Fisheries gear and biological context drive fishing-related incidental mortality in Pacific salmon spawning migrations

by

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Fisheries gear and biological context drive fishing-related incidental mortality in Pacific salmon spawning migrations

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Abstract

Most fisheries inadvertently capture non-target fish species or populations. In addition, fish may interact with and escape fishing gears without being observed. Regardless of whether fish are released or escape, negative impacts from interacting with fishing gears (including stress and injury) may lead to fishing-related incidental mortality (FRIM). However, FRIM is typically difficult to observe and therefore is not easily or well quantified. Further, FRIM is contingent on many biological and environmental factors, which have received little consideration in fisheries management. Specifically, infectious disease is expected to mediate FRIM, but has been studied little in this context. Because of their life histories, Pacific salmon are both particularly vulnerable to FRIM and ideal model animals for its study.

This dissertation sought to further the knowledge regarding FRIM in Pacific salmon during their freshwater spawning migrations by conducting *in situ* studies of capture methods and pertinent biological factors. Salmon received radio-frequency tags following exposure to fisheries gear so that migration survival could be determined. Quantitative PCR was performed on non-lethal biopsies to determine the presence and load of infectious agents, as well as immune system function. Sockeye salmon escaping or released from gillnets experienced elevated FRIM, slowed migration, and reduced spawning success. Although the impact of gears was consistent between years, the incidence of FRIM was reduced for mature fish. The high impact of severe injuries was indicated by low migration survival and the elevated expression of acute phase response genes. Mature Chinook salmon with infections of a blood-borne protozoan parasite experienced elevated mortality, regardless of a simulated fisheries gear exposure.

My dissertation indicates that salmon interacting with gillnets in freshwater experience significant FRIM, but biological factors can override these impacts. Future research should seek to estimate the rate of gillnet escapes, identify gears that minimize physical damage, localize the time in migrations when maturity confers resilience, and use repeated sampling to better define the role of infectious agents in FRIM. Fisheries managers could reduce FRIM by prioritizing lower impact gears, promoting fisheries in locations where salmon are more mature and pathogens are sparse, and conducting regular monitoring of infectious agents.

Lay Summary

During spawning migrations, Pacific salmon may interact with fishing gear in freshwater. Damage and stress from such an event may result in death days to weeks later, but this is difficult to observe. For this dissertation, I conducted original studies of fisheries impacts on salmon with specific focus on the impacts of different gears and fishing techniques, the role of maturity in mediating impact, and how infection might influence or be influenced by fisheries interactions. I found that:

- nets that entangle fish (gillnet) and cause damage to skin had a greater impact than nets that encircle fish (beach seine),
- more mature salmon were less impacted by fisheries interactions, and
- condition at capture (e.g., infection) can be more important than capture itself.

Gillnets cause elevated mortality, but factors including maturity and infection modulate mortality. I recommend that managers prioritize lower impact gears and consider biological factors when estimating fishing related mortality.

Preface

A version of Chapter 2 has been published as "Bass, A.L., Hinch, S.G., Patterson, D.A., Cooke, S.J., Farrell, A.P. (2018) Location-specific consequences of beach seine and gillnet capture on upriver-migrating sockeye salmon migration behavior and fate. Canadian Journal of Fisheries and Aquatic Sciences, DOI: 10.1139/cjfas-2017-0474". I performed the field work with the UBC Pacific Salmon Ecology and Conservation Laboratory (Hinch Lab), D. Patterson, and others. Field work was conducted on the territory of and with fishing assistance from the Kwantlen, Peters, and Skeetchestn First Nations. DNA analysis was conducted by T. Beacham's laboratory at the Fisheries and Oceans Canada (DFO) Pacific Biological Station. A. Bass conducted all data analysis and wrote the original draft of the manuscript. All co-authors contributed to writing and revision. This work was conducted under Animal Care and Use Program certificate A12-0250.

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List of Acronyms

AFT Accelerated Failure Time. 24

AICc Akaike's Information Criterion. 24

AME average marginal effect. 82

ANOVA Analysis of Variance. 81

APPs acute phase proteins. 11

APR acute phase response. 11

CJS Cormac Jolly Seber survival models. 23

CMR cell-mediated response. 11

DFO Fisheries and Oceans Canada. 17

FRIM fishing-related incidental mortality. 1

GCss Gates Creek sockeye salmon. 37

GLMs Generalized Linear Models. 24

GSE gross somatic energy. 38

HTqPCR high-throughput, quantitative polymerase chain reaction. 13

NTFI non-take fisheries interactions. 35

PIT passive integrated transponder. 38

RIB relative infectious burden. 60

VDD viral disease development. 78

Glossary

aerobic scope the maximum amount of oxygen available for any aerobic activity above routine. 18

anadromous conducting migrations between fresh and saltwater. 3

apoptosis programmed cell death, a strategy used in immune defense. 6

bycatch unwanted animals caught while fishing for another species. 1

chronic stress a negative physiological response to the presence of a persistent stressor . 3

cytokine a small protein that is involved in cell signaling (e.g., interleukin). 11

delayed mortality mortality occurring within days to weeks after a fisheries interaction. 1

escaped fish autonomously escaping from gears before being landed. 1

escapement the amount of a salmon population not caught by fisheries and returning to spawning habitat. 13

eustress minor, acute stress that has a stimulatory rather than negative effect. 3

fisheries interactions anytime a fish makes contact with fishing gear. 3

fishing fence a structure placed in a river that allows water to flow through, but guides upstream migrating salmon into a trap. 38

gillnet a into which fish swim and become entangled around the head, body, mouth, or fins. 1

immediate mortality mortality occurring during fisheries interaction. 1

immunostimulatory enhancing function of the immune system. 9

immunosuppression reduced function of the immune system. 1

infectious agents viruses, bacteria, fungi, protozoa, myxozoa, and helminths that cause disease in fish. 2

intrinsic factors pertaining to a fish that influence FRIM. 2

kype the protruding jaw of a mature male salmon, a secondary sexual characteristic useful for competition in spawning areas. 7

lymphocytes white blood cells, including natural killer, B- and T- cells, that have roles in immune function. 6

non-retention a fisheries interaction followed by either release or escape. 16

prespawn mortality when salmon successfully migrate to spawning areas but die before spawning. 3

redd nest where salmonids deposit eggs, constructed of gravel and cobbles. 7

released fish manually removed from gear and returned to water. 1

seine a net drawn around fish to prevent escape in a pelagic area (purse seine), or into shore (beach seine). 1

semelparous reproducing only once in a lifetime. 2

short-term mortality mortality occurring within 24 hrs following a fisheries interaction. 1

sub-lethal effect an impact that results in alterations to behavior or condition that are not lethal but may be detrimental. 12

telemetry the use of transmitting instruments to follow animal movements. 12

thermochron a small archival logger that records temperature. 20

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Hope I got everybody! Even though this dissertation must be written in first person, I clearly did not accomplish this alone.

Dedication

This dissertation is dedicated to all the salmon I harassed in the name of science. I hope that their sacrifice contributes to a positive future for their relatives and that they did not suffer in vain.

Chapter 1

Introduction

1.1 Fishing related incidental mortality

Almost all fisheries inadvertently capture non-target animals, which are collectively referred to as "bycatch". For example, the angler targeting salmon may hook a trout, the trawl fishery in Alaska targeting groundfish may catch juvenile halibut, longlines intended for fin fish may hook seabirds, and a purse seine set for tuna may encircle dolphins. Although bycatch may sometimes be released without any significant detriment, it can also experience physiological stress, serious injury, sublethal effects with downstream and delayed fitness consequences, or even mortality during or following the capture experience (DAVIS 2002).

While fauna such as turtles, marine mammals, and seabirds typically receive greater attention for conservation initiatives to reduce bycatch, non-target fish species constitute the largest component of bycatch and occur in most fisheries (ALVERSON *et al.* 1994). In 1994, the estimate of global bycatch of fishes (not including mammals, birds, etc.) was 20 – 40 million metric tons, or one-quarter of the worldwide fisheries catch (ALVERSON *et al.* 1994), but the amount of discarded bycatch has decreased slightly since then (PAULY and ZELLER 2016). Although fisheries bycatch is typically considered a problem in marine environments, it also occurs (and is understudied) in freshwater (RABY *et al.* 2011).

Depending upon the capture experience, bycatch fish can be categorized as immediate mortality, released, or escaped. As the name suggests, immediate mortality encompasses fish that expire during the capture process or are intentionally retained. Released fish, also known as discards, are removed from fishing gear by the fisher and returned to the water. Escaped fish, also known as drop-outs, encountered fishing gear but escaped without the aid of a fisher, before the gear was landed. Escaped fish can include the target species of a fishery as in the case of sockeye salmon, *Oncorhynchus nerka*, escaping the Bristol Bay gillnet fishery (BAKER and SCHINDLER 2009). For both the released and escaped categories, fish are alive at the time of release although their condition (and subsequent survival) can vary dramatically. However, the single study that experimentally compared released and escaped fish indicated that this distinction may be meaningful, since escaped fish experienced better survival (THOMPSON *et al.* 1971).

A fish that has been released or escaped from a fishery encounter may survive, or experience short-term mortality (within 24 h of encounter) or delayed mortality (days to weeks after encounter). Both short-term and delayed mortality, as well as immediate mortality, are encompassed by fishing-related incidental mortality (FRIM), a term coined by PATTERSON *et al.* (2017a). Short-term FRIM is usually caused by asphyxiation (KOJIMA *et al.* 2004), pH disequilibrium following prolonged burst swimming (WOOD *et al.* 1983), or severe injury (CHOPIN and ARIMOTO 1995). Delayed FRIM may result from immunosuppression caused by the stress response to capture (PICKERING and POTTINGER 1989), pathogen infections due to

disruption of the integument (SVENDSEN and BØGWALD 1997), and physiological imbalance caused by the loss of barrier function provided by the integument (MATEUS *et al.* 2017; OLSEN *et al.* 2012). Regardless of whether FRIM is short-term or delayed, mortality rates in the natural environment are rarely known since fish may appear healthy at release, which tends to be the last time they are seen (DAVIS 2002). The occurrence of FRIM has been related to aspects of the fisheries gear employed, the physical state of the animal experiencing capture, and aspects of the surrounding environment when and after capture occurs (PATTERSON *et al.* 2017a).

Some fish species are targeted in a single region by multiple gear types, and often these gear types inflict dissimilar FRIM. For example, sablefish (*Anoplopoma fimbria*) are fished off the United States west coast using trawls, longlines, and pots but these gears impose very different levels of FRIM (pot $<$ longline $<$ trawl) on non-target fish species and other organisms (JENKINS and GARRISON 2013; NEILSON *et al.* 1989). However, the way a gear is fished may supersede the type of gear chosen in terms of FRIM. For example, when large numbers of fish are caught in a seine or trawl net, mortality increases (DEPESTELE *et al.* 2014; NEILSON *et al.* 1989; RABY *et al.* 2014), and mortality increases with time as fish are left to struggle in a gillnet (BUCHANAN *et al.* 2002; TEFFER *et al.* 2017).

While the type of fisheries gear in use may influence the likelihood of FRIM, survival outcomes are also dependent upon factors intrinsic to the animal experiencing capture as well as factors of the surrounding environment (RABY *et al.* 2015). Intrinsic factors include body size (NEILSON *et al.* 1989), sex (RABY *et al.* 2015), maturity (RABY *et al.* 2013), physiological status (BROBBEL *et al.* 1996), and population of origin (DONALDSON *et al.* 2012). Extrinsic factors associated with FRIM include dissolved oxygen (SUSKI *et al.* 2006), salinity (BROADHURST *et al.* 2008), the presence of predators (RYER 2002), and temperature (GALE *et al.* 2013). An additional, important yet understudied, factor of the environment in which capture occurs is the presence of infectious agents, including viruses, bacteria, fungi, and microscopic parasites (MILLER *et al.* 2014). There are multiple examples in the literature of studies of FRIM that were confounded by elevated mortality stemming from the presence of infectious agents (OLSEN *et al.* 2012; ROBERTSON *et al.* 1987; THOMPSON *et al.* 1971), indicating their ability to enhance FRIM caused by capture stressors.

In fisheries where bycatch occurs, the release of non-target fish species is sometimes mandated by management, but this requirement is typically based on assumptions about the rate of FRIM, which are often based on studies of short-term FRIM. Proper estimation of FRIM on a population scale requires measurement of the rate of exposure to fisheries, as well as measurement of sensitivity to fisheries interactions (determined by intrinsic and extrinsic factors). A poor understanding of the impacts of FRIM on the population scale can result in an underestimation of the impact of a fishery (BAKER *et al.* 2014), and inadequate management as a consequence. Therefore, further understanding of (especially delayed) FRIM and the complex intersection of factors that influence FRIM is needed in the form of studies designed to test its elements.

