure. Migratory fate as IM for immediate mortality, ERM for en route mortality, and SUC for successful migration as determined by tracking fish using radiotelemetry post-release.

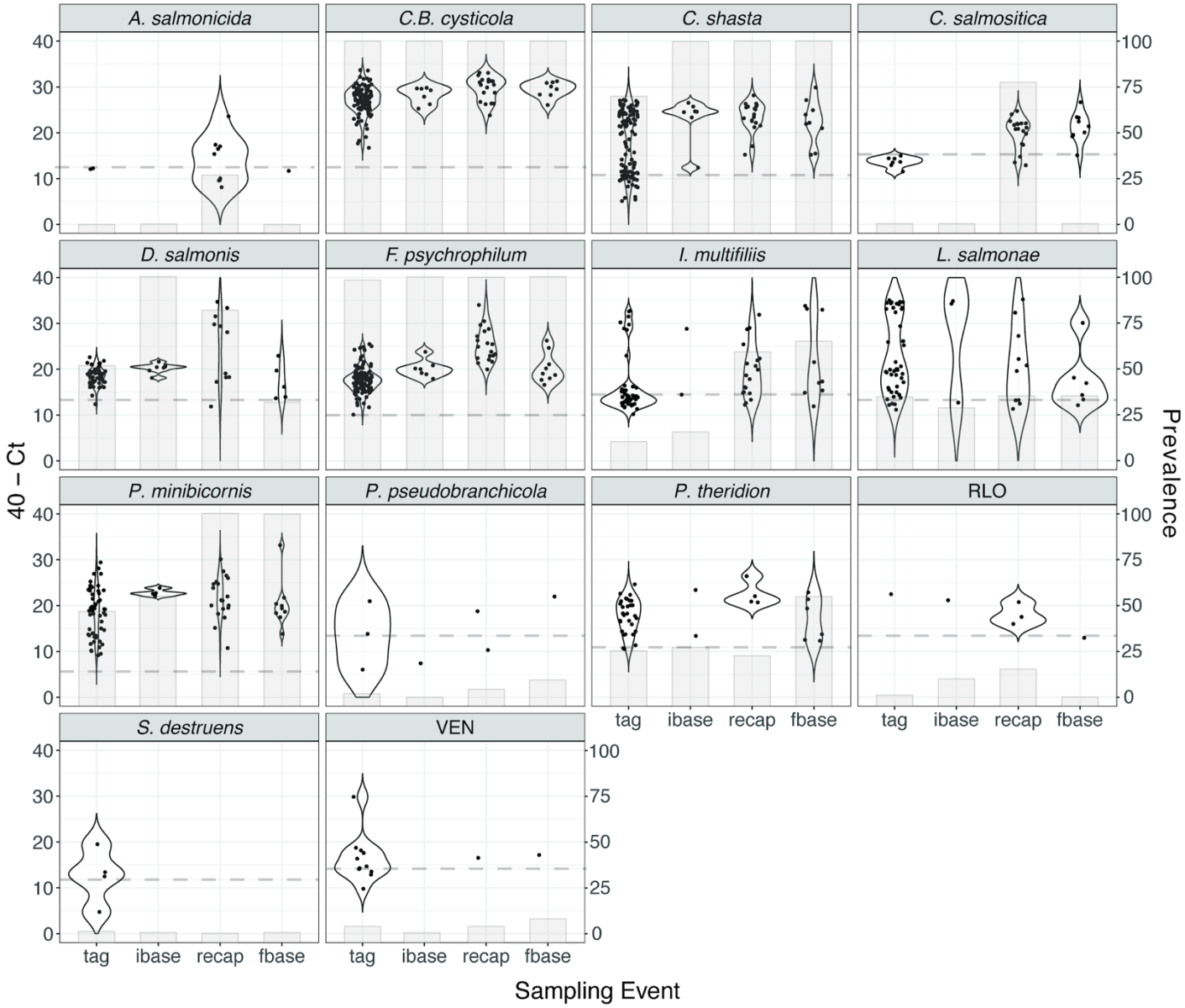

Figure 5-3 Relative loadings and prevalence of infectious agents detected at each sampling event. Prevalence values do not consider detections that were under the limit of detection (LOD), denoted by the dashed line. Sampling events are abbreviated as follows: samples taken at release (tag), initial baseline (ibase), upon recapture after migration (recap) and final baseline (fbase).

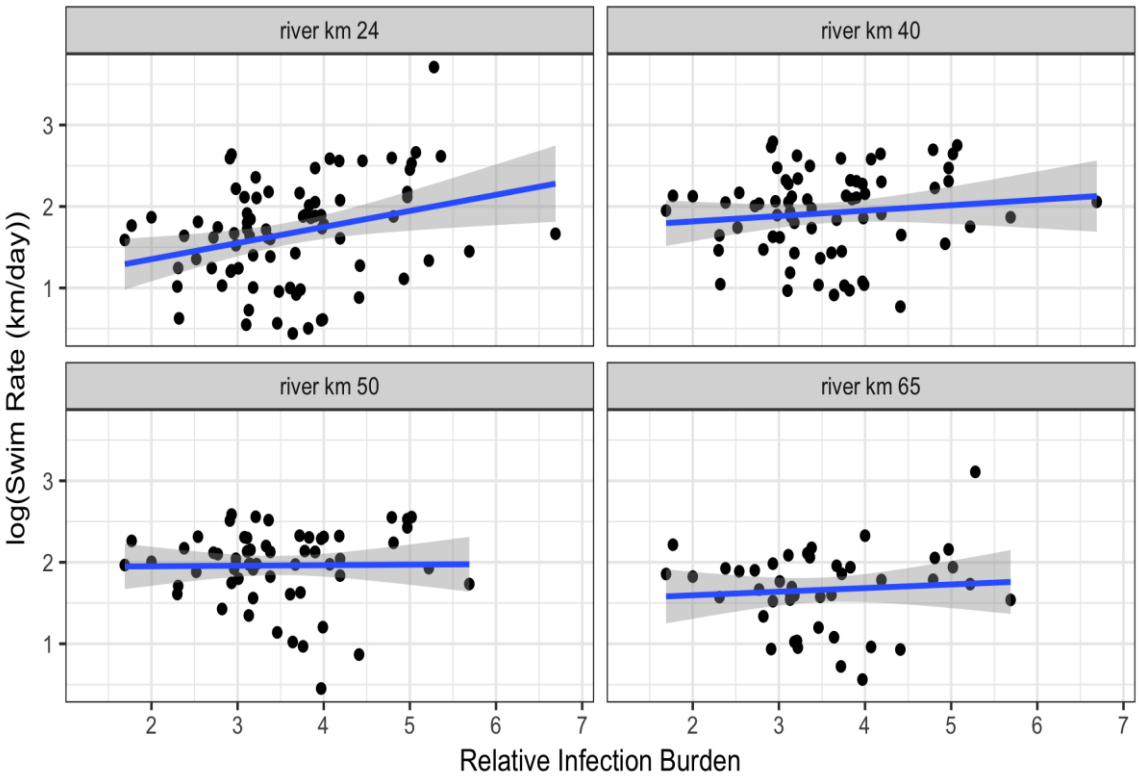

Figure 5-4 Up-river migration rate and Relative Infection Burden (RIB) of tagged coho from release point and between receivers in the Fraser/Chilliwack River system. A positive relationship between RIB and migration initiation post-release was found, however this relationship is not maintained for the remainder of the migration

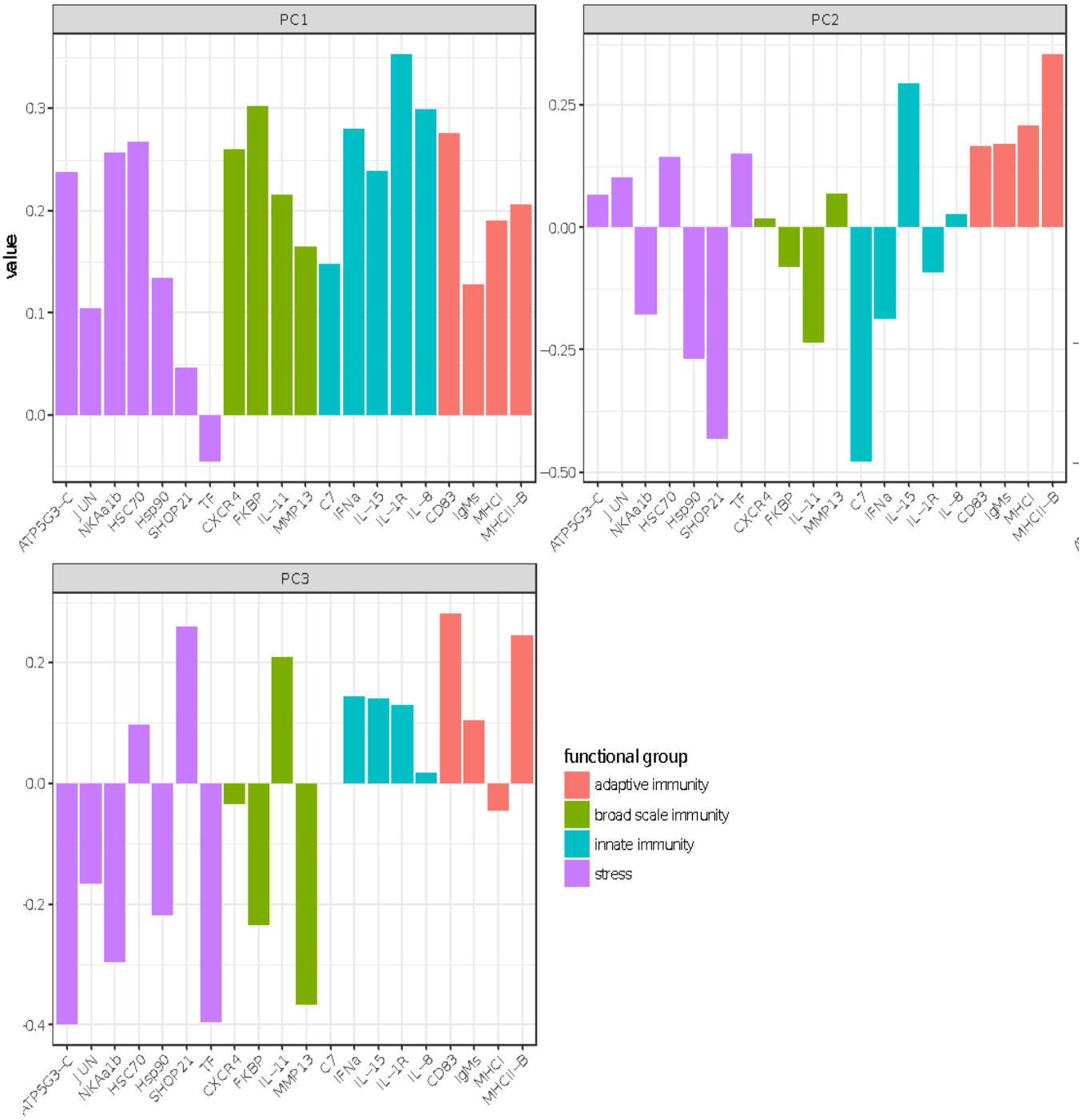

Figure 5-5 Eigenvalues from retained principal components of biomarker transcripts from migratory adult coho salmon, representing 32.2%, 16%, and 9% of the variation among the data respectively.