Pacific salmon, *Oncorhynchus spp.*, provide an excellent model organism for studies of FRIM. Their abundant spawning migrations bring them within close proximity of human populations and support large-scale fisheries that employ multiple gear types in marine, estuarine, and freshwater environments. They frequently travel in mixed-species and/or mixed-population aggregations, so bycatch and mandated releases are common phenomena. Pacific salmon provide an excellent opportunity to study the intersection between

fisheries interactions and disease since, by migrating into freshwater, they will likely be exposed to abundant infectious agents. Because they are cold-water species, temperature plays an important role in their survival and can be studied in relation to FRIM. They mature rapidly during migrations, enabling hypothesis tests regarding maturity, but over a short time scale. Their migrations are linear and thus fate can be tracked more easily than, for example, a marine species. The spawning habitats of Pacific salmon are generally more accessible to researchers than those of other fish species. Thus, spawning success, which has serious implications for lifetime fitness since Pacific salmon are semelparous, can be measured as a FRIM endpoint. The unique life history of Pacific salmon entails innate characteristics that will influence FRIM, sometimes to a greater degree than aspects of the capture experience itself. I will now provide a general description of the migrations of Pacific salmon, followed by a description of factors influencing FRIM.

1.2 Pacific salmon spawning migrations

Pacific salmon are keystone species in the many ecosystems that they bridge (WILLSON and HALUPKA 1995), and are of great cultural, nutritional, and economic value to countries surrounding the northern Pacific Ocean (LICHATOWICH 2013). Since spawning migrations bring a large amount of energy-rich biomass into small volumes of water on a predictable, annual basis, human populations have conducted fisheries targeting Pacific salmon during their migrations for millenia (MOSS *et al.* 1990). Due to the expansion of human populations on the west coast of North America leading to the advent of industrial fisheries, impoundment of rivers, destruction of spawning habitat, and well-intentioned but ineffective (and possibly detrimental) remediation efforts including artificial propogation (i.e., fish hatcheries), many populations of Pacific salmon have declined (KATZ *et al.* 2013; LICHATOWICH and LICHATOWICH 2001; RUCKELSHAUS *et al.* 2002). In fact, population declines persist and in some cases have accelerated in recent decades (BRADFORD and IRVINE 2000; GRANT *et al.* 2017; RIDDELL *et al.* 2013). For example, Fraser River sockeye salmon adult returns have declined in recent decades and 2016 witnessed the smallest run in recorded history (Figure 1.1, GRANT *et al.* 2017).

All five Pacific salmon species are anadromous migrators and semelparous breeders. Juvenile residence time in freshwater varies among species from days to years, but at some point after emerging from nests in gravel, all species eventually migrate to the ocean where they grow rapidly. Following months to years of ocean growth, Pacific salmon conduct spawning migrations back into freshwater, most returning to the breeding areas where they originated. The spawning migration is a tightly orchestrated process with little allowance for deviation in migratory timing and environmental conditions (QUINN 2011). A balance between swimming ability, immunocompetency, and reproductive investment must be maintained in order to migrate to spawning grounds, compete for mates, evade predators, and spawn successfully. In addition to these required challenges, migrating salmon may be further stressed by elevated water temperatures, abundant pathogens, aquatic contaminants, and contact with predators and fisheries (HINCH *et al.* 2012). The impact of these multiple stressors accumulates, so that fish interacting with fishing gears at high water temperatures may be quite vulnerable to sub-lethal and lethal effects (GALE *et al.* 2013; SCHRECK 2000).

The stressors experienced by Pacific salmon during their spawning migrations and the various outcomes

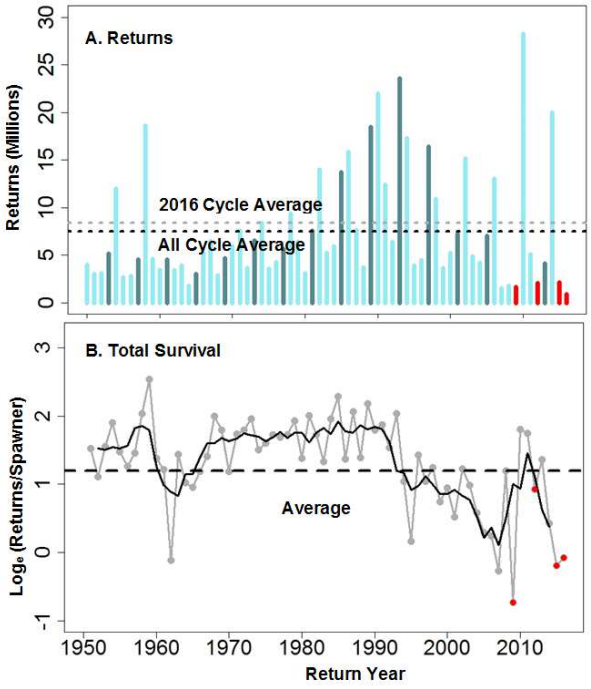

Figure 1.1: Total adult returns (A) and productivity (B) for Fraser River sockeye salmon from GRANT *et al.* (2017), reused with permission from the authors. Red bars and dots indicate low productivity across multiple populations.

of exposure to these stressors, including fisheries interactions, are presented in Figure 1.2. Migrating salmon initiate their spawning migration in a condition (energy reserves, physiology, infectious agent profile, maturity) that varies with species, populations, and individuals and will influence their recovery following a fisheries encounter. Entering freshwater, they will be exposed to abundant infectious agents, and they will be naive to some of these. Temperatures in the river might be higher than in the ocean, a common occurrence during spawning migrations of Pacific salmon that is increasing in frequency (MANTUA *et al.* 2010; PATTERSON *et al.* 2007a). In this theoretical example (Figure 1.2), 15% of the fish enter a state of chronic stress due to the accumulation of stressors (WENDELAAR BONGA 1997). Under chronic stress, immunosuppression occurs and infectious agents may proliferate (PICKERING and POTTINGER 1989). The remainder of salmon in this example have recovered from the stressors, or entered an elevated state of immunity known as "eustress" as a result of the stimulatory effects of the stressors (SCHRECK *et al.* 2016; TORT 2011). Next, all fish are exposed to an encounter with fisheries gear, and some fish die instantly due to acute injury or suffocation while others die in the next 24 hr (immediate and short-term mortality). Those that do survive must continue to contend with infectious agents and potentially elevated water temperatures. In this example, the majority (45% of all fish, here) move from a healthy state to a state of chronic stress or elevated infection. Survivors must contend with the energetic demands of the remaining migration and as a result, some of those in an impaired condition may migrate slowly or access cold water refugia. Delayed mortality will occur for the majority of impaired fish, whether they alter migratory behavior or not. In this example, 40% of the fish experiencing a fisheries encounter arrive at spawning grounds. After contending with the energetic costs of

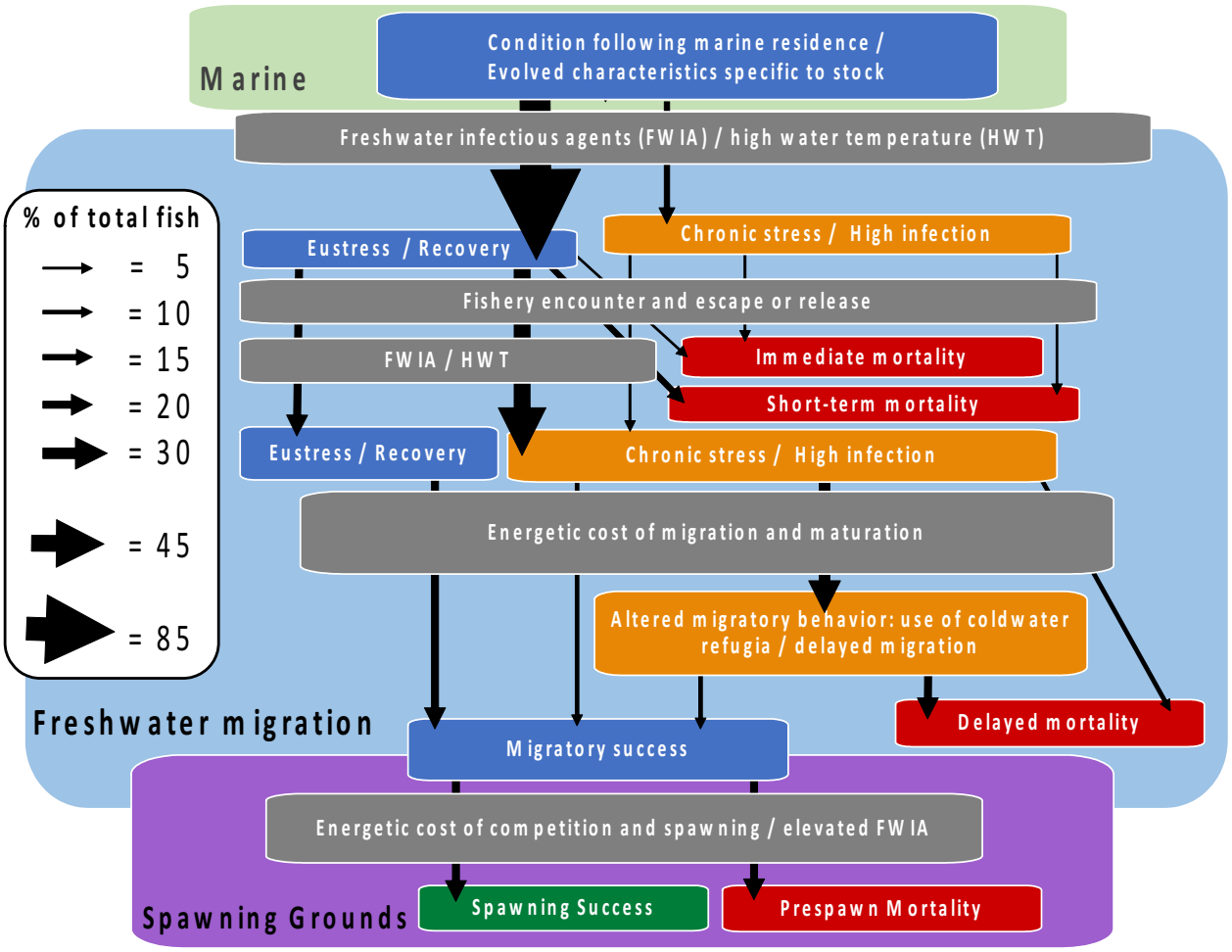

Figure 1.2: Conceptual diagram presenting how multiple stressors impact adult Pacific salmon experiencing fisheries interactions along their spawning migration. Status (blue and orange boxes) may be altered by migratory stressors (gray boxes), resulting in various fates (red and green boxes) across the different migratory environments (larger background boxes). Arrow thickness indicates the theoretical percentage of the population moving between status, stressors, and fates. For instance, 15% of fish become chronically stressed after entering freshwater and being exposed to infectious agents and elevated temperatures. In this diagram, arrow thickness is based upon a theoretical gillnet capture and subsequent release.

competition and spawning (including physical damage caused by competition), as well as extremely high densities of infectious agents, half of the remaining fish will experience prespawn mortality and the other half will successfully spawn (in this example, 20% of fish experiencing a fisheries encounter).

As one can imagine when viewing Figure 1.2, the percentage of salmon experiencing a particular fate (black arrows) following exposure to various stressors is highly dependent upon the context of the capture situation, including aspects of an individual fish's condition at capture and characteristics of the environment where fish are captured. In a review of FRIM in Pacific salmon, PATTERSON *et al.* (2017a) used the terms "intrinsic" and "extrinsic" to refer to factors related to the fish and its environment, respectively.

1.3 Intrinsic factors influencing FRIM for Pacific salmon

1.3.1 Species and population of origin

Due to the differences in size and morphology between the five species of Pacific salmon, one might expect that rates of FRIM should differ among species. For example, due to their larger size, Chinook salmon (*O. tshawytscha*) might survive a purse seine capture at higher rates than other smaller salmon species captured in the same set (DEPESTELE *et al.* 2014; NEILSON *et al.* 1989). Biochemical and physical characteristics of fish skin (including thickness) have been shown to differ between salmonid species (FAST *et al.* 2002), which could have implications for resistance to the disruption of the skin caused by a fisheries interaction, as well as subsequent infection. Few researchers have tested FRIM for multiple species of Pacific salmon, although a study of chum and pink salmon (*O. keta* and *O. gorbuscha*) on spawning grounds found that response to different capture stressors did not vary significantly between species (RABY *et al.* 2013). However, in studies of non-salmonids caught by trawl or longline, considerable inter-specific variation in FRIM has been documented (DEPESTELE *et al.* 2014; NEILSON *et al.* 1989). Considering literature that shows inter-species variation in response to non-fisheries stressors for Pacific salmon, PATTERSON *et al.* (2017a) concluded that inter-specific variation in FRIM is likely to exist.

Being one of the few groups of vertebrates that are tetraploid, salmonids have a great capacity for rapid evolution to diverse habitats (OTTO 2007; WAPLES *et al.* 2008) and have the capacity to form morphologically and physiologically distinct populations within a single species (BEACHAM *et al.* 2004; CROSSIN *et al.* 2004; ELIASON *et al.* 2011). Due to life history characteristics imposed by diverse environments, one population might be more tolerant of a particular stressor, such as temperature, than others (ELIASON *et al.* 2011). It might also be the case that different populations of the same salmon species have varying abilities to tolerate fisheries interactions.

Studies have been conducted with migrating Pacific salmon that demonstrate population differences in physiology and survival following fisheries interactions (DONALDSON *et al.* 2010, 2012). However, since these comparisons involved populations with substantially different migration strategies and morphologies, it can be hard to disentangle population-level differences from differences in maturity (see Section 1.3.4). Regardless of the underlying mechanism, if multiple populations are intercepted simultaneously (same gear, same location) and some populations respond poorly relative to others, there are serious implications for the impact to these populations and requirements for their management.

1.3.2 Physiological status

When faced with adversity, Pacific salmon mount a physiological stress response evolved to rapidly mobilize energy stores so that they can escape the stressor (WENDELAAR BONGA 1997). During the stress response, cortisol, a corticosteroid hormone released from the interrenal cells of the head kidney, rapidly converts hepatic glycogen into glucose, fueling escape or compensation (BARTON and IWAMA 1991). Elevated cortisol causes a decline in immune function by reducing production and circulation, but increasing apoptosis, of lymphocytes (HARRIS and BIRD 2000). Factors that can cause such a stress response include crowding (PICKERING and POTTINGER 1989), migrating through rapid water (WOODHEAD 1975), elevated

water temperature (TEFFER 2018), chase by a predator (DONALDSON *et al.* 2010), injury (NGUYEN *et al.* 2014), and infection (FAST *et al.* 2006). While elevated cortisol and its immunosuppressive effects are temporary in the case of acute stressors, stressors with a sustained presence are likely to cause chronic stress and accompanying immunosuppression (PICKERING and POTTINGER 1989; WENDELAAR BONGA 1997). A migrating Pacific salmon experiencing chronic stress at the time of capture will be less able to deal with the added stress of a fisheries encounter, and the energetic demands of recovering from such an encounter.

Prior to entering freshwater, salmon cease feeding. Therefore, the energy required for migration is strictly endogenous and migrating fish are essentially starving. In a study of Atlantic salmon, BROBBEL *et al.* (1996) found that fish with more glycogen reserves in white muscle were able to resist capture for longer and thus accumulate more white muscle and plasma lactate. Some mortality was seen in the higher energy group and white muscle intracellular pH remained depressed longer for these fish. These results indicate that more stored energy (which might intuitively be considered positive) may have a negative impact in a fisheries interaction scenario since it would enable prolonged struggle and further accumulation of stress and exercise metabolites, the clearance of which requires extended recovery (LEE *et al.* 2003a).

1.3.3 Sex

Several studies of migrating Pacific salmon have found elevated mortality for females relative to males (CROSSIN *et al.* 2008; JEFFRIES *et al.* 2012a; MARTINS *et al.* 2011, but see KEEFER *et al.* (2017)), and this has also been observed in studies of FRIM (ROBINSON *et al.* 2013; TEFFER *et al.* 2018, 2017). While the mechanism behind sex-biased mortality has not been demonstrated, some researchers have suggested that it could be related to the greater energetic investment in gonads by females (CROSSIN *et al.* 2008) while others have suggested that elevated basal stress levels in females may be responsible (FAGERLUND 1967; JEFFRIES *et al.* 2012a).

For some fisheries gears, such as gillnets, sex-based morphological differences could drive differences in FRIM. For example, due to the deeper bodies of male sockeye salmon, they may become more wedged into gillnets and thus receive more severe physical injury than females, which can more easily swim through (BAKER *et al.* 2011; PETERSON 1954). Or alternatively, the developed dorsal humps and protruding teeth of sexually mature males might interfere with entanglement while females from the same population could still be entangled firmly around the operculum (HAMLEY 1975). Clearly, either scenario is dependent upon the interplay between morphology and characteristics of the gear employed. Another factor potentially driving sex-biased FRIM is that males sometimes have thicker skin than females, which would make them more resistant to injuries occurring during fisheries interactions (MCBRIDE *et al.* 1986).

1.3.4 Maturity

As they migrate upstream, adult salmon undergo dramatic physiological and physical transformations that can alter their resilience to stressors (RABY *et al.* 2013). While the degree of transformation occurring throughout migration varies among species and populations, many Pacific salmon enter the river resembling ocean migrating fish, with silver, thin-skinned, sexually monomorphic, fusiform bodies, and arrive on spawning grounds brightly colored, with thickened skin, and distinct secondary sexual characteristics like a kype

and dorsal hump (males). As their body shapes change and they prepare for the challenges of the spawning area (e.g. courting mates, digging redds, fighting conspecifics, evading predators), the way that salmon (males especially) interact with gear may change (HAMLEY 1975). Thickened skin and absorbed scales towards the end of migrations (ROBERTSON and WEXLER 1960) are more difficult to damage than fragile skin closer to river entry. On spawning grounds, Pacific salmon demonstrate significantly elevated levels of plasma cortisol (BAKER and VYNNE 2014; FLORES *et al.* 2012; ROBERTSON *et al.* 1961). Although this phenomenon would appear maladaptive due the immunosuppressive effects of cortisol (MAULE *et al.* 1987), there is some speculation that the impacts of cortisol on homeostasis are reduced on the spawning ground (KUBOKAWA *et al.* 1999). Salmon close to spawning have decreased energy levels and so, in comparison to fish recently entering freshwater, may struggle less when entangled in fisheries gear, as described in Section 1.3.2. Since Pacific salmon generally mature during their upstream migration, especially in longer migrations, the location where fish from a given population are intercepted can serve as a proxy for maturity.

1.3.5 Infectious agents

Dozens of infectious agents (viruses, microscopic parasites, fungi, bacteria) play an important role in determining the survival of adult Pacific salmon on their spawning migrations, and many more are likely currently undiscovered (MILLER *et al.* 2014). While fish may enter the river with low levels of infectious agents (known as a "carrier" state) that infected them in seawater or during juvenile lifestages, in-river conditions provide abundant exposure to additional freshwater pathogens and the factors that promote their replication. Compared to the ocean, freshwater environments are spatially limited and the densities of infectious agents as well as their interaction with hosts are much higher. While salmon may be capable of resisting some infections through immune defenses, the added stress and damage from a fisheries interaction may result in infectious-agent-mediated mortality.

Although studies featuring infectious agents and stressors tend to focus on the vulnerability of fish to infectious agents after application of stressors, there is research that indicates how previously infected fish might respond to fisheries interactions. While the persistence of infectious agents contracted in freshwater (as juveniles) and saltwater environments is not well understood, it is known that some taxa remain present throughout life (BAILEY *et al.* 1989). Therefore, fisheries interactions could provide adequate conditions to facilitate disease mediated by previously latent infections. Fish with carrier state infections have been shown to experience an increase in infection following stressors, and this sometimes results in mortality (BERGMANN and KEMPTER 2011; TAKSDAL *et al.* 1998).

WAGNER *et al.* (2005) found a decreased ability to recover from swimming in sockeye salmon infected with *Parvicapsula minibicornis*. Given that fisheries interactions often involve vigorous swimming, this could indicate that infected migrating salmon experiencing such stressors would be more vulnerable than uninfected fish. In some cases, infections may be so pathogenic that the addition of a fisheries interaction does not cause differences in mortality between fish that do and do not experience the stressor. For example, CARUSO *et al.* (2002) found that sheatfish, *Silurs glanis*, infected with a bacteria and then subjected to repeated netting showed little difference in immune response and mortality compared to infected but unstressed fish, but mortality was high for both groups.

1.4 Extrinsic factors important for Pacific salmon

In addition to the many intrinsic factors governing a salmon's response to fisheries interactions, aspects of the environment prior to and following fisheries interactions can play a major role in FRIM. As mentioned in Section 1.1, these factors can include water temperature, salinity, dissolved oxygen, predator presence, and exogenous infectious agents (PATTERSON *et al.* 2017a). Since my thesis is primarily focused on intrinsic factors, I will limit the introduction of extrinsic factors here to temperature and infectious agents.

As water temperature is considered an "ecological master factor" for aquatic organisms (BRETT 1971), it governs the physiology and immune function of salmon, as well as the replication rate and persistence of the pathogens that colonize them. The temperature of migration routes used by Pacific salmon in the early portion of their freshwater migrations tend to be some of the warmest they will experience in their lives. Across a diversity of fish species in multiple environments, elevated water temperature tends to exacerbate the negative effects of fisheries interactions (GALE *et al.* 2013). However, for Pacific salmon, a cold-water obligate group of species, there is little evidence of the variable impact between fisheries interactions of different severity once temperatures are elevated (but see TEFFER 2018). In multiple experimental studies, elevated water temperature caused physiological imbalance and/or mortality regardless of capture experience (GALE *et al.* 2011; ROBINSON *et al.* 2013; TEFFER *et al.* 2018).

The presence of exogenous infectious agents after salmon experience fisheries interactions may lead to infection and subsequent mortality, and this phenomenon has been demonstrated experimentally (BADER *et al.* 2006; MAULE *et al.* 1989; SVENDSEN and BØGWALD 1997). Freshwater environments host abundant pathogens, and the number of taxa infecting salmon and the infectious load tend to increase as salmon migrate upstream (MILLER *et al.* 2014). Heterogeneity in the distribution of infectious agents often exists in freshwater (HALLETT *et al.* 2012), and since the period of greatest vulnerability following fisheries interactions is likely limited (BADER *et al.* 2006), the density of infectious agents in the water at the time of fisheries interaction is very important (PICKERING and WILLOUGHBY 1982; TEFFER 2018). Indeed, when salmon are held in captivity (a stressful environment for wild fish, with high potential for abrasion) with the goal of maintaining vitality for long periods, infectious agents must be chemically or physically removed from the water (BENDA *et al.* 2015; BRADFORD *et al.* 2010b). The aspects of fisheries interactions that render salmon vulnerable to infection are described below in Section 1.5.

For some infectious agents, elevated water temperature has a synergistic, negative effect for migrating salmon (and those experiencing fisheries interactions). First, infectious agents like *Ceratonova shasta*, *Tetracapsuloides bryosalmonae*, and *Ichthyophtherius multifiliis* can more rapidly complete their life cycles at elevated temperatures, resulting in more generations and higher production of infective units in one season (BJORK 2010; GRATZEK 1993; TOPS *et al.* 2006). Secondly, the chronic stress caused by elevated water temperature impacts immune function, leaving salmon more likely to contract infections (BRADFORD *et al.* 2010b; JEFFRIES *et al.* 2012b; TEFFER *et al.* 2018). Finally, some infectious agents are more pathogenic at elevated water temperatures (BETTGE *et al.* 2009; RAY *et al.* 2012).

1.5 Impacts of fisheries interactions

1.5.1 The physiological impacts of fisheries interactions

As described in Section 1.3.2, Pacific salmon faced with an acute stressor rapidly mobilize energy stores so that they can evade harm (SCHRECK 2010). It is well understood that the stress response results in imbalances of cortisol, lactate, and plasma osmolality (WENDELAAR BONGA 1997). Fisheries interactions tend to be short-lived and therefore more likely to cause acute stress, which can actually be immunostimulatory (TORT 2011), rather than chronic stress. However, the physical damage they cause coupled with intrinsic and extrinsic factors (e.g., infection or elevated water temperatures) can lead to chronic stress resulting in immunosuppression and delayed maturity (BAKER *et al.* 2013; TORT 2011).

Struggling in nets, sometimes coupled with air exposure, causes prolonged anaerobic swimming leading to exhaustion. This exhaustive struggle can result in a build up of lactate (FARRELL *et al.* 2000) and consumption of endogenous energy. Using rubber bands tied around rainbow trout, KOJIMA *et al.* (2004) mimicked the effect of constriction by gillnet and found that the half of test fish that perished exhibited plasma lactate levels 10-fold higher than, and blood pH levels lower than the survivors. To reoxidize excess lactate (which is never eliminated through excretion), deep respiration accompanied by minimal movement is required (HØGÅSEN 1998; LEE *et al.* 2003a). A failure to clear lactate will result in acidosis and subsequent mortality, as demonstrated by WOOD *et al.* (1983) who found considerable short-term mortality in rainbow trout *O. mykiss* following exhaustive swimming. The impact of anaerobic exercise on delayed mortality for migrating salmon has also been demonstrated *in situ* (BURNETT *et al.* 2014). Elevated lactate also impacts swimming performance until it is cleared (JAIN and FARRELL 2003), which could lead to vulnerability following a fisheries interaction, or delayed migration.