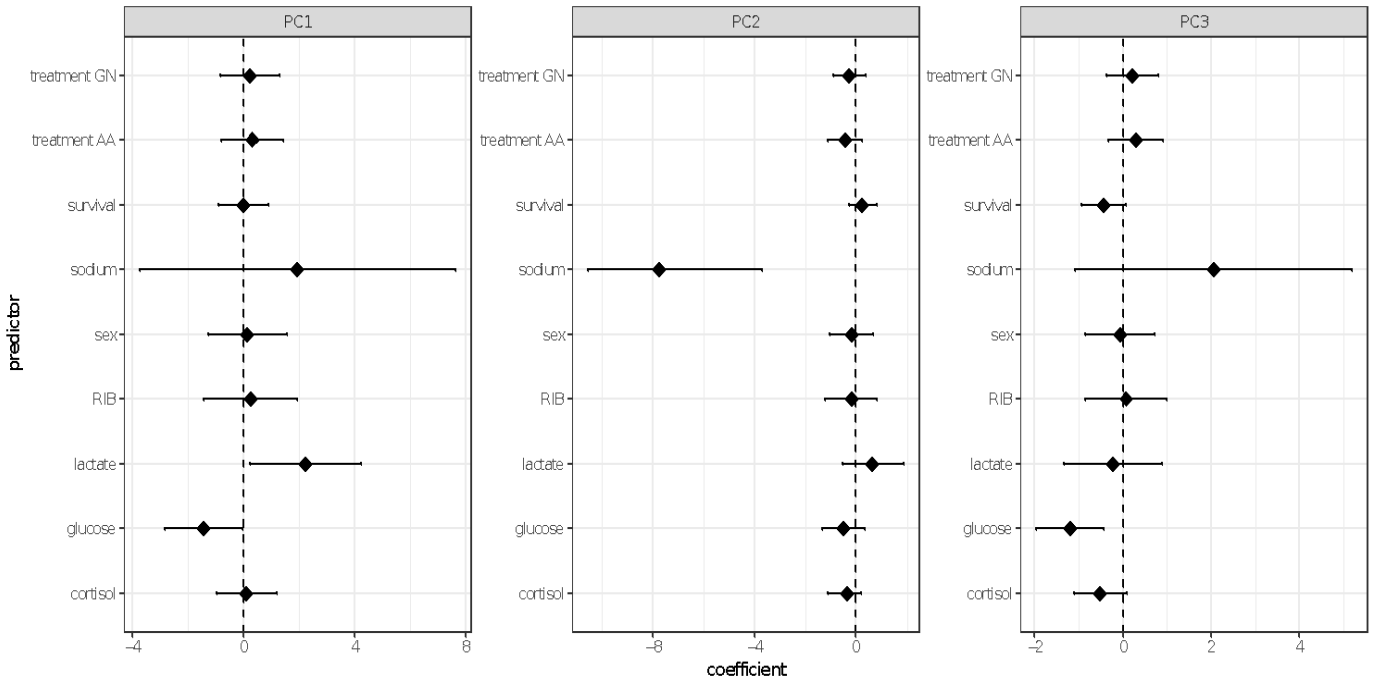

Figure 5-6: Forest plot of predictor coeffecients from generalized linear models for transcriptional profiles of adult migratory coho salmon represented by principal component analysis. Factors are abbreviated as follows: GN: gillnet; AA: angling with 1 min air exposure; RIB: relative infection burden.

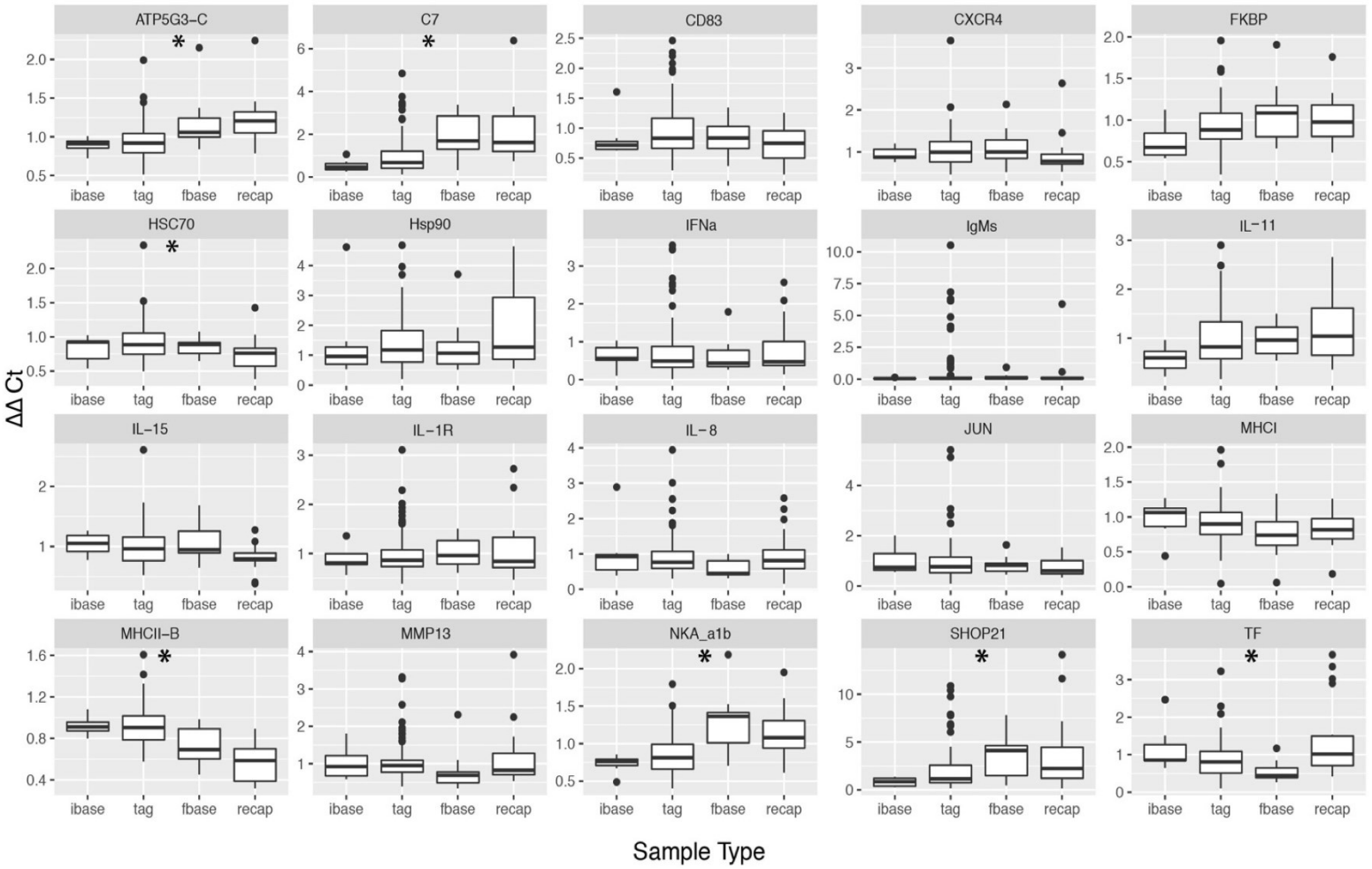

Figure 5-7: Gene expression of biomarkers associated with stress and immune function from migratory adult coho salmon collected from the Chilliwack hatchery in the Fraser River watershed. Axis labels represent type of sample: ibase (initial baseline sample n=7), tag (sample from experimentally relocated and fisheries treated fish n=112), recap (fish recaptured post-migration; n=19) and fbase (final baseline sample n=9). Asterisks represent significance based on Kruskal Wallis ranked sum with Bonferroni correction for multiple comparison at p = 0.0025.

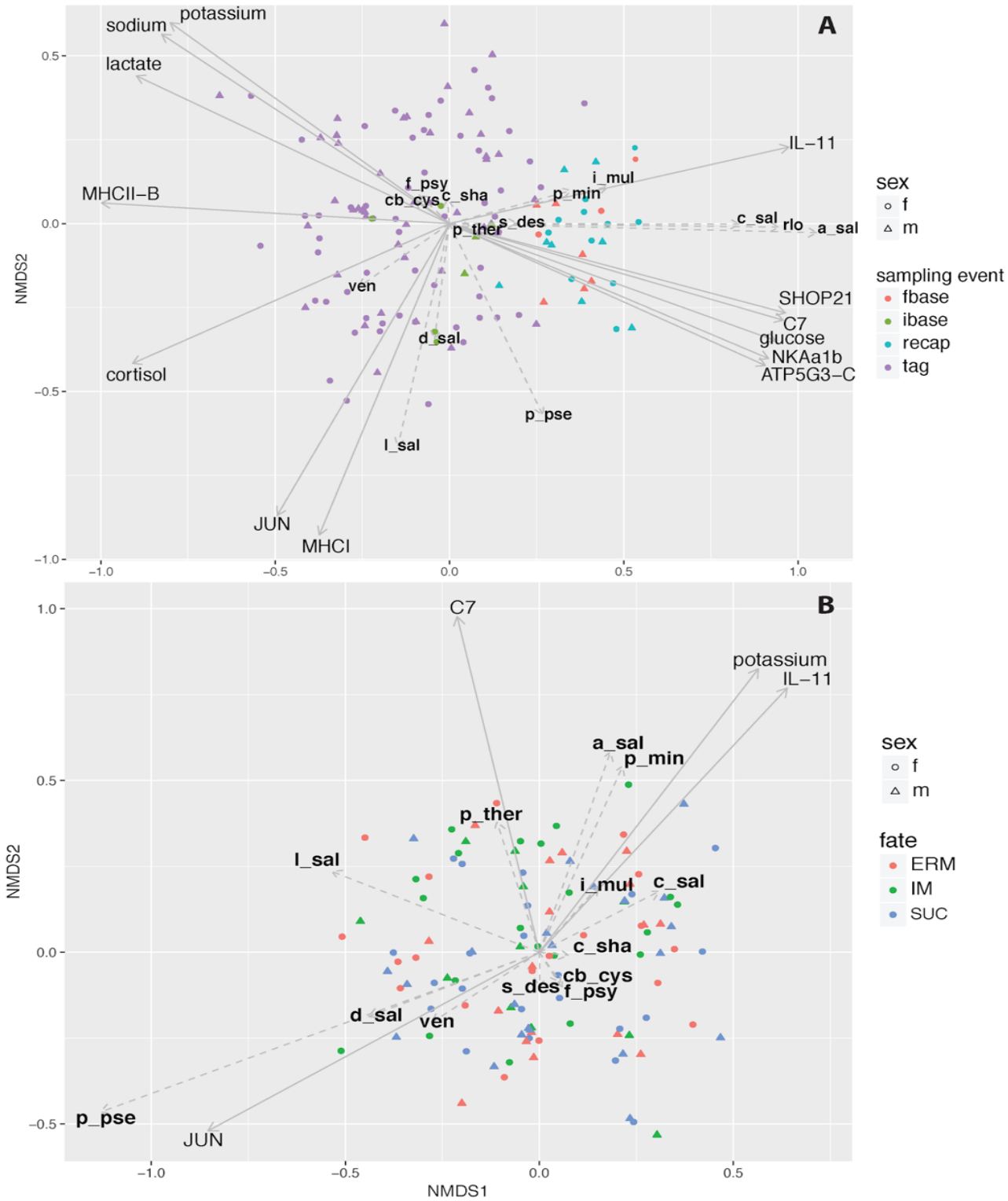

Figure 5-8: NMDS ordinations of infectious agent relative load for A. all samples, excluding initial samples from recaptured individuals and B. samples at tagging. Arrows represent biomarkers and blood parameters significantly correlated with the ordination ($p<0.05$). Ordination A was significantly related to the time of sampling (sample type: ibase = initial baseline, fbase = final baseline, tag = at release, recap = at recapture post-migration). Migratory fate (ERM = enroute mortality, IM = immediate mortality, SUC = successful migration) was not significantly related to ordination B.

Chapter 6: **General Conclusions**

While pathogens are ubiquitous in aquatic environments, infection dynamics are mediated by a multitude of factors including the condition, phenology, and distribution of the host, and availability of transmission pathways for the agent (Kotob et al. 2017; Sofonea et al. 2017). The over-arching hypothesis guiding research for this dissertation was that host response would be associated with species-specific phenology and infection burden, and that the observed response would be mediated by external stressors when present. The complexity of researching pathogens of wild fish was highlighted in Chapter 2, where a synthesis of the status and challenges of wild fish epidemiology was presented. Here, I also provided evidence that the understanding of wild fisheries ecology would be greatly improved by considering the potential influence pathogens have on fish behaviour and survival at a variety of scales (e.g. individual or population, spatiotemporal vatiation). The following data chapters provided support for this assertion and my central hypothesis that species- and context- specific factors are associated with infection dynamics, which can have outcomes important for fisheries conservation and management. While I hypothesized that individual genetic response would be associated with infection burden as previously observed in experimental studies (e.g. Teffer et al. 2018), biomarker profiles were more closely associated with sampling location (Chapter 3), temperature (Chapter 4), and handling and transport stress (Chapter 5). These data demonstrate the complexity inherent in isolating effects in naturally dynamic environments. Each data chapter provided unique evidence highlighting how species phenology and environment can potentially mediate host-pathogen dynamics; in Chapter 4, thermal stress was found to significantly impact host gene expression associated with

immunocompetency, however data did not support my hypothesis that fisheries stressors would mediate host resiliency to infection (Chapter 5).