Although the amount of energy consumed by adult salmon during net entanglement has not been directly quantified, studies of supercritical (burst) swimming by adult salmon indicate that it is energetically costly (STANDEN *et al.* 2002). Migrating Pacific salmon dedicate stored somatic energy to body maintenance, swimming, osmoregulatory changes, reproductive maturation, and spawning activity; sockeye salmon at the end of the migration were found to have used 95% of their energy stores (WOODHEAD 1975). Given this small margin of error, the energetic cost of a capture event (not to mention multiple fisheries interactions) may have a significant impact on migratory success. Following fisheries interactions, energy will also be expended during the wound healing process and immunological response (ARCHIE 2013; PANNEVIS and HOULIHAN 1992). Thus, energy dedicated to ameliorating the consequences of fisheries interactions is a decrease in energy available for maintenance, migration, maturation, and reproduction.

1.5.2 Physical damage caused by capture

The most external layer of fish skin provides a physical seal between a fish and its surroundings and expresses a great variety of antimicrobial peptides (RAKERS *et al.* 2010). The barrier function provided by skin plays an important role in osmoregulation, and studies featuring experimental removal of skin have found subsequent osmoregulatory imbalances (MATEUS *et al.* 2017; OLSEN *et al.* 2012). As the integument and its protective mucus covering provide the first line of defense in the salmon immune system (FAST *et al.* 2002),

damage revealing subdermal tissue can have important consequences for infection and survival (BAKER and SCHINDLER 2009; SVENDSEN and BØGWALD 1997; THOMPSON and HUNTER 1971). BAKER *et al.* (2014) found that 6 to 44 % of sockeye salmon that arrived at spawning grounds in 9 river systems (Bristol Bay, AK) over 5 years had injuries indicative of gillnet entanglement. Over half of these damaged fish reached spawning grounds but failed to reproduce and many presented fungal infections (BAKER and SCHINDLER 2009). In a comparison of different gillnet mesh sizes used to capture Chinook salmon (*O. tshawytscha*), VANDER HAEGEN *et al.* (2004) found that the largest mesh size, which also caused the most severe visible damage, was responsible for the highest rates of delayed mortality.

Mucus covering the skin, scales, stomach, and gills of fish is the first defense against invading pathogens (FAST *et al.* 2002), although some pathogens can actually use it as a growing medium (STAROSCIK and NELSON 2008). Mucus entraps microorganisms and continuously sloughs, inhibiting colonization of the integument (ROBERTS 2012). Mucus contains lysozymes, enzymes that destroy the cell walls of some bacteria (DALMO *et al.* 1997), and antibacterial proteins capable of forming lethal pores in the membranes of invading pathogens (EBRAN *et al.* 2000). Any significant removal of mucus is expected to expose fish to potential infection (PICKERING and WILLOUGHBY 1982), as demonstrated by SVENDSEN and BØGWALD (1997) with the experimental removal of mucus from Atlantic salmon (*Salmo salar*) coupled with exposure to and subsequent infection by *Vibrio anguillarum*. Although it has not been experimentally demonstrated, fisheries interactions, including even gentle handling, are expected to remove mucus to some degree.

1.5.3 Immunological response to capture

All vertebrates, including fish, react to stress, injury, and infection with a combination of the adaptive and innate arms of the immune system (MAGNADOTTIR 2010). While the two arms are now thought to work together, the adaptive arm is primarily considered responsible for the cell-mediated response (CMR) carried out by T- cells, and the antibody-mediated response, which is developed by B-cells in response to pathogens and preserves a "memory" useful in future infections (MAGNADOTTIR 2010). The innate arm, historically considered to be inborn, includes natural killer cells, macrophages, and humoral components including complement, metal-binding proteins, lysozymes, and antimicrobial peptides (BAYNE and GERWICK 2001). Components from the innate arm comprise the acute phase response (APR), which is the array of metabolic and physiological changes which occur in response to tissue injury or infection (KUSHNER 1982). Adaptive immunity in fish tends to be slow (around 2 weeks (MAGNADÓTTIR 2006)) relative to the APR, which induces instantly. The APR is initiated by plasma-borne cytokines, such as interleukins, which are released in repsonse to wounding or infectious agent presence (BAYNE and GERWICK 2001). These cytokines trigger the synthesis of plasma borne proteins (acute phase proteins (APPs)) involved in repairing tissue or fighting infection.

The APR can be considered an immunological analog of the physiological stress response. The role of the APR is to rapidly respond to injury or infection, and sometimes activate components of the CMR (MAGNADÓTTIR 2006). Therefore, salmon receiving wounds from fisheries interactions and subsequent infection are expected to demonstrate activation of the APR, and this has been the case in multiple studies of stressors from, and causing damage similar to, fisheries interactions (BAYNE and GERWICK 2001; SUTHERLAND

et al. 2014; TEFFER *et al.* 2017; WU *et al.* 2004). Because the addition of APPs to plasma increases the osmolarity and viscosity of blood and thus the work the heart must do to circulate it, other proteins are removed by plasma to maintain manageable osmotic pressure (BAYNE and GERWICK 2001). In addition, there is an energetic cost to protein synthesis so some proteins may be prioritized over others (PANNEVIS and HOULIHAN 1992). As an example of this prioritization of APP synthesis over other proteins, SUTHERLAND *et al.* (2014) found that APPs were upregulated in multiple salmon species challenged by exposure to sea lice, whereas some anti-viral proteins were downregulated.

1.5.4 Increased vulnerability to infectious agents

As a result of the combination of several factors described above, including physical damage to skin (Section 1.5.2), the immunosuppresive effect of chronic stress (Section 1.5.1), and the presence of endogenous (Section 1.3.5) or exogenous infectious agents (Section 1.4), fisheries interactions often promote infectious-agent-mediated mortality. For example, BAKER and SCHINDLER (2009) found that 93% of sockeye salmon with fungal infections (subsequent to receiving gillnet induced injuries) and 11% of non-infected fish arriving at spawning grounds experienced prespawn mortality. TEFFER *et al.* (2018) found that infection by the bacteria *Flavobacterium psychrophilum* was higher for Chinook salmon exposed to a simulated gillnet capture. Bacterial diseases of fish tend to be opportunistic, meaning that they are almost exclusively found in stressed fish (INGLIS *et al.* 1993). Fish held for research or aquaculture purposes are prime candidates for disease since, in addition to being held in high densities, they experience high stress and injury in artificial environments (BAKKE and HARRIS 1998). For wild fish, the literature relating fisheries interactions to disease development in a natural environment is limited due to the difficulties of following fish in the wild and collecting infectious agent data non-lethally (BAKKE and HARRIS 1998).

1.5.5 Modulation of migratory behavior following a capture event or infection

In addition to FRIM, Pacific salmon released or escaping from fisheries interactions may present behavioral, sub-lethal effects which can have negative fitness consequences (WILSON *et al.* 2014). The most commonly observed sub-lethal effect following fisheries interactions is an alteration in migration rate. Fish experiencing capture typically perform anaerobic burst swimming when trying to escape, which incurs an oxygen debt and associated recovery time (LEE *et al.* 2003a). This is commonly seen in fish telemetry studies as delayed migration following capture or "fall back" behavior (FRANK *et al.* 2009; MÄKINEN *et al.* 2000; RAND and HINCH 1998). The time required for recovery may increase with the severity of the capture event. Delays to migration may contribute to delayed mortality (CAUDILL *et al.* 2007), or negatively impact the ability to compete on spawning grounds (DICKERSON *et al.* 2005). RICHARD *et al.* (2014) radio tagged capture-release and control Atlantic salmon and found that fish that were angled showed diminished exploratory behavior prior to spawning, which might hinder the discovery of optimal spawning habitats or reduce the ability of the population to colonize new habitats.

Disease, which may be exacerbated by fisheries interactions, is another factor capable of altering migration behavior. Fish infected with *Parvicapsula minibicornis* recover more slowly after exhaustive exercise

(WAGNER *et al.* 2005), which would likely slow the subsequent swimming rate during migration. In contrast, YOUNG *et al.* (2006) found that aberrantly timed late-run Shuswap sockeye salmon (assumed to be suffering from disease or at least highly stressed) that perished en route traveled at higher rates than those that survived. A similar phenomenon was observed by TEFFER *et al.* (2018), who found that the intensity of infection was positively correlated with migration rate for Chinook salmon infected by multiple taxa. It has been hypothesized that migrating salmon utilize cool hypolimnion in stratified lakes to inhibit the progress of their infections. In a telemetry study, early arriving sockeye salmon only survived to spawning if they accessed lake hypolimnion as opposed to remaining in the warmer river (MATHES *et al.* 2009). The authors suggested that early arriving fish failing to access cold water perished from temperature-mediated *P. minibicornis* infections. There are also examples of fish moving into warmer water to accelerate the inflammatory response associated with an infection (ROBERTS 2012). Recently, JENSEN *et al.* (2015) demonstrated that experimentally inflicted wounds on Atlantic salmon heal significantly faster at $12°C$ than at $4°C$, although no related temperature preference by fish in such a situation has been demonstrated to date.

1.6 Knowledge gaps regarding FRIM in Pacific salmon

In an effort to prevent salmon populations from collapsing due to overfishing, fisheries managers are tasked with permitting an adequate number of salmon to pass unharmed through a given fishery so that escapement targets can be reached. However, the occurrence of FRIM complicates these efforts. In instances where stock assessment is conducted prior to spawning grounds and spawning success is not measured, FRIM can result in overestimation of effective spawners and underestimation of population productivity (BAKER *et al.* 2014). Managers often provide short-term openings under the assumption that escapement targets will be met because a manageable amount of FRIM will occur. However, an incomplete understanding of how FRIM functions in relation to intrinsic and extrinsic factors, as well as gears used, may result in greater than anticipated mortality, which may be difficult to detect (PATTERSON *et al.* 2007b).

Because FRIM can be context specific, studies that represent gear types across a diversity of species, populations, and different physiological states are needed to inform management decisions across diverse fisheries. Intrinsic and extrinsic factors can change anually for a population of fish, yet few studies have examined FRIM on a multi-year basis (but see BAKER *et al.* 2014). Even within a single season, intrinsic and extrinsic factors change throughout the migration period, and along the migratory pathway. Most studies of FRIM are based in a single location and do not compare the impacts of fisheries interactions at different points in the migration, even though fisheries may occur continuously along a migratory pathway. Estimates of FRIM for salmon intercepted early in the migration may not be applicable to those intercepted later. Studies of FRIM rarely compare multiple gears directly, so that the FRIM estimates that inform management of a single population of fish caught by multiple gears may be cobbled together from disparate studies set in extremely different contexts (DFO 2017). Finally, while frequently cited as the ultimate mechanism of mortality in studies of FRIM, infectious disease, as well as its governing factors and its role in FRIM, are not well understood. More knowledge regarding this topic should help identify fisheries, practices, or migration phases that are particularly prone to the impact of infectious agents.

1.7 Dissertation objectives

The overarching goal of this dissertation was to conduct novel, *in situ* research to fill knowledge gaps regarding FRIM during the freshwater portion of Pacific salmon spawning migrations. These studies all featured telemetry so that individual fate of salmon experiencing fisheries interactions could be determined and then scaled to investigate factors on a population level. The experimental component of these studies was variation in capture methods, application of simulated capture, or categorization of observed injury resulting from downstream fisheries. In some studies, biopsies were collected, including: fin tissue for DNA-based population identification, blood plasma for measurement of stress hormones and metabolites, and gill biopsies to determine infectious agent presence and load, as well as salmon immune gene expression. The latter two groups of parameters were determined using a recently validated high-throughput, quantitative polymerase chain reaction (HTqPCR) platform (MILLER *et al.* 2016). The major components of each study are displayed in Figure 1.3. Some of the specific questions that this dissertation attempted to address include:

1) How does FRIM vary between commonly used fisheries gears and techniques?

This question is addressed in Chapters 2 and 4 where migration survival and rate were compared to capture method. In Chapter 2, experienced fishing crews captured sockeye salmon using both beach seine and gillnet at the same location and same time. In Chapter 4, Chinook salmon received simulated gillnet capture with and without air exposure, to represent different ways fish might interact with gears or be released.

2) Does maturity influence the outcome of fisheries interactions?

Since late Shuswap sockeye salmon exhibit visible maturation during their freshwater migration, location of capture was used as a proxy for maturity in Chapter 2. The physical damage caused by capture and the occurrence of FRIM were contrasted between capture locations to elucidate the role of maturity in FRIM. In addition, Chinook salmon in Chapter 4 were intercepted at a late stage in their migration, and previous studies suggest that they might be refractory to fisheries interactions at this point (DONALDSON *et al.* 2012; RABY *et al.* 2013).

3) Is condition at capture associated with migratory success following release?

By collecting non-lethal biopsies immediately after application of experimental gillneting in Chapter 4, the importance of infectious agent presence, relative to simulated capture, could be determined. In addition, infectious agent load was compared to physiological parameters in blood plasma and immunological aspects of salmon gene expression to document impacts contributing to mortality.

4) Are visible injuries resulting from downstream fisheries predictive of FRIM?