6.1 Coinfection and Relative Infection Burden

Coinfection was observed in the vast majority of individuals screened across all three species, further demonstrating the need to account for coinfection dynamics in research on host-pathogen dynamics if data is intended to be used to inform conservation and management (Kotob et al. 2017). Relative infection burden also varied across species; Arctic char had the lowest pathogen burdens, an observation that is consistent with global patterns in biodiversity and the latitudinal species gradient (Allen et al. 2006). For coho and Atlantic salmon, I hypothesized that RIB would increase over time in chapters 3 and 4, where I was able to incorporate temporal sampling variation in pathogen dynamics in the study design. These hypotheses were supported, providing additional evidence that the recruitment of pathogens is a natural component of spawning migrations for both Pacific (Miller et al. 2014; Bass et al. 2017) and Atlantic salmon (Bakke and Harris 1997, Wallace et al. 2017). coho and Atlantic salmon were both sampled over the course of 30 days, however the diversity of infectious agents and increase in RIB during freshwater residency was far greater for coho compared to Atlantic salmon. Recruitment of infectious agents during spawning migrations is likely more pronounced in Pacific salmon as their condition rapidly deteriorates as part of their semelparous life-history strategy. While post-spawn survival in Atlantic salmon is low (Jonsson, Jonsson & Hansen 1991), they are not truly semelparous and so likely maintain higher levels of immunocompetency relative to Pacific salmon.

Fat content was the most significant predictor of relative infection burden in Atlantic salmon (Chapter 4). While fat content was not specifically measured for coho, anorexia and use of endogenous energy stores during migration results in similar reduction in lipid content and has been observed in other studies (Bass et al. 2018). Given the temporal changes observed in RIB of migratory coho, it is likely that lipid content would also be correlated with RIB in this species. For Arctic char however, not all anadromous migrants in a given year are expected to be in spawning condition during the return migration; individuals migrate to sea annually to feed, but only reproduce every 3-4 years (Harris et al. 2016), and migrations in this region are not long or particularly hydraulically challenging (Moore et al. 2017). How infection burden may be related to energy stores in this species is of interest given the predicted shifts in productivity (Campana et al. 2020) and thermal regimes that are anticipated to directly impact Northern fish metabolism (Reist et al. 2006). Research that incorporates nutritional status either using fatty acid analysis, blood physiology, or stable isotopes as a factor contributing to immunocompetency and/or infection dynamics could be highly informative for future scenario modelling.

Several species of pathogens were encountered in all three regions (bacteria *C*.B. cysticola, *F. psychrophilum*, ciliate *I. multifiliis,* and myxozoan *P. pseudobranchicola*). Microbial infectious agents of salmonids included in this screening panel are generalists, able to infect multiple species. However, data presented here did not address potential dynamics that occur doing concurrent infections, a component of infection properties that has been demonstrated to mediate transmission and virulence of pathogenic organisms (Seabloom et al. 2015; Karvonen et al. 2019). For example, the sequence of exposure to

pathogens can influence virulence (e.g. secondary infections from bacteria), or prevent infection through antagonistic pathways (Kotob et al. 2017). These processes may have influenced pathogen community structure or RIB metrics,

In Chapter 2, I outlined a number of considerations needed when implementing non-lethal sampling methods to investigate infectious agent communities. These considerations included the potential for false negatives, both for species that are present within the sample population but not included in screening, or in cases where genetic material of the infectious agent is not transient enough within the host to be contained within a small, non-lethal biopsy. It is also probable that the assay panel used to detect pathogens in Chapters 3-5 did not include microbial agents present in the screened populations, particularly for Arctic char where little work on wild microbial pathogens has been executed. This may include pathogen species that are highly host-specific, or those that are not highly pathogenic in endemic hosts and so have little importance for aquaculture. Omitting pathogen species reduces the capability to detect an association between RIB and transcription profiles because the estimated RIB would not reflect actual infection burden. Recent work has demonstrated the utility of challenge studies to identify molecular markers of disease response (Miller et al. 2017) for identification of novel microbial agents in wild populations (Mordecai et al. 2019). Several genes that are responsive to viral disease were included in the biomarker panels and could be used to identify individuals that may be harboring unknown agents. Targeted gene sequencing of these samples may reveal new pathogens of importance for conservation and future research and allow for more accurate testing of the relationship between RIB and gene profiles.

Interestingly, relative infection burden was an explanatory factor in post-release behaviour of coho in Chapter 5, however in a manner that did not support my hypotheses. Rather than negatively impacting migration success, RIB was associated with the rate at which up-river migration was initiated. As discussed in Chapter 2, the condition of hosts directly influences behaviour measured using tracking technologies. In senescing salmon, host condition is confounded with RIB; while it would be expected that higher infection burden would have a negative impact on recovery from fisheries interaction (Teffer et al. 2019a), RIB was more likely an indication of the senescence trajectory rather than a factor directly mediating post-release behaviour. Here, incorporating genetic based screening methods with telemetry revealed an indirect association that otherwise would have been overlooked.

6.2 Host Transcriptional Profiles

I hypothesized that transcription profiles would be correlated with RIB in all data chapters and mediated by the extraneous stressors in data Chapters 3 and 4. Biomarkers associated with the innate arm of immune response were consistently associated with RIB across all species including inflammatory interleukins (IL-11, IL-8), complement factors (C3, C7), and wound healing associated matrix metalloproteinases (MMP 13 and MMP25). The most prevalent pathogens, *F. psychrophilum* and *I. multifiliis,* cause damage to epithelial cell structure and so likely illicit an inflammatory response. Because gill was used for pathogen screening, biomarkers that were correlated with RIB may be representing the immune and metabolic state in response to pathogen infection within gill tissue. While C3 is involved in both the classical and alternative complement pathway,

C7 is a specific component of the membrane attack complex responsible for cytolysis of invading pathogen cells (Schmidt-Hempel 2011). Up-regulation of C7 in gill tissue supports gill as a major site of infection across species and potential transmission pathway for agents that infect internal organs. Gill tissue is a vulnerable area for fish and a potential entry point in to the body for many microbial pathogens, and the primary infection site for many of the species assayed in this research (Supplement Table 1). Gill is also an extremely delicate tissue and so inflammatory response and cell maintenance may occur at lower thresholds of disturbance than other tissues. While gill is a superior tissue to use for non-lethal screening of pathogens because it is highly vascular, metabolically responsive, and demonstrates high correspondence with internal organ pathogen detection (Teffer and Miller 2019), gene expression is likely to be site specific and so transcriptomic responses occurring at primary infection sites may be diluted and difficult to detect.

Adaptive immune components that were responsive to RIB across species were also negatively associated with senescence including the major histocompatibility complex I and II; MHC-I was positively associated with RIB in Arctic char and Atlantic salmon, yet down-regulated and negatively related to RIB in coho salmon. This phenomena has been observed in other Pacific salmon species during spawning migrations, including coho (Teffer et al. 2019a). Differential immune regulation associated with life-history and reproductive strategy may explain the species-specific responses, however teasing apart causal mechanisms is challenging given confounding environmental factors.

6.3 Stress as a Mediating Factor

Studying anadromous fish during spawning migrations is challenging because of the complexity of confounding factors and variation in naturally occurring cumulative stressors. Differentiating between endogenous stress associated with spawning migrations, naturally occurring variations in environmental conditions, and finally artificially imposed anthropogenic stress requires a larger sample size for isolation and identification of responses, or identification of highly sensitive and specific measurements of stress responsiveness. In data Chapters 3 and 4, transcription profiles were more closely associated with environmental (temperature) and anthropogenic (capture and transport) stress than infectious agent communities, and contrary to my hypothesis in Chapter 5, severity of fisheries stressor was not associated with transcriptional responses. Given the transient and variable nature of transcripts and potential for post-translational processes to obscure the ability to detect mechanistic relationships using RNA alone, general biomarker panels such as those used herein may be more suited to research on chronic stressors. Chronic stressors illicit prolonged transcriptional changes that are more repeatable and thus suitable for detection using transcripts as biomarkers of organism state (Houde et al. 2019). Incorporating post-translational modifications or investigations of epigenetic factors would provide an enhanced understanding of the proteomic response of fish to chronic stress and/or infection.

While questions remain regarding how Arctic char will be able to cope with increased temperatures, recent modeling has predicted that species with high phenotypic plasticity such as those in the genus *Salvelinus* may actually increase in biomass

(Campana et al. 2020), which has the potential to alter pathogen communities through increased density. At the same time, predicted changes in biodiversity associated with the invasion of closely related species are likely to increase pathogen loads with novel species. I have provided a modest baseline of infectious agent community data that can be used to inform future monitoring programs. This is particularly important for cold-water adapted species, where temperature shifts will likely facilitate the invasion of novel pathogens, or extend periods of enhanced virulence for endemic agents suppressed at lower temperatures (Marcos-López et al. 2010). Through collaborative work with test fisheries sampling teams in the Arctic, where fieldwork is especially costly and logistically challenging to implement, the utility of using small samples that are easy to transport and store was demonstrated. Future test fisheries could easily incorporate a gill sample within the current data collection techniques and in doing so provide comprehensive data that would allow relatively early detection of the impending shifts in aquatic pathogen communities.

The lack of association between fisheries stressors, relative infection burden and survival in coho salmon was counter to my hypothesis that fisheries stress would negatively influence survival (Chapter 5). Previous research on coho demonstrated sex-specific response to fisheries stress under elevated thermal temperatures (Teffer et al. 2019a). Given the lack of response to fisheries stressors and temperature-associated transcription observed in Atlantic salmon, these fish may be more sensitive to changes in thermal conditions than fisheries induced stress alone. However, the finding that the duration of handling was a significant predictor of fate, irrespective of fisheries treatment, relative infection burden, and sampling regiment, has significant implications for bycatch

management. Reducing bycatch mortality is an important conservation goal in salmonid fisheries (Davis 2002), where catch-and-release is a management tool that maximizes stakeholder participation while working to protect specific stocks at risk. Management actions that specifically address handling times, regardless of factors such as air exposure, in all fisheries sectors may have positive outcomes for bycatch survival.

6.4 Future Research Directions

Given the paucity of research addressing pathogens in wild aquatic organisms, many research opportunities exist that would contribute meaningfully to the understanding of disease ecology from both applied and theoretical perspectives. Conservation of the world's salmonid stocks is of critical importance due to their role in food security and economic stability. This is especially true for Arctic char, where much of local Inuit food security, culture, and now economic opportunity through emerging commercial fishery development relies on the health of these stocks. Yet Arctic char are facing significant pressure from invasive salmon species across their circumpolar distribution, and Inuit communities across the North are reporting changes to the appearance, texture, and taste of local Arctic char and in some cases are hesitant to consume abnormal fish (Chantal Langlois, Regional Dietician for the Kitikmeot Region, personal communication). Similarly, communities in Nunavik, Northern Quebec, are reporting diseased Arctic char that they have never seen before and will not eat (Monica Mulrennan, personal communication). While Pacific salmon are a major consideration in the Kitikmeot region that was sampled in Chapter 3, the Northward progression of wild Atlantic salmon has been observed (ICES 2018). Of perhaps greater concern is the

incidence of escaped aquaculture Atlantic salmon (Jensen et al. 2013) and Pacific pink salmon *O. gorbuscha* (Millane et al, 2019) now occurring in Northern latitudes (ICES 2018), which have the potential to act as vectors of disease. They will also directly compete for limited food resources and over-winter habitats, placing additional pressure on Arctic char and throwing off the balance of the small, highly constrained trophic structure of the low-diversity food webs. The reported phenomena may be an excellent opportunity to work with local Inuit communities to address research questions that are directly relevant for their community health and well-being. From an applied perspective, comprehensive pathogen screening of stocks important for local subsistence harvest would address local public health concerns and ensure consistent access to highly nutritious and culturally relevant food. Also screening captured non-native salmonid species may allow mitigative measures to be put in place if highly pathogenic agents (for either fish or humans) are detected. This invasion front also provides an opportunity to track and characterize the response of endemic host species to novel pathogens in a system with relatively low endemic pathogen and host diversity and relatively stable environmental conditions. The Arctic context reduces the number of confounding factors (e.g. coinfection dynamics, environmental stressors) compared to working in other systems and may allow the isolation of causal mechanisms for outcomes from the individual to population level.

As molecular techniques are more widely applied to infectious agent screening in wild fishes, more comprehensive data sets will be amassed and provide the potential to investigate more detailed characteristics of coinfection dynamic. Trends in pathogen community structures that indicate synergistic or antagonistic coinfection interactions, for

example, may be discernable by using analytical models currently used in community ecology (e.g. stochastic metacommunity models; Seabloom et al. 2015). Further combining this with host transcriptome data may reveal mechanisms underlying coinfection dynamics, as changes observed at the pathogen level may be a consequence of either host or pathogen response (e.g. pathogen-pathogen competition or host immunosuppression). Such advances in analytical methods would enhance the predictive abilities of future scenario models relevant for wildlife epidemiologists, salmon management and conservation (Johnson et al. 2015).

When addressing host response to diverse pathogen infection, the responsiveness of MHC expression may provide an interesting avenue for future research. MHC I and II are responsible for recognizing and binding peptides of pathogens for recruitment of the cellular immune response. Consequently, allelic polymorphisms in MHC sequences represents selection pressures associated with pathogen diversity (Eizaguarrie and Lenz 2010) and potential heritable resistance to pathogens (Kronenburg et al. 1994; Siddle et al. 2010) which has been demonstrated in coho (Gomez et al. 2011) and Atlantic salmon (Miller et al. 2004; Gomez et al. 2011) but not Arctic char. The diversity of MHC alleles is believed to be directly associated with selection pressure from pathogens (Wegner et al. 2003), particularly those that occur during the freshwater phase of anadromous life cycles (McClelland et al. 2013). Wild Arctic char may provide an interesting model for additional research in fish MHC alleles because of the low pathogen diversity and high species polymorphism may result in a pronounced variation if localized adaptation is present. Also, better characterization of MHC diversity may reveal how increased pathogens likely to come from invasive species will affect char populations.

6.5 General Conclusions

This thesis has contributed to the current body of research investigating the phenological associations between microbial infections agents and adult salmonids and provided some of the first comprehensive screening for wild Arctic char, and first temporal pathogen data for wild Atlantic salmon. Species-specific adaptations in reproductive strategies is closely linked with immunocompetency and thus infection profiles during spawning migrations. Further research is required to solidify the potential effects of human and climate induced shifts on host-pathogen dynamics, however novel research methods and experimental designs such as those proposed in Chapter 2 and employed in the field studies detailed in Chapters 3-5 demonstrate the potential for infectious agent screening to be incorporated in to fisheries research. All data presented in this thesis was obtained from gill samples, highlighting the utility of non-lethal sampling for both pathogen screening and profiling of host condition by using genetic biomarkers of stress and immune response that was presented in Chapter 2. A great deal of insight can be gained from a small, non-invasive sample, insight that may be able to improve the predictive capacity of ecological models as more is revealed about how fish condition and infectious agents are influenced by human and climate induced changes. Combining field research methods with laboratory analysis in collaboration with disease experts will greatly improve the understanding of aquatic animal ecology by considering all potential factors relevant for fish fitness and survival

Appendix A Supplementary Tables

Appendix A: Table 1: Microbial infectious agents, associated disease, and sampling locations previously reported from wild populations of Arctic char *Salvelinus alpinus*

Microbial pathogen species	Disease associated with pathogen	Locations detected	Citation
Renibacterium salmoninarum	Bacterial Kidney Disease	Kitikmeot, Qiqitaaluk, and Kivaliq regions of Nunavut; Scotland; Iceland	Souter et al. 1987, Jónsdóttir et al. 1998, Wallace et al. 2017 Meyers et al. 1993
Infectious Pancreatic Necrosis Virus		Northwest Territories, Canada; Scotland; Switzerland	Souter et al. 1984, Knussel et al. 2003; Wallace et al. 2017
Aeromonas salmonicida	Furunculosis	Scotland	Wallace et al. 2017
Salmonid alphavirus	Pancreas disease	Scotland	Wallace et al. 2017
Viral Haemorhagic Septicemia Virus (VHSV)	Viral haemorrhagic septicaemia	Switzerland; Scotland	Knussel et al. 2003; Wallace et al. 2017
Spironucleus salmonicida	Systemic spironucleosis	Norway	Jørgensen et al. 2011a
Spironucleus barkhanus	Systemic spironucleosis	Norway	Jørgensen et al. 2011a
Parvicapsula pseudobranchicola		Norway	Jørgensen et al. 2011b
Myxobolus arcticus		Ungava Bay, Quebec	Desdevises et al. 1998
*Haemogregarina irkalukpiki**		Ungava Bay, Quebec	Laird 1961

Appendix A: Table 2: Summary of all infectious agents included in qPCR screening in this study. Prevalence for each species is listed out of all individuals examined. Primary infection site and associated disease is listed but not exclusive as many agents may be associated with a given pathology. Source sequences listed as 'in house' indicates that the assay was designed by staff at the Molecular Genetics Laboratory in Nanaimo, BC.

Group	Infectious Agent	O. kisutch	S. alpinus	S. salar	Classification	Intracellular/ extracellular	Primary Infection Site	Associated Disease	Environment	Taqman Primer Sequences (5'–3') Probe Sequence (FAM-5'–3'-MGB)	Source sequence
bacteria	Aeromonas hydrophila	0	0	0	Gammaproteobacteria	Extracellular	Systemic	Hemorrhagic septicemia	Freshwater	F—ACCGCTGCTCATTACTCTGATG / R—CCAACCCAGACGGGAAGAA / P—TGATGGTGAGCTGGTTG	Lee et al. 2006
bacteria	Aeromonas salmonicida	0.03	0.02	0	Gammaproteobacteria	Extracellular	Systemic	Furunculosis	Freshwater	F—TAAAGCACTGTCTGTTACC / R—GCTACTTCACCCTGATTGG / P—ACATCAGCAGGCTTCAGAGTCACTG	Keeling et al. 2013
bacteria	Candidatus Branchiomonas cysticola	1	0.82	0.96	Chlamydiaceae	Intracellular	Gills	Epitheliocystis	Marine, Freshwater	F—AATACATCGGAACGTGTCTAGTG / R—GCCATCAGCCGCTCATGTG / P—CTCGGTCCCAGGCTTTCCTCTCCCA	Mitchell et al. 2013
bacteria	Candidatus Piscichlamydia salmonis	0	0	0.07	Chlamydiaceae	Intracellular	Gills	Proliferative Gill Disease	Marine, Freshwater	F—TCACCCCCAGGCTGCTT / R—GAATTCCATTTCCCCCTCTTG / P—CAAAACTGCTAGACTAGAGT	Nylund et al. 2008
bacteria	Flavobacterium psychrophilum	0.99	0.07	0.74	Flavobacteria	Extracellular	Systemic	Bacterial Coldwater Disease	Freshwater	F—GATCCTTATTCTCACAGTACCGTCAA / R—TGTAAACTGCTTTTGCACAGGAA / P—AAACACTCGGTCGTGACC	Duesund et al. 2010
bacteria	Gill chlamydia	0	0.04	0	Chlamydiaceae	Extracellular	Gills	Contirbuting to Proliverative Gill Inflammation	Freshwater	F—GGGTAGCCCGATATCTTCAAAGT / R—CCCATGAGCCGCTCTCTCT / P—TCCTTCGGGACCTTAC	Duesund et al. 2010
bacteria	Piscirickettsia salmonis	0	0	0	Chlamydiaceae	Intracellular	Systemic	Salmonid Rickettisal Septicemia	Marine	F—TCTGGGAAGTGTGGCGATAGA / R—TCCCGACCTACTCTTGTTTCATC / P—TGATAGCCCCGTACACGAAACGGCATA	Corbeil et al. 2003
bacteria	Renibacterium salmoninarum	0	0	0	Actinobacteria	Intracellular	Kidney	Bacterial Kidney Disease	Marine, Freshwater	F—CAACAGGGTGGTTATTCTGCTTTC / R—CTATAAGAGCCACCAGCTGCAA / P—CTCCAGCGCCGCAGGAGGAC	Powell et al. 2005
bacteria	Rickettsia-like organism	0.03	0	0	Chlamydiaceae	Extracellular	Systemic	Red Mark Syndrome/Cold water Strawberry Disease	Marine, Freshwater	F—GGCTCAACCCAAGAACTGCTT / R—GTGCAACAGCGTCAGTGACT / P—CCCAGATAACCGCCTTCGCCTCCG	Lloyd et al. 2011
bacteria	Vibrio anguillarum	0	0	0	Gammaproteobacteria	Extracellular	Systemic	Vibriosis	Marine	F—CCGTCATGCTATCTAGAGATGTATTTGA / R—CCATACGCAGCCAAAAATCA / P—TCATTTCGACGAGCGTCTTGTTCAGC	In house

bacteria	Vibrio salmonicida	0	0	0	Gammaproteobacteria	Extracellular	Systemic	Cold-water Vibriosis	Marine	F—GTGTGATGACCGTTCCATATTT / R—GCTATTGTCATCACTCTGTTTCTT / P—TCGCTTCATGTTGTGTAATTAGGAGCGA	In house
bacteria	Yersinia ruckeri	0	not run	not run	Gammaproteobacteria	Extracellular	Buccal cavity	Enteric Redmouth Disease/yersiniosis	Freshwater	F—TGCCGCGTGTGTGAAGAA / R—ACGGAGTTAGCCGGTGCTT / P—AATAGCACTGAACATTGAC	Glenn et al. 2011
platyhelminth	Gyrodactylus salaris	0	0	0	Monogenea	Extracellular	External surfaces		Freshwater	F—CGATCGTCACTCGGAATCG / R—GGTGGCGCACCTATTCTACA / P—TCTTATTAACCAGTTCTGC	Collins et al. 2010
platyhelminth	Nanophyetus salmincola	0	0	0	Trematoda	Extracellular	Systemic		Freshwater	F—CGATCTGCATTTGGTTCTGTAACA / R—CCAACGCCACAATGATAGCTATAC / P—TGAGGCGTGTTTTATG	In house
protozoa	Ceratonova shasta	0.79	0	0	Myxosporea	Extracellular	Intestine	Ceratomyxosis	Freshwater	F—CCAGCTTGAGATTAGCTCGGTAA / R—CCCCGGAACCCGAAAG / P—CGAGCCAAGTTGGTCTCTCCGTGAAAAC	Hallett and Bartholomew 2006
protozoa	Cryptobia salmositica	0.2	0	0	Kinetoplastida	Extracellular	Blood	Cryptobiasis	Freshwater	F—TCAGTGCCTTTCAGGACATC / R—GAGGCATCCACTCCAATAGAC / P—AGGAGGACATGGCAGCCTTTGTAT	In house
protozoa	Dermocystidium salmonis	0.51	0	0	Microsporidia	Extracellular	Oral cavity, external surface	Cysts on gill tissue	Marine, Freshwater	F—CAGCCAATCCTTTCGCTTCT / R—GACGGACGCACACCACAGT / P—AAGCGGCGTGTGCC	In house
protozoa	Facilispora margolisi	0	0	0	Microsporidia	Extracellular	Systemic		Marine	F—AGGAAGGAGCACGCAAGAAC / R—CGCGTGCAGCCCAGTAC / P—TCAGTGATGCCCTCAGA	In house
protozoa	Ichthyophonus hoferi	0	0	0	Mesomycetozoea	Extracellular	Muscle	Granulomatous Systemic Disease	Marine	F—GTCTGTACTGGTACGGCAGTTTC / R—TCCCGAACTCAGTAGACACTCAA / P—TAAGAGCACCCACTGCCTTCGAGAAGA	White et al. 2013
protozoa	Ichthyophthirius multifiliis	0.46	0.35	0	Oligohymenophorea	Extracellular	Oral cavity, gills, external surfaces	White Spot, Ich	Freshwater	F—AAATGGGCATACGTTTGCAAA / R—AACCTGCCTGAAACACTCTAATTTTT / P—ACTCGGCCTTCACTGGTTCGACTTGG	In house
protozoa	Kudoa thyrsites	0	0	0	Myxosporea	Intracellular	Muscle	Myoliquifaction	Marine	F—TGGCGGCCAAATCTAGGTT / R—GACCGCACACAAGAAGTTAATCC / P—TATCGCGAGAGCCGC	Funk et al. 2007
protozoa	Loma salmonae	0.38	0	0	Microsporidia	Intracellular	Gills	Microsporidial Gill Disease of Salmon	Freshwater	F—GGAGTCGCAGCGAAGATAGC / R—CTTTTCCTCCCTTTACTCATATGCTT /	In house