By intercepting Gates Creek sockeye salmon after they passed through gillnet fisheries, visible injuries

could be identified and categorized based on severity (Chapters 3 and 5). By conducting these studies in a closed and well-monitored system, with minimal handling impacts and large sample sizes, robust estimates of FRIM could be obtained (Chapter 3). Furthermore, three years of study provided an opportunity to investigate annual variance in FRIM. In Chapter 5, the relationship between the severity of gillnet injury and FRIM was investigated, with a focus on the potential mechanisms underlying mortality (infectious agents, immune gene expression) and behavioral modulation in response to injury (temperature selection in a stratified lake).

	Experimental		Intrinsic factors			Response / Endpoint		
Dissertation section	Simulated or real capture	Observed injuries	Infectious agents	Physiological condition	Immune response	Behavioral modulation	Migration survival	Spawning success
Chapter 2	X					X	X	
Chapter 3		X		X			X	X
Chapter 4	X		X	X	X	X	X	
Chapter 5		X	X	X	X	X	X	X
Appendix A			X					
Appendix B			X					

Figure 1.3: Diagram indicating the organization of the dissertation sections with regards to the experimental manipulation upon which each section was based, intrinsic factors measured to determine their potential relationships with delayed mortality, and the response variables or endpoints that were assessed.

Chapter 2

Location-specific consequences of beach seine and gillnet capture on upriver-migrating sockeye salmon migration behavior and fate

2.1 Synopsis

Fish released after capture, or fish interacting with gear but escaping, sometimes experience fishing-related incidental mortality (FRIM). For adult Pacific salmon migrations, knowing the magnitude of FRIM is important to accurately estimate escapement, and the total impact of a specific fishery. To determine how multiple gear types are associated with FRIM at different levels of maturity, sockeye salmon (*Oncorhynchus nerka*) were captured by both gillnet and beach seine at three locations along their migration route (10, 26, and 72% of a 500 km freshwater migration) and their migratory success determined using telemetry. FRIM was higher for fish captured by gillnet except at the location closest to spawning grounds. In addition, salmon captured by gillnet at the lower river locations temporarily delayed migration, potentially indicating a requirement for lengthier recovery time compared to beach-seined fish. These results provide the first empirical and parallel comparison of these two common in-river fishing methods for salmon, revealing clear differences in FRIM between the two fishing methods in lower river fisheries and the importance of maturity.

2.2 Introduction

As Pacific salmon (*Oncorhynchus spp.*) undertake migrations to natal spawning areas, they are fished in marine and freshwater (hereafter "in-river") fisheries. Prior to and during spawning migrations, fisheries managers are tasked with estimating total mortality (natural mortality plus fisheries captures) to ensure that a sufficient number of salmon return to spawning grounds (spawning escapement) and perpetuate populations (PATTERSON *et al.* 2017a). While the number of fish removed by in-river fisheries can be directly estimated, delayed mortality resulting from damage inflicted during unobserved encounters with and escape from fishing gear (BAKER and SCHINDLER 2009) is difficult to estimate. Likewise, spawning escapements for species or populations that co-mingle with those targeted for harvest, but for which release is mandated due to conservation concerns, can also be impacted by delayed mortality. Regardless of whether fish are intentionally released or experience unobserved entanglement, we hereafter refer to the phenomenon of con-

tact with fisheries gear followed by continued migration as "non-retention" and the associated mortality as "fishing-related incidental mortality", or FRIM (PATTERSON *et al.* 2017a). Without accurately accounting for FRIM, anticipated spawning escapement targets for populations may not be achieved, negatively affecting future production (BAKER *et al.* 2014) and accurate accounting of total mortality for a given population is not possible (PATTERSON *et al.* 2017b).

Among the in-river Pacific salmon fishery gears (angling, drift and set gillnets, fish wheels, dipnets, beach seines, and fish weirs) used in the Fraser River watershed, BC, (Canada's largest producer of Pacific salmon) fishers use gillnets and beach seines to capture the majority of fish. Historically (and during abundant years in modern times), commercial fishers used gillnets in the Lower Fraser River and both gillnets and beach seines are commonly employed by Indigenous Peoples in their in-river fisheries. Gillnets entangle fish, which often results in suffocation, lacerations, removal of mucus, and constriction (BAKER and SCHINDLER 2009; KOJIMA *et al.* 2004). Estimates of successful arrival at spawning areas for fish captured by gillnet in the lower sections of large rivers range from 43% (sockeye salmon [*O. nerka*] in the Fraser River (DONALDSON *et al.* 2010)) to 57% (Chinook salmon [*O. tshawytscha*] in the Columbia River (VANDER HAEGEN *et al.* 2004)). Beach seines corral fish into shallow water and, provided the net is kept at adequate depth while fish are removed, handling time can be minimal and little more than removal of mucus is expected to occur (RABY *et al.* 2014). Even so, local oxygen is depleted in nets if fish density is high (RABY *et al.* 2014). Researchers have found survival rates in large rivers to range from 52% (sockeye salmon in the Fraser River (DONALDSON *et al.* 2011)) to 74% (coho salmon [*O. kisutch*] in the Fraser River (RABY *et al.* 2012)) for salmon captured by beach seine and subsequently released. However, Fisheries and Oceans Canada (DFO) estimates post-release survival at 40 and 95% for gillnet and beach seine caught salmon, respectively (DFO 2017). To date, no study has simultaneously compared FRIM resulting from beach seine and gillnet capture.

Sublethal effects, alterations to behavior, growth, or reproduction resulting from stress or injury (WILSON *et al.* 2014), are underemphasized compared to physiological changes and mortality rates in studies of fisheries gear impacts. For semelparous salmon with uni-directional migrations, a behavioral alteration such as migratory delay is a measurable sub-lethal impact that can limit reproductive opportunities (DICKERSON *et al.* 2005) or prolong exposure to unfavorable river conditions (e.g., temperatures (MARTINS *et al.* 2011), infectious agents (WAGNER *et al.* 2005), fisheries). Salmon often delay migration following capture and tagging, likely due to recovery from that stressful experience (BERNARD *et al.* 1999; LIEDTKE and RUB 2012). In several studies, migratory delay varied among multiple fisheries gear used to capture fish (DONALDSON *et al.* 2011; MÄKINEN *et al.* 2000; NGUYEN *et al.* 2014). While FRIM is an obvious measurement of the negative potential consequences of non-retention, the total impact of a stressor cannot be assessed without acknowledgment of sublethal effects (PATTERSON *et al.* 2017a).

The occurrence of FRIM and sublethal effects depends upon the biological context under which capture occurs, including the physiological condition, disease state, and maturity of the captured fish (BROBBEL *et al.* 1996; PATTERSON *et al.* 2017a; RABY *et al.* 2015). For example, the morphology and physiology of a migrating salmon changes rapidly and substantially along its migration route, e.g., absorption of scales (KACEM *et al.* 1998), consumption of endogenous energy (GILHOUSEN 1980), thickening of the epidermis (ROBERTSON and WEXLER 1960), development of secondary sexual characteristics, alterations to immune

function (DOLAN *et al.* 2016), and changes in physiological parameters such as hormones and ions (BAKER and VYNNE 2014; SHRIMPTON *et al.* 2005). Indeed, mature fish in their spawning areas are highly tolerant of net entanglement and air exposure (RABY *et al.* 2013). Thus, the distance along the migration as a proxy for maturity and physiological condition must be considered as a factor potentially influencing FRIM. However, no studies have evaluated the survival of released salmon captured across the migratory corridor for a single population complex during a single spawning migration, although the impact of non-retention has been experimentally compared between salmon at different states of maturity (BROBBEL *et al.* 1996).

Temperature is a key environmental factor influencing the physiology of migrating salmon (GONIEA *et al.* 2006; LEE *et al.* 2003b). For example, while the thermal optimum for aerobic scope of migrating adult sockeye salmon varies by population (ELIASON *et al.* 2011), and mortality increases above 18°C (MARTINS *et al.* 2011), many salmon pathogens become virulent above 16°C (RICHTER and KOLMES 2005) and pathogen-associated mortality and sub-lethal impacts have been demonstrated in multiple studies featuring high water temperature (BENDA *et al.* 2015; WAGNER *et al.* 2005). Nevertheless, the impact of multiple fishing gears has, to our knowledge, only once been compared under high water temperature scenarios (DONALDSON *et al.* 2011).

In view of the above knowledge gaps that are important to FRIM, I compared the impacts on survival and migration rates of capture by beach seine and gillnet for late-run Fraser River sockeye salmon at three locations along the migration route. In addition, I repeated the study with summer-run sockeye salmon at the second location to examine the influence of high water temperature. I visually assessed injury after capture and used radio telemetry to determine the survival and migration rate to the natal spawning area. The null hypothesis for each fishing location was that visible injury, survival to spawning grounds, and migration rate would not differ between sockeye salmon captured by beach seine or gillnet. I also tested the null hypothesis that visible injury would not vary for each fishing gear among capture locations.

2.3 Methods

2.3.1 Fish collection, biopsy, and tagging

Handling, biopsy, and tagging were performed according to the UBC animal care and use permit, A12-0250. The late-run Shuswap sockeye salmon population was studied in 2014 (N = 348) and the predominantly summer-run mixture of populations in 2015 (N = 281). Microsatellite analysis was conducted at the DFO Pacific Biological Station (Nanaimo, B.C.) to determine population origin by variation in the major histo-compatability complex (BEACHAM *et al.* 2004). This molecular technique required a 6 mm tissue punch from the adipose fin was taken of each fish (BEACHAM *et al.* 2004). Populations tagged in 2015 included: Chilko (50%), Nadina (30%), Stellako (10%), Tachie (5%), Bowron (3%), other small populations (2%).

In 2014, fishing crews collected sockeye salmon at McMillan Island (48 river kilometers [hereafter, rkm] from the mouth of the Fraser River, Sept 23 – 25), Peters Road (131 rkm, Sept 30 – Oct 2), and at Savona, immediately downstream of Kamloops Lake (363 rkm, Oct 7 – 8) (Figure 2.1). In 2015, fish were only captured at Peters Road (July 29 – 31 & Aug 4 – 6). The rarity of river characteristics that allowed use of both beach seine and gillnet, paired with the ability to find local fishing crews that employ both

gears, partially dictated the choice of locations. Nevertheless, the three locations represent recent entry to freshwater (McMillan Island), transition from the lower river to the Fraser canyon and faster moving water (Peters Road), and close proximity to the natal river where maturity should be relatively advanced (Savona). The number of fish radio-tagged at each capture location are presented by sex, capture method, average fork length, and average netscore (see below) in Table 2.1.

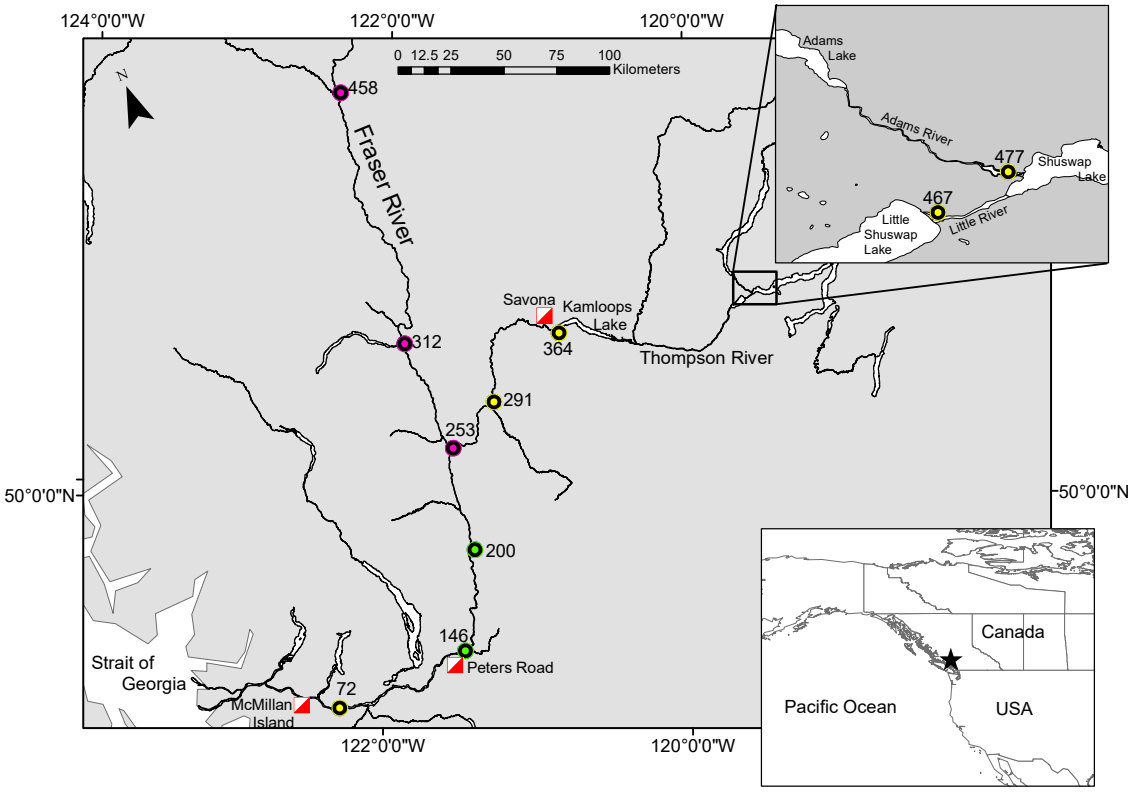

Figure 2.1: Map of stationary radio receivers (circles) and tagging locations (red and white icons) used for studies of migrating adult sockeye salmon in the Fraser River, BC in 2014 and 2015. Yellow circles represent receivers used in only 2014, green circles represent those used in 2014 and 2015, and pink circles represent those used in only 2015. Distances from the ocean (km) are provided for each receiver location. Geospatial data used to create this map are from the British Columbia Freshwater Atlas (www2.gov.bc.ca).