protozoa	Myxobolus arcticus	0	0	0	Myxosporea	Extracellular	Nervous tissue		Freshwater	P—TGCCTGAAATCACGAGAGTGAGACTACCC F—TGGTAGATACTGAATATCCGGGTTT / R—AACTGCGCGGTCAAAGTTG / P—CGTTGATTGTGAGGTTGG	In house
protozoa	Myxobolus cerebralis	0	0	0	Myxosporea	Extracellular	Cartilage, Brain	Whirling Disease	Freshwater	F—GCCATTGAATTTGACTTTGGATTA / R—ACCATTCATGTAAGCCCGAACT / P—TCGAAGCCTTGACCATCTTTTGGCC	Kelley et al. 2004
protozoa	Myxobolus insidiosus	0	0	0	Myxosporea	Extracellular	Striated muscle		Freshwater	F—CCAATTTGGGAGCGTCAAA / R—CGATCGGCAAAGTTATCTAGATTCA / P—CTCTCAAGGCATTTAT	In house
protozoa	Neoparamoeba perurans	0	0	0	Amoebozoa	Extracellular	Gills	Amoebic Gill Disease	Marine	F—GTTCTTTCGGGAGCTGGGAG / R—GAACTATCGCCGGCACAAAAG / P—CAATGCCATTCTTTTCGGA	Fringuelli et al. 2012
protozoa	Nucleospora salmonis	0	0.02	0	Microsporidia	Intracellular	Hematopoetic cells	Chronic Severe Lymphoblastosis, Leukemia-like Condition	Marine, Freshwater	F—GCCGCAGATCATTACTAAAAACCT / R—CGATCGCCGCATCTAAACA / P—CCCCGCGCATCCAGAAATACGC	Foltz et al. 2009
protozoa	Paranucleospora theridion	0.25	0	0.13	Microsporidia	Intracellular	Gills, other organs	Proliferative Gill Disease; Proliferative Gill Inflammation	Marine	F—CGGACAGGGAGCATGGTATAG / R—GGTCCAGGTTGGGTCTTGAG / P—TTGGCGAAGAATGAAA	Nylund et al. 2010
protozoa	Parvicapsula kabatai	0	0	0	Myxosporea	Extracellular	Kidney	emaciation disease (?)	Marine, Freshwater	F—GTCGGATGATAAGTGCATCTGATT / R—ACACCACAACTCTGCCTTCCA / P—TGCGACCATCTGCACGGTACTGC	In house
protozoa	Parvicapsula minibicornis	0.51	0	0	Myxosporea	Extracellular	Pseudobranch, Kidney		Marine, Freshwater	F—AATAGTTGTTTGTCGTGCACTCTGT / R—CCGATAGGCTATCCAGTACCTAGTAAG / P—TGTCCACCTAGTAAGGC	Hallett and Bartholomew 2009
protozoa	Parvicapsula pseudobranchicola	0.03	0.02	0	Myxosporea	Extracellular	Kidney, Pseudobranchs, Gill	Parvicapsulosis	Marine	F—CAGCTCCAGTAGTGTATTTCA / R—TTGAGCACTCTGCTTTATTCAA / P—CGTATTGCTGTCTTTGACATGCAGT	Jørgensen et al. 2011
protozoa	Sphaerothecum destruens	0.01	0	0	Mesomycetozoea	Intracellular	Macrophages		Freshwater	F—GCCGCGAGGTGTTTGC / R—CTCGACGCACACTCAATTAAGC / P—CGAGGGTATCCTTCCTCTCGAAATTGGC	In house
protozoa	Spironucleus salmonicida	0	0.05	0	Diplomonadida	Extracellular	Systemic		Marine, Freshwater	F—AACCGGTTATTCGTGGGAAAG / R—TTAACTGCAGCAACACAATAGAATACTC / P—TGCCAGCAGCCGCGGTAATTC	In house

protozoa	Tetracapsuloides bryosalmonae	not run	0	0.55	Malacosporea	Extracellular	Kidney, spleen	Proliferative Kidney Disease	Freshwater	F—GCGAGATTTGTTGCATTTAAAAAG / R—GCACATGCAGTGTCCAATCG / P—CAAAATTGTGGAACCGTCCGACTACGA	Bettge et al. 2009
virus	Atlantic salmon paramyxovirus	0	0	0	Paramyxoviridae	Intracellular	Gill	Contirbuting agent to Proliverative Gill Inflammation	Marine	F—CCCATATTAGCAAATGAGCTCTATCTT / R—CGTTAAGGAACTCATCATTGAGCTT / P—AGCCCTTTTGTTCTGC	Nylund et al. 2008
virus	Infectious Haematopoietic Necrosis Virus	0	0	0	Rhabdoviridae	Intracellular	Systemic	IHNV	Freshwater	F—AGAGCCAAGGCACTGTGCG / R—TTCTTTGCGGCTTGGTTGA / P—TGAGACTGAGCGGGACA	Purcell et al. 2013
virus	Pacific salmon parvovirus	0	0	0	Parvoviridae	Intracellular	Systemic		Marine, Freshwater	F—CCCTCAGGCTCCGATTTTTAT / R—CGAAGACAACATGGAGGTGACA / P—CAATTGGAGGCAACTGTA	In house
virus	Piscine myocardial virus	0	0	0	Totiviridae	Intracellular	Heart	Cardiomyopathy Syndrome	Marine	F—TTCCAAACAATTCGAGAAGCG / R—ACCTGCCATTTTCCCCTCTT / P—CCGGGTAAAGTATTTGCGTC	Løvoll et al. 2010
virus	Piscine Orthoreovirus	0	0	0	Reoviridae	Intracellular	Heart	Heart and Skeletal Muscle Inflammatory Syndrome	Marine	F—TGCTAACACTCCAGGAGTCATTG / R—TGAATCCGCTGCAGATGAGTA / P—CGCCGGTAGCTCT	Wiik-Nielsen et al. 2012
virus	Salmonid herpesvirus	0	0	0	Alloherpesviridae	Intracellular	Skin, liver	OMV disease	Freshwater	F—GCCTGGACCACAATCTCAATG / R—CGAGACAGTGTGGCAAGACAAC / P—CCAACAGGATGGTCATTA	In house
virus	Viral encephalopathy and retinopathy virus	0	0	0	Nodaviridae	Intracellular	Nervous tissue	Piscine Nodavirus Disease	Marine	F—TTCCAGCGATACGCTGTTGA / R—CACCGCCCGTGTTTGC / P—AAATTCAGCCAATGTGCCCC	Korsnes et al. 2005
virus	Viral erythrocytic necrosis virus	0.08	0	0	Iridoviridae	Intracellular	Blood	Viral Erythrocytic Necrosis	Marine	F—CGTAGGGCCCCAATAGTTTCT / R—GGAGGAAATGCAGACAAGATTTG / P—TCTTGCCGTTATTTCCAGCACCCG	James Winton, pers. comm.
virus	Viral haemorrhagic septicaemia virus	0	0	0	Rhabdoviridae	Intracellular	Systemic	VHS	Freshwater	F—ATGAGGCAGGTGTCGGAGG / R—TGTAGTAGGACTCTCCCAGCATCC / P—TACGCCATCATGATGAGT	Garver et al. 2011

Appendix A: Table 3: Assay and gene names for biomarkers of stress, immune function, and viral disease response run on gill tissue collected from Atlantic salmon and Arctic char in Chapters 2 and 3. Only biomarkers were efficiency was between 80-120% were used in analyses. NA for efficiency indicates a failure to amplify. Primer and probe sequences were designed at the molecular genetics lab in Nanaimo, BC unless otherwise stated.

Assay name	S.salar efficiency	S.alpinus efficiency	Gene name	Functional group	Functional group general	Primer and probe sequences	Source
B2M	1.13	0.72	Beta 2-microglobulin	Acquired Immunity	immune	F—TTTACAGCGCGGTGGAGTC /R—TGCCAGGGTTACGGCTGTAC /P—AAAGAATCTCCCCCCAAGGTGCAGG	Haugland et al. (2005)
C1Qc	0.94	2.53	complement C1q subcomponent subunit C	Innate Immunity	immune	F—CGCCGGTGAGTGGAATCTA/R—CTTCTCCATCATGTGGTGTGCTA/P—ACCTCCAAACATAGAAGAG	
C3	1.05	0.88	Complement component 3	Innate Immunity	immune	F—ATTGGCCTGTCCAAAACACA /R—AGCTTCAGATCAAGGAAGAAGTTC /P—TGGAATCTGTGTGTCTGAACCCC	Raida and Buchmann (2009)
C7	0.90	0.88	Complement factor	Innate Immunity	immune	F—ACCTCTGTCCAGCTCTGTGTC /R—GATGCTGACCACATCAAACTGC /P—AACTACCAGACAGTGCTG	
CCL4	0.93	0.9	CC chemokine 4	Innate Immunity	immune	F— TCTCTTCATTGCAACAATCTGCTT/ R—ACAGCAGTCCACGGGTACCT/ P—CTACGCAGCAGCATT	
CD8a	1.03	0.65	T-cell surface glycoprotein CD8 alpha chain	Acquired Immunity	immune	F—ACACCAATGACCACAACCATAGAG/R—GGGTCCACCTTTCCCACTTT/P—ACCAGCTCTACAACTGCCAAGTCGTGC	Raida et al. (2011)
DEXH	0.99	0.82	DEXH box helicase, DNA ligase-associated	Multi-function Immunity	immune	F—CCATAAGGAGGGTGTCTACAATAAGAT/R—CTCTCCCCCTTCAGCTTCTGT/P—TGGCGCGCTACGTG	
GAL3	1.12	5.21	Galectin-3-binding protein precursor	Innate Immunity	immune	F—TTGTAGCGCCTGTTGTAATCATATC/R—TACACTGCTGAGGCCATGGA/P—CTTGGCGTGGTGGC	
HBA	0.98	NA	Hemoglobin subunit alpha	Multi-function Immunity	immune	F—GCCCTGGCTGACAAATACAGA/R—GAGCAGGAACTGGAGTCCAATG/P—ACCATCATGAAAGTCC	
Hep	1.21	0.97	Hepcidin	Multi-function Immunity	immune	F—GAGGAGGTTGGAAGCATTGA /R—TGACGCTTGAACCTGAAATG /P—AGTCCAGTTGGGGAACATCAACAG	Raida and Buchmann (2009)
HERC6	0.96	0.85	Probable E3 ubiquitin-protein ligase	Multi-function Immunity	immune	F—AGGGACAACTTGGTAGACAGAAGAA/R—TGACGCACACACAGCTACAGAGT/P—CAGTGGTCTCTGTGGCT	
IFI44A	0.86	0.9	IFN-induced protein 44-1	Innate Immunity	immune	F—GCTAGTGCTCTTGAGTATCTCCACAA/R—TCACCAGTAACTCTGTATCATCCTGTCT/P—AGCTGAAAGCACTTGAG	
IFIT5	0.91	0.86	Interferon-induced protein with tetratricopeptide repeats 5	Innate Immunity	immune	F—CCGTCAATGAGTCCCTACACATT/R—CACAGGCCAATTTGGTGATG/P—CTGTCTCCAAACTCCCA	

IFNa	NA	NA	Interferon alpha	Multi-function Immunity	immune	F—CGTCATCTGCAAAGATTGGA/R—GGGCGTAGCTTCTGAAATGA/P—TGCAGCACAGATGTACTGATCATCCA	
IgMs	1.50	0.98	Immunoglobulin	Acquired Immunity	immune	F—CTTGGCTTGTTGACGATGAG /R—GGCTAGTGGTGTTGAATTGG /P—TGGAGAGAACGAGCAGTTCAGCA	Raida et al. (2011)
IgT	1.07	4.91	Immunoglobulin tau	Acquired Immunity	immune	F—CAACACTGACTGGAACAACAAGGT/R—CGTCAGCGGTTCTGTTTTGGA/P—AGTACAGCTGTGTGGTGCA	Tadiso et al. 2011
IL-11	0.61	0.98	Interleukin 11	Innate Immunity	immune	F—GCAATCTCTTGCCTCCACTC /R—TTGTCACGTGCTCCAGTTTC /P—TCGCGGAGTGTGAAAGGCAGA	Raida and Buchmann (2008)
IL-15	1.14	1	Interleukin 15	Innate Immunity	immune	F—TTGGATTTTGCCCTAACTGC /R—CTGCGCTCCAATAAACGAAT /P—CGAACAACGCTGATGACAGGTTTTT	Raida et al. (2011)
IL-17D	1.34	2.29	interleukin 17-delta	Innate Immunity	immune	F— CAACAGAAGTGCGAACGATG /R—GATGCCACATCGCATAACAG /P—TGGTCGAGTATCTTTCGTGTGTTTGC	
IL-1B	0.93	0.85	Interleukin 1-beta	Innate Immunity	immune	F—AGGACAAGGACCTGCTCAACT/R—CCGACTCCAACTCCAACACTA/P—TTGCTGGAGAGTGCTGTGGAAGAA	Raida et al. (2011)
IL-8	1.04	2.03	Interleukin 8	Innate Immunity	immune	F—GAGCGGTCAGGAGATTTGTC/R—TTGGCCAGCATCTTCTCAAT/P—ATGTCAGCGCTCCGTGGGT	
IRF1	1.12	NA	Interferon regulatory factor 1	Innate Immunity	immune	F—CAAACCGCAAGAGTTCCTCATT /R—AGTTTGGTTGTGTTTTTGCATGTAG /P—CTGGCGCAGCAGATA	
MHCI	1.06	1.1	Major histocompatibility complex I	Acquired Immunity	immune	F—GCGACAGGTTTCTACCCCAGT /R—TGTCAGGTGGGAGCTTTTCTG /P—TGGTGTCCTGGCAGAAAGACGG	Ingerslev et al. (2009)
MHCII-B	1.00	0.97	Major histocompatibility complex IIβ	Acquired Immunity	immune	F—TGCCATGCTGATGTGCAG /R—GTCCCTCAGCCAGGTCACT /P—CGCCTATGACTTCTACCCCAAACAAAT	Raida and Buchmann (2008)
MMP13	1.02	0.96	Matrix Metallopeptidase 13	Multi-function Immunity	immune	F—GCCAGCGGAGCAGGAA /R—AGTCACCTGGAGGCCAAAGA /P—TCAGCGAGATGCAAAG	Tadiso et al. (2011)
MMP25	0.93	1.1	Matrix Metallopeptidase 25	Multi-function Immunity	immune	F—TGCAGTCTTTTCCCCTTGGAT/R—TCCACATGTACCCACACCTACAC/P—AGGATTGGCTGGAAGGT	
Mx	0.86	0.82	Antiviral protein	Innate Immunity	immune	F—AGATGATGCTGCACCTCAAGTC /R—CTGCAGCTGGGAAGCAAAC /P—ATTCCCATGGTGATCCGCTACCTGG	Eder et al. (2009)
NFX	0.91	NA	Zinc finger NFX1-type	Multi-function Immunity	immune	F—CCACTTGCCAGAGCATGGT/R—CGTAACTGCCCAGAGTGCAAT/P—TGCTCCACCGATCG	
RIG-I	NA	1.07	Retinoic acid-inducible gene I	Innate Immunity	immune	F - ACAGCTGTTACACAGACGACATCA /R - TTTAGGGTGAGGTTCTGTCCGA/P - TCGTGTTGGACCCCACTCTGTTCTCTC	