At each location, fish were captured using both gillnet and beach seine. Fishers were instructed to capture and handle fish using these gears as they normally would if fishing during a period when bycatch is expected and release would be mandated for some species (for example, when regulations require the release of the Early Stuart River sockeye salmon population during the opening period for summer Chinook salmon, *Oncorhynchus tshawytscha*). At McMillan Island and Peters Rd, gillnets were drifted with the current from boats (drift net). At McMillan Island, the gillnet was attached to a large boat and a smaller boat moved along the net, with fishers removing fish soon after (5 - 10 min) they were captured and placing them in a tote full of river water. At Peters Rd, a single boat drifted a gillnet for 5 min, then retrieved the net and disentangled salmon were placed in a tote full of river water. At Savona, the river conditions required the use of a gillnet

Table 2.1: Summary information for releases of radio-tagged late-run Shuswap sockeye salmon captured by beach seine or gillnet in the Fraser and Thompson Rivers, BC (2014 & 2015). Netscore represents the severity of observable wounds caused by the capture experience (0 = no injury, 1 = minimal net marking, 2 = moderate net marks around head, no exposed flesh, 3 = extensive net marks from head to dorsal fin, exposed flesh).

Year	Location	Dates	Fishing gear	Sex	N	mean fork length (cm ± SD)	mean netscore (0–3 ± SD)
2014	McMillan Island (rkm 48)	Sept 23 - Sept 25	beach seine	female	27	59.1 (1.8)	0.37 (0.69)
			beach seine	male	9	61.6 (1.6)	0.22 (0.44)
			gillnet	female	37	58.7 (2.5)	1.86 (0.71)
			gillnet	male	36	62.2 (2.5)	1.03 (0.77)
	Peter's Rd (rkm 131)	Sept 30- Oct 2	beach seine	female	52	58.5 (2.4)	0.23 (0.42)
			beach seine	male	22	61.2 (2.2)	0.45 (0.51)
			gillnet	female	42	58.5 (1.8)	1.79 (0.61)
			gillnet	male	16	61.3 (2.1)	1.38 (0.81)
	Savona (rkm 363)	Oct 7- Oct 8	beach seine	female	35	59.7 (2.3)	0.06 (0.24)
			beach seine	male	19	63.6 (2.1)	0.11 (0.46)
			gillnet	female	33	58.2 (1.9)	1.52 (0.67)
			gillnet	male	20	62.3 (2.4)	0.45 (0.60)
2015	Peter's Rd (rkm 131)	July 29-31 & Aug 4-6	beach seine	female	106	57.5 (2.2)	0.69 (0.61)
			beach seine	male	59	59.1 (3.0)	0.66 (0.66)
			gillnet	female	62	58.1 (1.9)	2.29 (0.76)
			gillnet	male	25	59.3 (2.4)	2.00 (0.87)

fixed in place in a large eddy (set net). After the floats on the gillnet were noticed to bob (indicating an entangled fish), the gillnet soaked for an additional 5 min and then fish were removed from the net. Gillnet mesh size at all locations was 13.3 cm. At each location, beach seines were drawn using motor boats and nets were pulled by hand into knee deep water, where fish were removed using dipnets. The mesh size of the beach seines was such that sockeye salmon did not become entangled behind the operculum. After capture, fish were held in pens in flowing water (1 m deep) prior to biopsy, tagging, and release. Median holding times, from after capture to tagging were 84 min for beach seine (range: 4 – 230 min) and 44 min for gillnet (range: 3 – 130 min). Since as many as 80 fish were caught in a single beach seine (Savona), more time was required to process all fish at this site.

The biopsy and tagging process was identical for all fish and very similar to TEFFER *et al.* (2017). The median biopsy time required was 2.5 min (range: 1.4 – 6.6 min). Fish could not be anesthetized because of the possibility of recapture and human consumption. During biopsy and tagging, a technician held a fish in the trough while other technicians performed the biopsy and tagging procedure. Fork length was measured and sex was determined from secondary sexual characteristics. Blood was drawn from the caudal vasculature using a heparinized vacutainer and a small gill sample (2-3 mm of 3-4 gill filament tips) was removed with sterilized end clippers. Biopsies were taken for another study and the data are not present herein. A Pisces

5® radio tag (43 mm length × 16 mm diameter, 15.2 g in air, 5 s burst rate; Sigma Eight Inc, Newmarket, ON) was placed in the stomach, immediately behind the esophageal sphincter with the wire antenna hanging out of the fish's mouth. A haphazardly distributed proportion of the radio tags (64 in 2014 and 37% in 2015) had an Ibutton® thermochron model DS1921Z logger (6 mm height × 16 mm diameter, 3.3 g in air, 30 min recording; Maxim Integrated, San Jose, CA) affixed to them to record the water temperature experienced by each fish. Acquisition of these data require recovery of the ibutton. To provide an indication of water temperature experienced by fish prior to tagging, water temperature collected by DFO near Hope (rkm 150) was incorporated (see PATTERSON *et al.* (2007a)).

Damage caused in the capture process (netscore) was assessed for each fish (0 = no visible damage, 1 = minimal net marking, 2 = moderate net marks around head, no exposed flesh, 3 = extensive net marks from head to dorsal fin, exposed flesh). An elastic visual identification tag (aka "spaghetti" tag, Northwest Marine Technology, Shaw Island, WA) was looped through the musculature posterior to the dorsal fin to identify fish in spawning areas or those captured by fishers. Radio and spaghetti tags were labeled with contact information so that tags could be returned and information regarding date and location of capture could be relayed.

2.3.2 Radio Telemetry

In 2014, fixed radio telemetry receivers (Orion®, Sigma Eight Inc or SRX600, Lotek), each equipped with either a 3 or 4 element Yagi antenna, were positioned at strategic locations along the Fraser and Thompson Rivers (Figure 2.1, Table 2.2). In 2015, some of the same sites were used but additional receivers were

Table 2.2: Stationary telemetry receivers used to detect migrating sockeye salmon in the Fraser and Thompson Rivers, BC (2014 & 2015). Table includes distance from ocean (river km), location names, and probability of detection for each release group as determined by CJS modelling. Asterisks indicate that receiver efficiency was "fixed" to permit parameter identification in Program MARK.

	2014		Estimated ρ		2015		Estimated ρ
Site #	River km	Description	McMillan 2014	Peters 2014	River km	Description	Peters 2015
1	72	Mission	0.17	–	–	–	–
2	146	Hope	0.51	0.61	146	Hope	0.90
3	199	Hell's Gate	1.00*	1.00*	199	Hell's Gate	1.00*
4	291	Spence's Bridge	1.00*	1.00*	253	Lytton	0.52
5	364	Savona	0.57	0.32	312	Lillooet	0.83
6	467	Little & Adams Rivers	1.00*	1.00*	458	Chilcotin River	1.00*

positioned along the mainstem Fraser River (Figure 2.1, Table 2.2). Reception range was tested by placing a radio tag 1 m underwater on both sides of the river channel where possible. For the late-run Shuswap population (2014), the Little River (rkm 467) and Adams River (rkm 477) receivers were considered in aggregate as the final receiver location since spawning occurs upstream of both of these receivers and in other tributaries to Shuswap Lake (Inset Figure 2.1, Table 2.2). In 2014, mobile tracking was performed in

the lower 10 km of the Adams River by boat using a Lotek SRX 600 (Newmarket, Ontario, Canada) with a 3-element Yagi antenna.

Radio detections were filtered for each individual fish so that detections at a given receiver separated by more than 2 minutes (likely false positives) or less than 5 seconds (pulse rate of radio tags) were removed. The detection history for each fish was plotted against receiver location so that false detections could be identified and removed. For a given fixed receiver "X", detection efficiency was determined by the mark-recapture analysis described below as the number of fish detected at receiver X, divided by the total number of fish known to have passed receiver X, independent of detection at receiver X (by release, detection, or capture). Low detection efficiencies ($< 80\%$) typically occurred when receivers malfunctioned, or when the river was deep and wide (Site 1, Table 2.2) or receivers were positioned too far from the river (Site 4, 2015). Individuals detected during mobile tracking in 2014 on the Adams River (N > 100) were used to determine that detection efficiency for the Little River and Adams River receivers combined was 100%. Similarly, detection efficiency for the Chilcotin confluence receiver in 2015 was also 100%, based on upstream detections and tag returns.

I recovered tags during mobile tracking on spawning grounds and from voluntary reports by fishers. In 2014, 11 sockeye salmon tagged at the Lower Fraser River release locations were reported from recaptures in net and recreational fisheries in the Fraser and Thompson Rivers. The Kamloops Lake (rkm 364) purse seine fishery run by the Secwepemc Fisheries Commission captured another 12. The sockeye salmon tagged in 2015 experienced substantially more fishery openings and effort (Table 2.3) and fishers reported 37 tags, with 81% of these captured between the Hope and Hell's Gate receivers (rkm 146 - 200).

Table 2.3: Weekly effort for the combination of First Nations Food, Social, and Ceremonial and Economic Opportunity fisheries during the periods of late-run Shuswap (2014) and summer-run (2015) Fraser River tagged sockeye salmon migration, provided by DFO catch monitoring. Effort represents a count of the nets participating multiplied by the number of hours licensed. Gillnet is a combination of drift and set nets. Dashes indicates that the fishery was either closed or there were no observed participants. NA indicates that the river reach was not applicable to the population monitored in that year given its migration route.

Week ending date	Hope to Sawmill Ck			Sawmill Ck to Texas Ck		Texas Ck to Kelly Ck		Thompson River	
	Gillnet	Dipnet	B.Seine	Gillnet	Dipnet	Gillnet	Dipnet	Gillnet	Dipnet
Sept 28, 2014	–	82	8	252	–	NA	NA	–	252
Oct 5, 2014	–	–	–	–	–	NA	NA	34	269
Oct 12, 2014	–	–	–	–	–	NA	NA	5	118
Aug 2, 2015	180	–	–	1320	24	240	144	NA	NA
Aug 9, 2015	6645	–	–	8702	134	2100	756	NA	NA
Aug 16, 2015	315	–	–	4848	–	1488	336	NA	NA

2.3.3 Statistical Analyses

Visible Injury

As netscore is an ordinal variable, rank sum tests were conducted for between group comparisons. Netscore was compared for each sex, year, and location combination. Pairwise comparisons of rank sums (Kruskal-Wallis test) with p values adjusted for multiple comparisons were implemented using Dunn's test with the R statistical software (R CORE TEAM 2017) package "dunn.test" (DINNO 2017). The Benjamini-Hochberg adjustment was used to control the false discovery rate (BENJAMINI and HOCHBERG 1995).

Survival

The explanatory variable of interest was fishing method (beach seine vs gillnet). In 2014, survival to spawning was defined as detection at the last receiver group. In 2015, survival was defined as detection at the Chilcotin receiver. Although spawning habitat is still several hundred rkm upstream of these locations, this length of migration was adequate for determining survival differences between groups. Differences in survival between seine and gillnet capture was tested with a series of Cormac Jolly Seber survival models (CJS) using Program MARK within the RMARK interface in R statistical software (LAAKE 2013; R CORE TEAM 2017; WHITE and BURNHAM 1999). CJS models allow the estimation of apparent survival (ϕ) as a function of the probability of detection (ρ) and based on maximum likelihood estimation. Three sets of models were run, one for each Lower Fraser River tagging location and year combination. Due to an inadequate number of receivers between release and the spawning area, CJS modeling could not be applied to the Savona tagging location. Here, a series of generalized linear models were fit (see below).

For the McMillan Island tagging location, receiver sites 1 through 6 were included in the analysis and for the Peters Road group, sites 2 through 6 were included. Fish that were captured and reported by in-river fisheries were censored at the receiver downstream of (prior to) their capture location. For each model set I used the RELEASE goodness of fit function within Program MARK to test two assumptions: every marked animal present in the population at time i has the same probability of recapture, and every marked animal in the population immediately after time i has the same probability of surviving to time i + 1. Overdispersion was evaluated using the median $\hat{c}$ method in Program MARK, with 100 replicates at 15 points (WHITE and BURNHAM 1999).