RSAD	0.95	0.78	Radical S-adenosyl methionine Domain-containing protein 2	Innate Immunity	immune	F—GGGAAATTAGTCCAATACTGCAAAC/R—GCCATTGCTGACAATACTGACACT/P—CGACCTCCAGCTCC	
SAA	1.04	0.7	Serum amyloid protein alpha	Innate Immunity	immune	F—GGGAGATGATTCAGGGTTCCA/R—TTACGTCCCCAGTGGTTAGC/P—TCGAGGACACGAGGACTCAGCA	
SERPIN	4.17	NA	serpin H1-Precursor (heat shock protein 47)	Multi-function Immunity	immune	F—ACTATGACCACTCGAAGATCAACCT/R—CCCATTCGTTGATGGAGTTCA/P—AGGGACAAGAGGAGC	
SRK2	0.92	0.74	Tyrosine-protein kinase FRK	Multi-function Immunity	immune	F—CCAACGAGAAGTTCACCATCAA/R—TCATGATCTCATACAGCAAGATTCC/P—TGTGACGTGTGGTCCT	
STAT1	3.70	0.77	Signal transducer and activator of transcription 1-alpha/beta	Innate Immunity	immune	F—TGTCACCGTCTCAGACAGATCTG/R—TGTTGGTCTCTGTAAGGCAACGT/P—AGTTGCTGAAAACCGG	
TCRa	1.02	NA	T cell receptor alpha chain	Acquired Immunity	immune	F—ACAGCTTGCCTGGCTACAGA/R—TGTCCCCTTTCACTCTGGTG/P—CAGCGCACACAAGGCTAATTCG	
TF	1.46	0.97	Transferrin	Innate Immunity	immune	F—TTCACTGCTGGAAAATGTGG /R—GCTGCACTGAACTGCATCAT /P—TGGTCCCTGTCATGGTGGAGCA	Raida and Buchmann 2009
TNF	0.79	0.8	Tumour necrosis factor	Innate Immunity	immune	F—CCCACCATACATTGAAGCAGATT/R—GGATTGTATTCACCCTCTAAATGGA/P—CCGGCAATGCAAAA	Ching et al 2010
ZAP7	0.91	0.91	Tyrosine-protein kinase (ZAP-70)	Acquired Immunity	immune	F—TCACCTCCGGACCTTTCATT/R—CCATGTGGGAAGCCTTTTCTT/P—TCTTGTATGGTTTTCCTCC	
CFTR-I_v1	NA	NA	Cystic fibrosis transmembrane conductance regulator I	Osmoregulation	osmoregulation	F—GAGCTGTCAGAGAGGAAGTTCTCA /R—GCAGCGACTCTTCAACCTGAT /P—TGGTGCCCGAGGAC	
NKA_a3	1.15	1.85	Na/K ATPase α-3a (freshwater)	Osmoregulation	osmoregulation	F—GGAGACCAGCAGAGGAACAG /R—CCCTACCAGCCCTCTGAGT /P—AAGACCCAGCCTGAAATG	Stefansson et al. 2007
NKAa1-a	1.09	0.72	Na/K ATPase α-1a (freshwater)	Osmoregulation	osmoregulation	F—TGGAATCAAGGTTATCATGGTCACT/R—CCCACACCCTTGGCAATG/P—ATCATCCCATCACTGCGA	
NKAa1-b	1.01	1	Na/K ATPase α-1b (saltwater)	Osmoregulation	osmoregulation	F—GCCTGGTGAAGAATCTTGAAGCT/R—GAGTCAGGGTTCCGGTCTTG/P—CCTCCACCATTTGCTCA	
SHOP21	1.00	0.74	Salmon hyperosmotic protein 21	Osmoregulation	osmoregulation	F—GCGGTAGTGGAGTCAGTTGGA /R—GCTGCTGACGTCTCACATCAC /P—CCTGTTGATGCTCAAGG	
786d16.1P	NA	1.08	39S ribosomal protein L40, mitochondrial precursor	Reference	reference	F—CCCAGTATGAGGCACCTGAAGG /R—GTTAATGCTGCCACCCTCTCAC /P—ACAACAACATCACCA	
COIL	0.88	0.79	Coiled-coil domain-containing protein 84	Reference	reference	F—GCTCATTTGAGGAGAAGGAGGATG /R—CTGGCGATGCTGTTCCTGAG /P—TTATCAAGCAGCAAGCC	
MRPL40	0.83	0.85	39S ribosomal protein L40, mitochondrial precursor	Reference	reference	F—CCCAGTATGAGGCACCTGAAGG /R—GTTAATGCTGCCACCCTCTCAC /P—ACAACAACATCACCA	

ACTB	1.30	0.78	Beta-actin	wound healing/growth/cell structure	stress	F—GAAATCGCCGCACTGGTT/R—CGGCGAATCCGGCTTT/P—TTGACAACGGATCCGGT
CA4	1.27	1.01	Carbonic anhydrase 4	Stress	stress	F—GGTCATTTTGGTTTTGTACACAGTCT/R—CCTAGATATAGCTATCCACGTACTCACCTA/P—TGATACGTGGTATAGAAAAG
EF-2	0.87	0.66	Elongation factor 2	Stress	stress	F—AGGTCACAGCCGCCCTTAG /R—ACACAGTCTCTGTCTGCACACACA /P—CGACTGCGTCTCAGGT
HIF1A	1.00	0.86	Hypoxia-inducible factor 1-alpha	Stress	stress	F—TGGCAAATCTGCCTACGAATT/R—GCAGGCTCTTGGTCACATGA/P—ATCATGCCCTGGACTC
HIF1A_6	0.92	0.83	Hypoxia-inducible factor 1-alpha-like	Stress	stress	F—AGAGGAGGCAGTGCTGTATTCAA/R—GGGACAAGGCCCTCCAAT/P—AGGGCCCTGACCATG
HSC70	1.25	0.86	Heat shock cognate 70	Stress	stress	F—GGGTCACACAGAAGCCAAAAG /R—GCGCTCTATAGCGTTGATTGGT /P—AGACCAAGCCTAAACTA
HSP90a	1.05	0.79	Heat shock protein 90-alpha	Stress	stress	F—AGTACCCTGTTGCACTGAGTTTTAAA/R—GAATGTTTCATTTCCCATTGTTCA/P—ATTGGACTGGTAGATGTGT
HSP90ab1	1.14	1.42	heat shock protein 90-beta	Stress	stress	F—GACACGGTGTTGGGTTGGTT /R—TTGCAGTCAACTCTCCATGCA /P—TCATGTGCAACATAACAT
JUN	0.88	0.77	AP-1 Transcription Factor Subunit	Stress	stress	F—TTGTTGCTGGTGAGAAAACTCAGT/R—CCTGTTGCCCTATGAATTGTCTAGT/P—AGACTTGGGCTATTTAC
KRT8	1.70	1.42	Keratin, type II cytoskeletal 8	Stress	stress	F—CGATTGAGCGGCTGGATAA/R—GCATTGTTTACCTTTGACTTGAATTG/P—CCCCCTTCTCTACTCTCTTGCTCACCATTC
LDHB	0.93	NA	lactate dehydrogenase B	Stress	stress	F—GTCACTGCTCCCATTTTACACTCTAG/R—CCCAAACTCCCTCCCAGATAAC/P—CTGTTCTTAGCTTCCC
PDIA4	0.92	1.02	protein disulfide isomerase associated 4	Stress	stress	F—TGAGGTGCAGGACTTTTTTAAGAA /R—TCGTTGCTCTGTTTCCTGTGA /P—ACATCCTGCCACTGGT
Tuba1a	0.85	0.8	Tubulin alpha-1A chain	wound healing/growth/cell structure	stress	F—CTCTGCTGAGAAGGCCTACCAT /R—AGCAGGCGTTGGTGATGTC /P—AGCAGCTGTCTGTTGC
UBE2Q2	0.87	0.96	ubiquitin-conjugating enzyme E2 Q2-like	wound healing/growth/cell structure	stress	F—GGCAGGACCACTTGAACGTAA /R—AGGCCTGCACTGAACCAGAT /P—TGCTCATTCGGGTGCG
CA054694	0.87	0.86	Mitochondrial ribosomal protein (VAR1)	Viral Disease Related	viral disease associated	F—CCACCTGAGGTACTGAAGATAAGACA /R—TTAAGTCCTCCTTCCTCATCTGGTA /P—TCTACCAGGCCTTAAAG

CD9	0.96	NA	CD9 antigen	Viral Disease Related	viral disease associated	F—CGCCACCACAACCAAGGT /R—TCCTCAGCCTCTTCTTCTTGAAG /P—AGATCCCCAAGACTCTGTCAGACGCCT
RPL6	1.07	2.82	Neoplasm-related protein C140	Viral Disease Related	viral disease associated	F—CGCCACCACAACCAAGGT /R—TCCTCAGCCTCTTCTTCTTGAAG /P—AGATCCCCAAGACTCTGTCAGACGCCT
VHSV-P10	0.96	1.05	VHSV-inducible protein-10	Viral Disease Related	viral disease associated	F—GCAAACTGAGAAAACCATCAAGAA/R—CCGTCAGCTCCCTCTGCAT /P—TGTGGAGAAGTTGCAGGC
VHSVI-P4	0.95	0.72	VHSV-inducible protein-4	Viral Disease Related	viral disease associated	F—TGGCTTCCCACATTGCAA /R—CCTCCTCCCCCCTGCAT /P—AGATGGAGACAGGAATG

Appendix B Abstracts of publications resulting from graduate course work

1. Lennox, RJ, **Chapman, JM**, Souliere, CM, Tudorache, C, Wikelski, M, Metcalfe, JD, and Cooke, SJ. 2016. Conservation physiology of animal migration. Conservation Physiology 4:10.1093/consphys/cov072.

Migration is a widespread phenomenon among many taxa. This complex behaviour enables animals to exploit many temporally productive and spatially discrete habitats to accrue various fitness benefits (e.g. growth, reproduction, predator avoidance). Human activities and global environmental change represent potential threats to migrating animals (from individuals to species), and research is underway to understand mechanisms that control migration and how migration responds to modern challenges. Focusing on behavioural and physiological aspects of migration can help to provide better understanding, management and conservation of migratory populations. Here, we highlight different physiological, behavioural and biomechanical aspects of animal migration that will help us to understand how migratory animals interact with current and future anthropogenic threats. We are in the early stages of a changing planet, and our understanding of how physiology is linked to the persistence of migratory animals is still developing; therefore, we regard the following questions as being central to the conservation physiology of animal migrations. Will climate change influence the energetic costs of migration? Will shifting temperatures change the annual clocks of migrating animals? Will anthropogenic influences have an effect on orientation during migration? Will increased anthropogenic alteration of migration stopover sites/migration corridors affect the stress physiology of migrating animals? Can physiological knowledge be used to identify strategies for facilitating the movement of animals? Our synthesis reveals that given the inherent challenges of migration, additional stressors derived from altered environments (e.g. climate change, physical habitat alteration, light pollution) or interaction with human infrastructure (e.g. wind or hydrokinetic turbines, dams) or activities (e.g. fisheries) could lead to long-term changes to migratory phenotypes. However, uncertainty remains because of the complexity of biological systems, the inherently dynamic nature of the environment and the scale at which many migrations occur and associated threats operate, necessitating improved integration of physiological approaches to the conservation of migratory animals.

2. **Chapman, JM**, Algera, D, Dick, M, Hawkins, EE, Lawrence, MJ, Lennox, RJ, Rous, AM, Souliere, CM, Stemberger, HLJ, Struthers, DP, Vu, M, Ward, TD, Zolderdo, AJ, and Cooke, SJ. 2015. Being relevant: Practical guidance for early career researchers interested in solving conservation problems. Global Ecology and Conservation 4:344-348.

In a human-altered world where biodiversity is in decline and conservation problems abound, there is a dire need to ensure that the next generation of conservation scientists have the knowledge, skills, and training to address these problems. So called "early career researchers" (ECRs) in conservation science have many challenges before them and it is clear that the status quo must change to bridge the knowledge–action divide. Here we identify thirteen practical strategies that ECRs can employ to become more relevant. In this context, "relevance" refers to the ability to contribute to solving conservation problems through engagement with practitioners, policy makers, and stakeholders. Conservation and career strategies outlined in this article include the following: thinking 'big picture' during conservation projects; embracing various forms of knowledge; maintaining positive relationships with locals familiar with the conservation issue; accepting failure as a viable (and potentially valuable) outcome; daring to be creative; embracing citizen science; incorporating interdisciplinarity; promoting and practicing pro-environmental behaviours; understanding financial aspects of conservation; forming collaboration from the onset of a project; accepting the limits of technology; ongoing and effective networking; and finally, maintaining a positive outlook by focusing on and sharing conservation success stories. These strategies move beyond the generic and highlight the importance of continuing to have an open mind throughout the entire conservation process, from establishing one's self as an asset to embracing collaboration and interdisciplinary work, and striving to push for professional and personal connections that strengthen personal career objectives.

Appendix C : Abstracts of publications resultant from doctoral studies not included in thesis

1. Brownscombe, JW, Griffin, LP, **Chapman, JM,** Morley, D, Acousta, A, Crossin, GT, Iverson, SJ, Adams, AJ, Cooke, SJ, Danylchuk, AJ. 2019. A practical method to account for variation in detection range in acoustic telemetry to accurately quantify the spatial ecology of aquatic animals. Methods in Ecology and Evolution. 11:82-94.

1. Acoustic telemetry is a popular tool for long-term tracking of aquatic animals to describe and quantify patterns of movement, space use, and diverse ecological interactions. Acoustic receivers are imperfect sampling instruments, and their detection range (DR; the area surrounding the receiver in which tag transmissions can be detected) often varies dramatically over space and time due to dynamic environmental conditions.
2. Therefore, it is prudent to quantify and account for variation in DR to prevent telemetry system performance from confounding the understanding of real patterns in animal space use. However, acoustic receiver DR consists of a complex, dynamic, three-dimensional area that is challenging to quantify.
3. Although quantifying the absolute DR of all receivers is infeasible in the context of most acoustic telemetry studies, we outline a practical approach to quantify relative variation among receiver DR over space and time. This approach involves selecting a set of sentinel receivers to monitor drivers of variation in detection range. Each sentinel receiver is subject to a range testing procedure to estimate detection efficiency (DE; the proportion of total transmissions detected by the receiver), at a range of distances from the receiver, to derive the maximum range (MR; distance from the receiver where DE is 5%) and Midpoint (distance from the receiver where DE is 50%). A reference transmitter is then placed at the Midpoint, providing a standardized measure of long-term variation in DE, with each station having similar freedom of variance. Variation in reference tag DE is then combined with MR to calculate a DR correction factor (DRc). A modelling approach is then used to estimate DRc for all receivers in the array at spatial and temporal scales of ecological interest, which can be used to correct animal detection data in various ways.
4. We demonstrate this method with a hypothetical dataset, as well as empirical data from an acoustic telemetry array to delineate spatio-temporal patterns of fish habitat use. This is a flexible and practical approach to account for variation in acoustic receiver performance, allowing more accurate spatial and temporal patterns in aquatic animal spatial ecology to be revealed.

2. Twardek, WM, **Chapman, JM**, Miller, KM, Meere, MC, Li, S, Kaukinen, KH, Danylchuk AJ, Cooke, SJ. 2019. Evidence of a hydraulically challenging reach serving as a barrier for the upstream migration of infection-burdened adult steelhead. Conservation Physiology doi.org/10.1093/conphys/coz023.

Anadromous fishes such as steelhead trout, Oncorhynchus mykiss, are exposed to a suite of infectious agents and migratory challenges during their freshwater migrations. We assessed infectious agent load and richness and immune system gene expression in gill tissue of Bulkley River (British Columbia, CA) steelhead captured at and upstream of a migratory barrier to evaluate whether infectious burdens impacted migration success. We further considered the potential influences of water temperature, sex and fish size on host infectious agents and transcription profiles. There were eight infectious agents detected in steelhead gill tissue, with high prevalence of the bacteria Candidatus Branchiomonas cysticola (80%) and Flavobacterium psychrophilum (95%) and the microparasite Sphaerothecum destruens(53%). Fish sampled at the falls had significantly greater relative loads of Ca. B. cysticola and F. psychrophilum, higher infectious agent richness and differential gene expression compared to fish captured upstream. Flavobacterium psychrophilum was only associated with immune gene expression (particularly humoral immunity) of fish sampled at the falls, while water temperature was positively correlated with genes involved in the complement system, metabolic stress and oxidative stress for fish captured upstream. This work highlights interesting differences in agent–host interactions across fisheries and suggests that hydraulic barriers may reduce the passage of fish with the heaviest infectious agent burdens, emphasizing the selective role of areas of difficult passage. Further, this work expands our knowledge of infectious agent prevalence in wild salmonids and provides insight into the relationships between infectious agents and host physiology.

3. Lennox, RJ, **Chapman, JM**, Twardek, WM, Broell, F, Bøe, K, Whoriskey, FG, Fleming, IA, Robertson, MJ, Cooke, SJ. 2019. Biologging in combination with biotelemetry reveals behavior of Atlantic salmon following exposure to capture and handling stressors. Canadian Journal of Fisheries and Aquatic Sciences. doi.org/10.1139/cjfas-2018-0477.

We investigated the response of Atlantic salmon (Salmo salar) to capture and handling stressors by analyzing fine-scale locomotor activity using accelerometer data loggers and broader-scale movements by tracking migration with radiotelemetry. Half the sample population was exposed to experimental exercise and air exposure and released with a control group to simulate fisheries handling. All but two of the surviving fish (both in the treatment group) returned to the counting fence to resume the 2016 spawning migration (survival = 86%–91%). There were no differences in postrelease locomotor activity, measured by an index of total body action (jerk), between control and treatment salmon ($p = 0.81$). Comparison of mean time to return to the counting fence against a null model revealed that treatment salmon were significantly delayed in returning to the counting fence ($p < 0.01$), whereas control fish were not ($p = 0.24$). Both the abiotic environment and human interactions influenced locomotor activity of the migratory fish and synchrony of the migration with untreated conspecifics.

4. Cook, KV, Reid, AJ, Patterson, DA, Robinson, KA, **Chapman, JM**, Hinch, SG, Cooke, SJ. 2019. A synthesis to understand responses to capture stressors among fish discarded from commercial fisheries and options for mitigating their severity. Fish and Fisheries. 20:25-43.

Discarding non-target fish from commercial fisheries is controversial and has been a persistent concern for fisheries managers globally. Discard management strategies typically begin by understanding mortality rates among discarded fish, a challenging task given the dynamic, highly context-specific nature of fisheries. An alternative is to develop our knowledge of how stressors operate by first understanding the causes of mortality that drive this context dependence. Particularly relevant to mitigation efforts is an understanding of how fish respond to the physical factors of fishing, such as the gear itself and methods of fishing and handling the gear. We provide a synthesis of how commercial fishing methods may influence discard mortality and outline means by which capture-induced stress and injury can be mitigated for common commercial gear types, emphasizing method variants or alternatives during capture, handling, and release that could improve survival. This synthesis identifies exhaustion and injury as the most detrimental and ubiquitous stressors experienced by discarded fish, with few options for mitigating their effects. Trawls and hanging net fisheries are identified as the most harmful gears for by-catch, characterized by high stress regardless of method variants and limited options for mitigation. Irrespective of gear type and type of stressor, minimizing durations of capture and handling and encouragement of good handling behaviour (e.g., during landing and sorting) will reduce the magnitude of stress and injury in fish, and ultimately increase survival.

5. Brooks, JL, **Chapman, JM**, Barkley, AN, Kessel, ST, Hussey, NE, Hinch, SG, Patterson, DA, Hedges, KJ, Cooke, SJ, Fisk, AT, Gruber, SH, Nguyen, VM. 2018. Biotelemetry informing management: case studies exploring successful integration of biotelemetry data into fisheries and habitat management. Canadian Journal of Fisheries and Aquatic Sciences. doi.org/10.1139/cjfas-2017-0530.

Biotelemetry data have been successfully incorporated into aspects of fishery and fish habitat management; however, the processes of knowledge mobilization are rarely published in peer-reviewed literature but are valuable and of interest to conservation scientists. Here, we explore case examples from the Ocean Tracking Network (OTN), including Pacific salmon (*Oncorhynchus* spp.) in British Columbia, Canada; Greenland halibut (*Reinhardtius hippoglossoides*) in Cumberland Sound, Canada; and lemon sharks (*Negaprion brevirostris*) in Florida, USA, to document key processes for science integration. Typical recommendations documented in the literature (e.g., co-production of knowledge, transdisciplinary methodologies, applied research questions) were recorded to have had successful fisheries management integration, although we documented some exceptions. In each case, it was early, active, and ongoing communication outside of traditional science communication and the visual evidence of fish movement that were

critical in engaging all parties with a vested interest. Networks offer forums for knowledge sharing on lessons learned and development of skills to engage in active communication. Greater investments and attention to develop these skills are needed to foster positive and active relationships that can impart real change in management and conservation.

6. **Chapman, JM***, Moore, JS*, Mazerolle, MJ, Harris, LN, Taylor, EB. 2018. Premature alarm on the impacts of climate change on Arctic Char in Lake Hazen. Nature Communications. Doi:10.1038.s41467-018-06479-5

A recent paper by Lehnherr et al.[1] reported on a long-term study of the ecological impacts of climate change in the world's largest high Arctic lake: Lake Hazen on Canada's Ellesmere Island. The paper made a convincing case that climate change has had a dramatic and significant impact on the watershed of this important freshwater ecosystem. Some of these changes have clearly impacted the ecology of Lake Hazen. We disagree, however, with the conclusion that such ecological changes have resulted in a significant decline in the condition of Arctic Char (*Salvelinus alpinus*) from the lake based on the presented data. It is critical to examine the evidence for changes in the condition of Arctic Char given the importance of this species to communities throughout Canada's Arctic as a valued food resource and because changes to condition could impact its management.

7. Lizee, TW, Lennox, RJ, Ward, TD, Brownscombe, JW, **Chapman, JM**, Danylchuk, AJ, Nowell, LB, Cooke, SJ. 2018. Influence of landing net mesh type on handling time and tissue damage of angled brook trout. North American Journal of Fisheries Management 38:76-83.

Recreational catch-and-release angling is a popular activity. Anglers often use landing nets to shorten fight times, reduce stress on the line and rod, restrict fish movement to facilitate dehooking of the fish, and protect fish from undue harm caused by handling or dropping. Landing nets are constructed using a variety of netting materials that could have varied consequences when coming in contact with fish. Salmonids are among the most targeted fishes in the world, but little is known about how landing nets contribute to postcapture tissue damage. We compared handling time and instances of fin fraying, scale loss, and mucus loss sustained by Brook Trout *Salvelinus fontinalis* landed by four net mesh types (i.e., large, knotless rubber mesh; knotless nylon micromesh; large, knotted polypropylene mesh; and small, knotless rubber-coated nylon mesh) or by using bare wet hands in a recreational fishery. The knotted polypropylene mesh resulted in the greatest extent of fin fraying, whereas the bare wet hands method, knotless nylon micromesh, and rubber-coated nylon mesh resulted in the most scale loss. Interestingly, extended handling times were noted for several mesh types (i.e., knotless nylon micromesh and rubber-coated nylon mesh) relative to bare wet hands because of hook

entanglement in the netting material. However, using bare wet hands to land Brook Trout resulted in higher odds of the fish being dropped into the bottom of the boat. We concluded that the large, knotless rubber mesh was the least damaging to Brook Trout. Changes to angler practices, such as using appropriate landing tools, can benefit fish welfare in catch-and-release fisheries.

8. Patterson, DA, Robinson, KA, Lennox, RJ, Nettles, TL, Donaldson, LA, Eliason, EJ, Raby, GD, **Chapman, JM**, Cook, KV, Donaldson, MR, Bass, AL, Drenner, SM, Reid, AJ, Cooke, SJ, and Hinch, SG. 2017. Review and evaluation of fishing-related incidental mortality for pacific salmon. DFO Canadian Science Advisory Secretariate Research Document. 2017/010.ix+ 155p.