Sex and fork length (FL) were included as covariates because sex can be associated with survival (JEFFRIES *et al.* 2012a; MARTINS *et al.* 2012; TEFFER *et al.* 2017) and body size is associated with vulnerability to gillnets (PETERSON 1954) and other capture-and-release scenarios (DAVIS 2002). In a single-cohort CJS model with detections over a spatial extent (e.g., a salmon migration), the parameter "time" represents variability occurring between detection locations. For all models, both survival and probability of detection were varied by time since it was expected that detection probability varied between sites and I was interested in determining how survival varied between river reaches. For each tagging location, a full model was fit that included capture method, sex, body size, and time as explanatory variables for both ϕ and ρ. This model was used to plot survival estimates and determine overdispersion (without fork length, since individual covariates can not be included in the determination of median $\hat{c}$) (WHITE and BURNHAM 1999). The full model and

all nested models were compared using Akaike's Information Criterion for small populations, adjusted for overdispersion (QAICc) (BURNHAM and ANDERSON 2003).

Because parameter estimates that approach boundaries (0 or 1) can confound analyses in Program MARK and result in unidentified parameters, especially in the case of small sample sizes, several model parameters were fixed for each set of models. I fixed ρ to 1 for the last time interval based on 100% detection efficiency at site 6 in both years. This allowed the estimation of the last ϕ parameter, which is usually not possible in CJS models since the final ρ is generally unknown. In addition, ρ was fixed to 1 for Hell's Gate and Spence's Bridge (sites 3 and 4 in 2014) due to perfect efficiency at these locations (Table 2.2).

For the Savona tagging location in 2014, a series of Generalized Linear Models (GLMs) were compared by Akaike's Information Criterion (AICc) for migratory success to the final receiver group. A full model included fishing method, sex, and body size to all nested models and a null model containing no explanatory variables. To meet the assumption of multicollinearity, I estimated the variance inflation factor to be sure that it was below 4 for all variables (O'BRIEN 2007). I determined that overdispersion for the full model was negligible (1.04). A Hosmer Lemeshow goodness of fit test indicated that the model fit was appropriate (HOSMER *et al.* 1997).

Migration Time and Rate

The same explanatory variables (fishing method, sex, body size) were applied to Accelerated Failure Time (AFT) models (WEI 1992) of migration time to determine whether fishing method was associated with migration time. For the Lower Fraser tagging locations in 2014 and 2015, migration time from release to the Hell's Gate receiver (rkm 200, the first receiver with 100% detection efficiency) was modeled. Migration time was calculated as the difference between time of release and the first detection at Hell's Gate. For the 2014 groups, migration time from Spence's Bridge (rkm 291) to Little River (rkm 467) was also modeled (insufficient data for modeling migration time of 2015 fish beyond Hell's Gate). This was calculated as the difference between the first detection at Spence's Bridge and the first detection at Little River. Migration time to Hell's Gate was investigated separately from migration time through the Thompson River because I was interested in whether delay occurred shortly after capture, and also if there was a difference in delay by treatment further along the migration when many unsuccessful fish had already been removed. For the Savona tagging group, migration time was from release (rkm 363) to Little River.

To meet the assumption of the appropriate error distribution for all AFTs, distributions were selected via AIC after each model was fit using the log logistic, log normal, logistic, exponential, Weibull, and Gaussian distributions (SWINDELL 2009). The two distributions with the lowest AIC values were visually assessed by plotting the negative log of the Cox-Snell residuals against time and the distribution demonstrating better agreement with a line through the origin with a slope of 1 was selected.

In addition to the AFT analysis, I created boxplots of migration rates comparing fishing method for river reaches between receivers with relatively abundant detections. Migration rate was calculated as the distance between a given receiver and the next upstream receiver divided by the time difference between first detection at the two receivers. The group means were compared using Welch Two Sample t-tests for each year, reach, and tagging group combination.

2.4 Results

Water temperature data collected at Hope and temperature profiles from fish tagged at the Peter's Road location indicate the temperatures experienced by fish prior to and following tagging in 2014 and 2015 (Figure 2.2). The late-run Shuswap population (2014) rarely experienced water temperatures above 16°C while the mixture of summer-run populations (2015) consistently experienced water temperatures above 18°C between river entry and the Chilcotin River receiver.

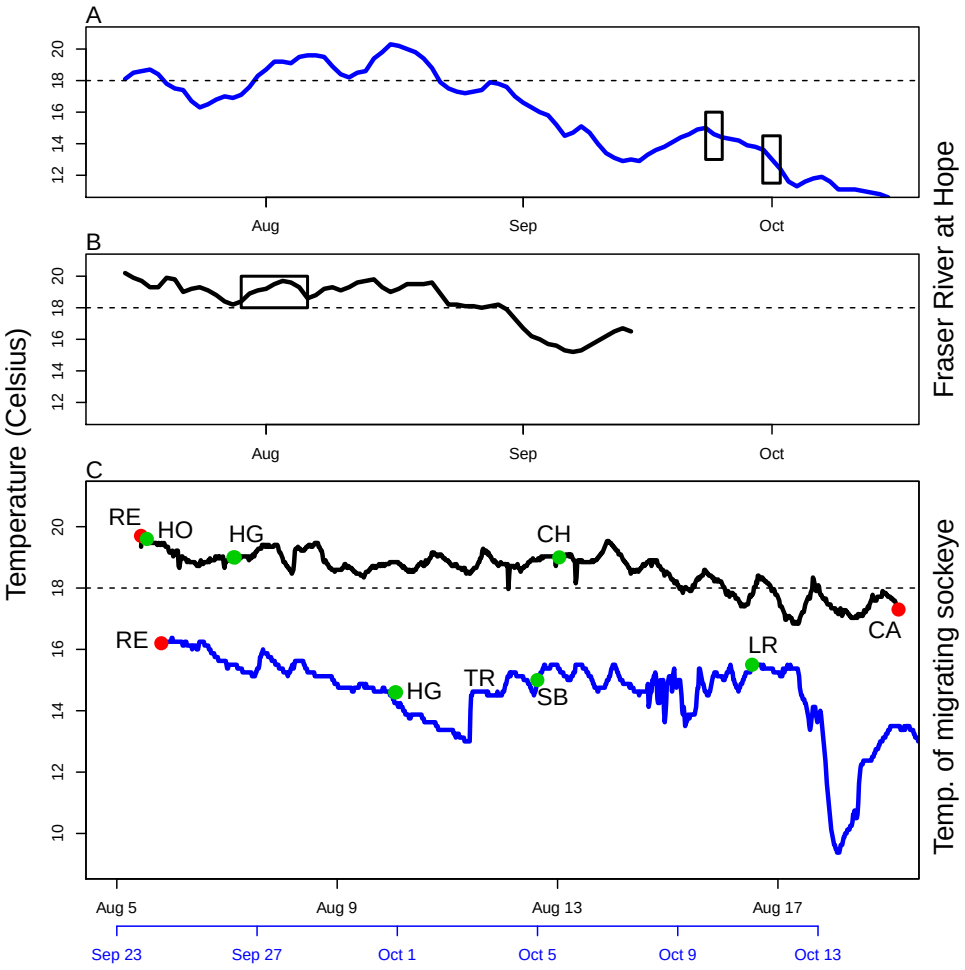

Figure 2.2: Temperatures experienced by sockeye salmon migrating through the Fraser River in 2014 and 2015. Panels A (2014) and B (2015) show water temperature recorded at Hope, rectangles indicate tagging periods (McMillan Island and Peter's Road in 2014, Peter's Road in 2015). Panel C shows water temperatures recorded by thermal loggers attached to gastric radio tags in individual sockeye salmon in 2014 (blue line) and 2015 (black). Locations determined by radio telemetry are indicated by points. For 2014, RE = release at McMillan Island, HG = Hell's Gate, TR = entry to Thompson River, SB = Spence's Bridge, LR = Little River. For 2015, RE = release at Peter's Road, HO = Hope, HG = Hell's Gate, CH = Chilcotin River, CA = capture by gillnet at river km 702. In all panels, 18°C, the temperature at which sockeye salmon begin to experience elevated mortality (MARTINS *et al.* 2011), is indicated by a dashed line.

Controlling for capture location and sex, gillnet capture always resulted in a significantly higher netscore

than beach seine ($p \leq 0.01$) except in the case of male sockeye salmon at Savona ($p = 0.12$) (Table D.1). Netscore differed by sex only for gillnet capture at McMillan Island and Savona, where females had significantly higher netscore than males ($p < 0.001$ for both). In 2014, netscore for gillnet-captured male sockeye salmon was lower at Savona compared to gillnet-captured males at both McMillan Island and Peters Road ($p = 0.02$, 0.002, respectively). Although netscore at Peters Road was higher in all groups for 2015 compared to 2014 (Table 2.1), the only significant difference was beach seine capture for females ($p < 0.001$).

2.4.1 Survival

For all the experimental fishing performed in the Lower Fraser River, capture by gillnet was associated with lower survival than beach seine. For all tagging locations, ρ for the top model was always time (Table 2.4), which reflects the variation in detection probability between receivers (Table 2.2). Based on uncorrected

Table 2.4: Top five models (determined by QAICc rank) of survival for four sockeye salmon release groups. ϕ parameters were associated with survival probability and ρ parameters were associated with detection probability. Since time consists of multiple detection sites, it adds > 1 parameter to the total number of model parameters (# parameters), but sites where ρ was fixed (perfect detection) do not contribute to this number. Cormack Jolly Seber models were created for the Lower Fraser River locations, and logistic regression models were created for the Savona tagging location.

	Model Structure					
Tagging location	ϕ parameters	ρ parameters	# parameters	QAICc	Δ QAICc	weight
McMillan Island, 2014	fork length + fishing method + time	time	9	422.44	0.00	0.24
	sex + fork length + fishing method + time	time	10	422.94	0.51	0.19
	fork length + fishing method + time	sex + time	10	424.24	1.80	0.10
	fork length + fishing method + time	fork length + time	10	424.28	1.84	0.10
	fork length + fishing method + time	fishing method + time	10	424.51	2.07	0.09
Peters Rd, 2014	time	time	6	684.05	0.00	0.19
	fishing method + time	time	7	685.16	1.10	0.11
	fork length + time	time	7	685.76	1.71	0.08
	time	fishing method + time	7	685.82	1.76	0.08
	sex + time	time	7	686.03	1.98	0.07
Peters Rd, 2015	fishing method + time	time	8	318.09	0.00	0.26
	fishing method + time	fishing method + time	10	319.73	1.64	0.11
	sex + fishing method + time	time	9	319.75	1.66	0.11
	fork length + fishing method + time	time	9	319.94	1.86	0.10
	fishing method + time	sex + time	9	320.15	2.06	0.09
Savona, 2014 (logistic regression)	~ 1	—	1	122.9	0.00	0.32
	sex	—	2	124.0	1.10	0.18
	fishing method	—	2	124.5	1.55	0.15
	fork length	—	2	125.0	2.06	0.11
	sex + fishing method	—	3	125.6	2.72	0.08

detections and accounting for fisheries captures, 71 of beach seined and 35% of gillnetted fish captured and tagged at McMillan Island were detected at the final receiver group. The top model for fish tagged at McMillan Island included body size (β = 0.27, 95% C.I. = 0.14 – 0.41), fishing method (β = -1.73, 95% C.I. = -2.54 – -0.92), and time (Table 2.4, Figures 2.3, 2.4). Although a competing model (ΔQAICc = 0.51) for this tagging location included sex (Table 2.4), males and females had a similar likelihood to survive (β =