The number of fish that encounter fishing gear is greater than the number of fish retained as catch. The proportion of this difference that die from the encounter is defined as fishing-related incidental mortality (FRIM). FRIM estimates are required for improved stock assessments, but they are difficult to attain and vary across fisheries. To cope with this challenge we review and evaluate the scientific knowledge on FRIM. First, we review the different mortality components of FRIM (i.e., avoidance, escape, depredation, drop-out, on-board, short-term release, and delayed mortality) in relation to how a fish responds to different aspects of a fishery encounter (e.g., handling). To better understand how fish respond to a fishing encounter, different fishing factors (e.g., gear type) that act in consort with extrinsic (e.g., water temperature) and intrinsic (e.g., fish size) factors elicit different fish responses that can lead to the different types of mortality (e.g., acute) were examined. A fish response to a stressor (i.e., factor) is a combination of the magnitude and duration of the stressor itself. The initial fish response includes acute physiological stress and injury, followed by behaviour changes, chronic stress, and increased risk of infection. Next, a review was done to provide an up-to-date accounting of the mortality rate information available on estimates of FRIM for Pacific salmon (Oncorhynchus spp.). We created an interactive and searchable catalogue of evidence from predominantly primary literature using standardized systematic mapping protocols, with a focus on coding information to determine study reliability and relevance. Next, we synthesize the factor and mortality information to provide recommendations on the use of five major mortality risk factors that are linked to FRIM. Each factor (capture, handling, injury, water temperature, and predators) is scaled to a mortality risk to provide guidance on evaluating FRIM estimates. The recommendations from this work are focussed on addressing the current knowledge gaps and examining FRIM in broader physiological and ecological context. Ideas for future work include researching cumulative impacts, sub-lethal effects, drop-off mortality, and predation. We have chosen a fish-centric hybrid approach that focusses first on understanding factors that drive mortality, and then on mortality estimates. As such, this paper is not meant as the definitive guide on FRIM but a transparent, defensible, and rigorous evaluation of the primary evidence base for making future decisions about FRIM. Further guidance on how to use the information herein is part of an accompanying CSAS research document.

9. Birnie-Gauvin, K, Walton, S, Delle Palme, CA, Manouchehri, BA, Venne, S, Lennox, RJ, **Chapman, JM**, Bennett, JR, and Cooke, SJ. 2017. Conservation physiology can inform threat assessment and recovery planning processes for threatened species. Endangered Species Research 32:507-513.

Conservation physiology has emerged as a discipline with many success stories. Yet, it is unclear how it is currently integrated into the activities of the IUCN and other bodies which undertake international, national, or regional species threat assessments and work with partners to develop recovery plans. Here we argue that conservation physiology has much to offer for the threat assessment process and we outline the ways in which this can be operationalized. For instance, conservation physiology is effective in revealing causal relationships and mechanisms that explain observed patterns, such as population declines. Identifying the causes of population declines is a necessary precursor to the design of actions to reverse or mitigate such threats. Conservation physiology can also identify complex interactions and support modeling activities that consider emerging threats. When a population or species is deemed threatened and recovery plans are needed, physiology can be used to predict how organisms will respond to the conservation intervention and future threats. For example, if a recovery plan was focused on translocation, understanding how to safely translocate organisms would be necessary, as would ensuring that the recipient habitat provides the necessary environmental characteristics to meet the fundamental physiological needs/tolerances of that organism. Our hope is that this paper will clarify ways in which physiological data can make an important contribution to the conservation activities of bodies like the IUCN that are engaged in threat assessment and recovery of endangered organisms. Although we focus on activities at the international scale, these same concepts are relevant and applicable to national and regional bodies.

10. Brownscombe, JW, Danylchuck, AJ, **Chapman, JM**, Gutowsky, LFG, and Cooke, SJ. 2017. Best practices for catch-and-release recreational fisheries – angling tools and tactics. Fisheries Research DOI: 10.1016/j.fishres.2016.04.018.

Catch-and-release angling is an increasingly popular conservation strategy employed by anglers voluntarily or to comply with management regulations, but associated injuries, stress and behavioural impairment can cause post-release mortality or fitness impairments. Because the fate of released fish is primarily determined by angler behaviour, employing ‘best angling practices’ is critical for sustainable recreational fisheries. While basic tenants of best practices are well established, anglers employ a diversity of tactics for a range of fish species, thus it is important to balance science-based best practices with the realities of dynamic angler behaviour. Here we describe how certain tools and tactics can be integrated into recreational fishing practices to marry best angling practices with the realities of angling. While the effects of angling practices vary considerably across contexts and conditions, we also outline available methods for

assessing fish condition by examining physical injuries and reflexes, which enable recreational anglers to make educated real-time decisions related to angling practices, as well as when, where, and whether to release captured fish based on their probability of survival. In cases where fish are in poor condition, there are recovery tactics available that can improve survival, although this is among the most understudied aspects of angling practices.

11. Kerr SM, Ward TD, Lennox RJ, Brownscombe JW, **Chapman JM**, Gutowsky LFG, Logan JM, Twardek WM, Elvidge CK, Danylchuk AJ, Cooke SJ. 2017. Influence of hook type and live bait on the hooking performance of inline spinners in the context of catch-and-release brook trout Salvelinus fontinalis fishing in lakes. Fisheries Research 186:642-647.

The objective of catch-and-release angling is for the fish to survive with minimal fitness consequences. However, fish survival can be compromised by a number of factors, especially anatomical hooking location. To evaluate whether hook type or bait influence hooking outcomes, we tested different combinations of hook (treble or single siwash hooks) and bait (hook tipped with worm or no worm) while angling for brook trout (*Salvelinus fontinalis*) with inline spinner-style fishing lures. The study was conducted at spring water temperatures (~20 °C) in small lakes stocked with trout in southwestern Quebec, Canada. Incidences of hooking in the interior of the mouth (i.e. internal hooking) were uncommon (19%), did not differ significantly between hook types or bait treatments, and occurred independently of fish size. Reflex impairments after hook removal were not related to hook or bait treatment. Short-term mortality was quantified with 24 h holding in net pens and was determined to be infrequent for all treatment groups (treble/worm: 6%; treble/no worm: 5%; single/worm: 2%; single/no worm: 0%). Although no fish were hooked in the gills, esophagus, stomach, odds of mortality increased by 14.21 when fish were hooked internally, which is consistent with the position that hook placement is an important predictor of the fate of fish released by anglers. However, our finding that neither hook nor bait type significantly increased the odds of internal hooking, bleeding, reflex impairment, or mortality in this study suggests that restrictions imposed on the use of baited lures or certain hook types attached to lures when fishing may have little influence on short-term catch-and-release mortality of brook trout at these temperatures.

12. Cooke, SJ, **Chapman, JM**, and Vermaire, JC. 2015. On the apparent failure of silt fences to protect freshwater ecosystems from sedimentation: a call for improvements in science, technology, training, and compliance monitoring. Journal of Environmental Management 164:67-73.

Excessive sedimentation derived from anthropogenic activities is a main factor in habitat and biodiversity loss in freshwater ecosystems. To prevent offsite movement of soil

particles, many environmental regulatory agencies mandate the use of perimeter silt fences. However, research regarding the efficiency of these devices in applied settings is lacking, and fences are often ineffective due to poor installation and maintenance. Here, we provide an overview of the current state of research regarding silt fences, address the current culture surrounding silt fence installation and maintenance, and provide several recommendations for improving the knowledge base related to silt fence effectiveness. It is clear that there is a need for integrated long-term (i.e., extending from prior to fence installation to well after fence removal) multi-disciplinary research with appropriate controls that evaluates the effectiveness of silt control fences. Through laboratory experiments, in silico modelling and field studies there are many factors that can be experimentally manipulated such as soil types (and sediment feed rate), precipitation regimes (and flow rate), season, slope, level of site disturbance, fence installation method, type of fence material, depth of toe, type and spacing of support structures, time since installation, level of inspection and maintenance, among others, that all require systematic evaluation. Doing so will inform the practice, as well as identify specific technical research needs, related to silt fence design and use. Moreover, what constitutes "proper" installation and maintenance is unclear, especially given regional- and site-level variation in precipitation, slope, and soil characteristics. Educating and empowering construction crews to be proactive in maintenance of silt fencing is needed given an apparent lack of compliance monitoring by regulatory agencies and the realities that the damage is almost instantaneous when silt fences fail. Our goal is not to dismiss silt fences as a potentially useful tool. Instead, we question the way they are currently being used and call for better science to determine what factors (in terms of fence design, installation and site-characteristics) influence effectiveness as well as better training for those that install, maintain and inspect such devices. We also encourage efforts to "look beyond the fence" to consider how silt fences can be combined with other sediment control strategies as part of an integrated sediment control program.

Appendix D : Abstracts of publications resultant from professional development related work at Carleton University

1. **Chapman, JM,** Schott, S. 2020. Knowledge co-evolution: generating new understanding through bridging and strengthening distinct knowledge systems and empowering local knowledge holders. Sustainability Science. https://doi.org/10.1007/s11625-020-00781-2

The effective and appropriate bridging of Western science with traditional or Indigenous knowledge is an ongoing discussion in the literature and in practice. The discourse transitioned from separate knowledge system to knowledge integration and most recently to knowledge co-production. We argue it is the moral and ethical responsibility of Western scientists working in and with Indigenous communities to make a concerted effort to collectively create mutually advantageous new knowledge while strengthening traditional knowledge and considering the normative impacts of Western science methods. Our knowledge coevolution framework provides guidance for achieving this in a flexible manner that can be applied to an array of research programs. Project governance structure, steps for implementation, checks and balances, and challenges are presented within the context of research project execution. We then illustrate application of the model throughout a harvest study conducted in Gjoa Haven, Nunavut, Canada.

2. Raby, G, **Chapman, JM**, de Bruijn, R, Eliason, EJ, Elvidge, CK, Hasler, CT, Madliger, CL, Nyboer, EA, Reid, AJ, Roche, DG, Rytwinski, T, Ward, TD, Wilson, ADM, Cooke, SJ. 2020. Teaching post-secondary students in ecology and evolution: Strategies for early-career researchers. Ideas in Ecology and Evolution 13:doi.org/10.24908/iee.2020.13.3.e

Teaching can be a rewarding, yet challenging, experience for early career researchers (ECRs) in fields like ecology and evolution. Much of this challenge arises from the reality that ECRs in ecology and evolution typically receive little, if any, pedagogical training or advice on how to balance teaching, research (which can include extended field work), and other demands on their time. Here, we aim to provide accessible, pragmatic advice for ECRs in ecology and evolution who are given the opportunity to teach (as instructor of record). The advice is based on the authors' collective experiences teaching in ecology and evolution as ECRs and is meant to help ECRs address two challenges: a) balancing the demands of teaching against one's research, service, and personal life, and b) being effective in the classroom while doing so. The guidance we provide includes practical steps to take when teaching for the first time, including carefully refining the syllabus (course planning), adopting 'non-traditional' teaching methods, and taking advantage of free teaching resources. We also discuss a range of 'soft skills' to consider including guarding against imposter syndrome (i.e., self-doubt and fear of being exposed

as a fraud), managing expectations, being empathetic, compassionate, authentic, and fostering an inclusive classroom. Lastly, we emphasize the need to focus on developing students' critical thinking skills, integrating research and teaching where possible, and setting limits on class preparation time to maintain balance with your research and personal life. Collectively, we hope the examples provided herein offer a useful guide to ECRs new to teaching.

3. Schott, S, Qitsualik, J, van Coeverden de Groot, P, Okpakok, S, **Chapman, JM**, Lougheed, S, Walker, V. In Press. Operationalizing knowledge coevolution: Towards a Sustainable Fishery for Nunavummiut. Arctic Science. XXX-XXX.

Knowledge coevolution is the process through which information is generated by joining knowledge systems in an inclusive and iterative way to facilitate self-determination of communities and promote cultural resilience. A central and practical component of this framework is the fostering of progress towards improved co-management and community led research. Here we illustrate a knowledge coevolution framework in the context of a major five-year genomics and food security fishery research project in Gjoa Haven, Nunavut. We highlight the process, changes in research objectives, logistical requirements, mutual benefits, and challenges associated with Northern collaborative research, and what lessons we have learned from the process. Knowledge coevolution could be linked to more inclusive and effective fishery co-management in Nunavut and possibly elsewhere. Further, the research process appears to have reinforced Indigenous Knowledge and western science without merging these distinct knowledge systems. Here we strive to provide readers with concrete examples of knowledge coevolution and encourage research groups to incorporate and improve these practices in future projects and in adaptive fishery co-management. We further call on funding agencies to place more value, and thus budgetary priority, on activities related to ongoing consultation, engagement, dissemination, and implementation of project outcomes.

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