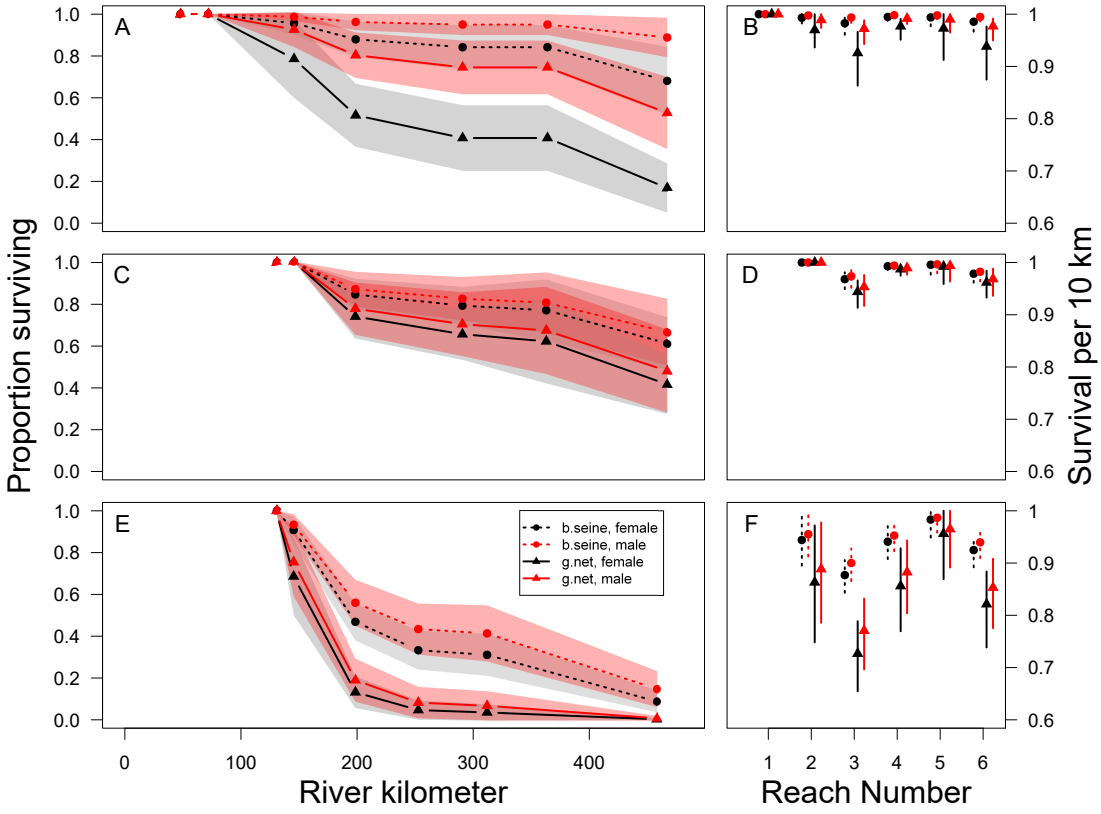

Figure 2.3: Plots of cummulative survival (left) and distance normalized survival across river reaches (right) for female and male Fraser River sockeye salmon captured by beach seine or gillnet at McMillan Island (A,B), Peters Road in 2014 (C,D), and Peters Road in 2015 (E,F). To show survival estimates across multiple river reaches, grouped by sex and capture method, but with confidence intervals, a full Cormack Jolly Seber model was created for each tagging group (ϕ = fishing method + sex + time, ρ = fishing method + sex +time). These estimates were generated using the "deltamethod.special" function in RMark.

0.16, 95% C.I. = -0.66 – 0.99). Sex was not a statistically significant factor in any of the top models, despite a consistent (and sometimes large) numerical difference (Figure 2.3).

For the Peters Road tagging location in 2014, 62 and 46% of beach seined and gillnetted fish (calculated as above) were detected at the final receiver group. The top CJS model only included time, indicating that differences among the river reaches accounted for more variation in survival than fishing method at Peters Road. As seen in CJS modeling for all tagging releases (Figure 2.3), survival was lowest between the Hope (rkm 146) and Hell's Gate (rkm 200) receivers, followed by the final river reaches monitored (Savona receiver [rkm 364] to Little River [rkm 467] receiver in 2014, Lillooet receivers [rkm 312] to Chilcotin receiver [rkm 458] in 2015). The next competing model (ΔQAICc = 1.10) included fishing method as a predictor of ϕ, and the effect of gillnetting was significantly negative (β = -0.57, 95% C.I. = -0.01 – -1.13).

For the Peters Road tagging location in 2015, survival was very low with only 9 and 2% of beach seined and gillnetted fish (calculated as above) detected at the Chilcotin receiver (rkm 458). The top model included fishing method (β = -1.95, 95% C.I. = -2.46 – -1.44) and time (Table 2.4). The next competing model (ΔQAICc = 1.64) included fishing method as a significant predictor of the probability of detection,

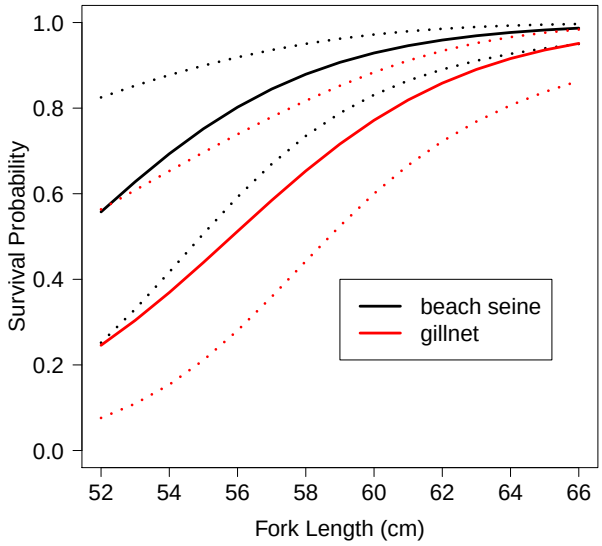

Figure 2.4: Predicted survival estimates based on fork length for sockeye salmon captured at McMillan Island by beach seine or gillnet. These estimates are derived from the best model (by QAICc rank), ϕ = fishing method + fork length + time, ρ = time. Survival was estimated for the reach from the Hope receiver (rkm 146) to the Hell's Gate receiver (rkm 199) which was the river reach with the highest mortality and greatest difference in survival between beach seined and gillnetted fish.

with the effect of gillnetting being significantly negative. In another competing model including sex as a predictor of survival, sex was not significant.

For the Savona tagging location in 2014, 82 and 75% of beach seined and gillnetted fish (calculated as above) were detected at the final receiver group. For GLM models (instead of CJS) compared by AICc, the null model had the lowest AIC, suggesting none of the explanatory variables adequately explained the probability of survival to Little River (rkm 467). The next three models by AIC included one of each of the explanatory variables used to create the full model, but none were significantly associated with survival (Table 2.4).

2.4.2 Migration Rate

For the Lower Fraser River release groups in 2014, gillnet capture significantly slowed migration to Hell's Gate (rkm 200) but not beyond to the spawning areas (Figure 2.5, Table 2.5). Neither body size nor sex were significantly associated with migration rate in any models.

To complete migration from the tagging location to Hell's Gate, late-run sockeye salmon tagged at McMillan island in 2014 took a median 6.88 (95 % C.I. = 6.78 – 7.74) and 7.64 (7.12 – 8.18) days for beach seine and gillnet caught fish, respectively. The same values for fish tagged at Peters Road in 2014 were 4.82 (4.74 – 4.95) and 5.91 (5.52 – 6.88) days. While parameter estimates were similar for the Peters Road location in 2014 and 2015, there was no significant effect of fishing method on migration time to Hell's Gate in 2015 (Table 2.5)). AFT models for the Lower Fraser River tagging locations indicated that gillnetted

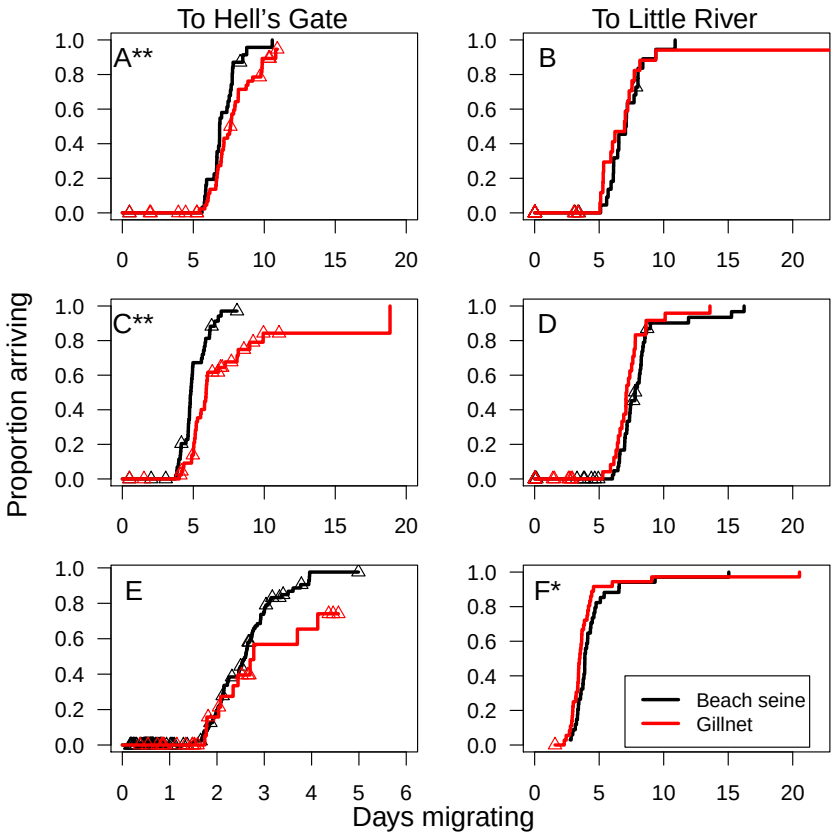

Figure 2.5: Kaplan-Meier plots of migration times to Hell's Gate (left) and Little River (right) for beach seined and gillnetted sockeye salmon tagged at McMillan Island (A,B), Peter's Road in 2014 (C,D), Peter's Road in 2015 (E), and Savona (F). The starting point was the Spence's Bridge receiver for panels B and D, and the tagging location for all other panels. An asterisk next to the title letter of a panel indicates significance at $p < 0.05$ and two asterisks indicate $p \leq 0.001$.

fish would take from 15% (McMillan 2014, 95% C.I. = 6 – 27%) to 21% (Peter's Road 2014, 14 – 35%) longer than beach seined fish to complete this section of migration. The observed difference for migration times in the lower Fraser River did not persist in the Thompson River for the groups tagged in the lower Fraser River in 2014. In contrast, beach seined fish migrated from the Savona tagging location to the Little River 13% (0 –28%) slower than gillnetted fish (Table 2.5, Figure 2.5).

A comparison of migration rates (Figure 2.6) showed that sockeye salmon caught by beach seine at both release locations in both years migrated significantly faster to the Hope receiver (rkm 146) than fish caught by gillnet (McMillan: $p = 0.04$; Peters Rd, 2014: $p < 0.001$; Peters Rd, 2015: $p < 0.001$). Thereafter, migration rate was not different between the groups except for the Peters Road group where in the following river reach, gillnet caught fish migrated significantly faster than beach seine caught fish ($p = 0.001$).

Table 2.5: Model results for accelerated failure time models applied to sockeye salmon captured by beach seine and gillnet. *P* values for explanatory variables significant at $p < 0.05$ are indicated in boldface.

				Explanatory Variables		
Tagging Group to Receiver	Survival Distribution	N obs (events)	Model Parameter	Sex (M)	Fishing Method (GN)	Fork Length (cm)
McMillan Island, 2014	lognormal	109	β	-0.08	0.15	-0.02
to Hell's Gate		(76)	SE	0.05	0.05	0.01
			p	0.14	**0.001**	0.09
Peter's Rd, 2014	loglogistic	131	β	-0.06	0.21	-0.01
to Hell's Gate		(105)	SE	0.05	0.04	0.01
			p	0.25	**< 0.001**	0.26
Peter's Rd, 2015	lognormal	180	β	-0.09	0.15	-0.004
to Hell's Gate		(86)	SE	0.06	0.08	0.01
			p	0.13	0.08	0.70
McMillan Island, 2014	loglogistic	68	β	-0.02	-0.05	-0.01
Spence's Bridge		(37)	SE	0.07	0.07	0.02
to Little River			p	0.80	0.40	0.63
Peter's Rd, 2014	loglogistic	94	β	-0.03	-0.06	0.003
Spence's Bridge		(63)	SE	0.05	0.04	0.01
to Little River			p	0.53	0.12	0.76
Savona, 2014	loglogistic	107	β	-0.09	-0.13	-0.003
to Little River		(70)	SE	0.09	0.06	0.02
			p	0.34	**0.04**	0.84

2.5 Discussion

Although it is often assumed that gillnet capture is a more deleterious fishing method than beach seining for adult Pacific salmon encountering these gear-types during their freshwater spawning migration (DFO 2017; RABY *et al.* 2015), this study provides the first side-by-side, empirical comparison of the two fishing methods where treatments were true capture experiences (as opposed to simulations). Similar to a study where gillnet capture was simulated (NGUYEN *et al.* 2014), we found that sockeye salmon released following capture by gillnet in the lower Fraser River had elevated mortality, higher physical damage, and slowed migration rate relative to those captured by beach seine. In contrast to those captured early in their freshwater migrations, fish captured by gillnet at the furthest upstream location experienced survival similar to, migrated to spawning grounds faster than, and, in the case of male fish, displayed physical damage indistinct from that of beach seined fish. These results indicate that gillnets generally cause more damage and mortality than beach seines, but the relative impact of these gears changes as salmon undergo physical and physiological alterations during migration. In light of these results, I suggest that FRIM can be reduced substantially under certain conditions, for example, the use of beach seines instead of gillnets will, in most cases, reduce FRIM until Pacific salmon have reached a level of maturity where they have greater resilience to capture (i.e.

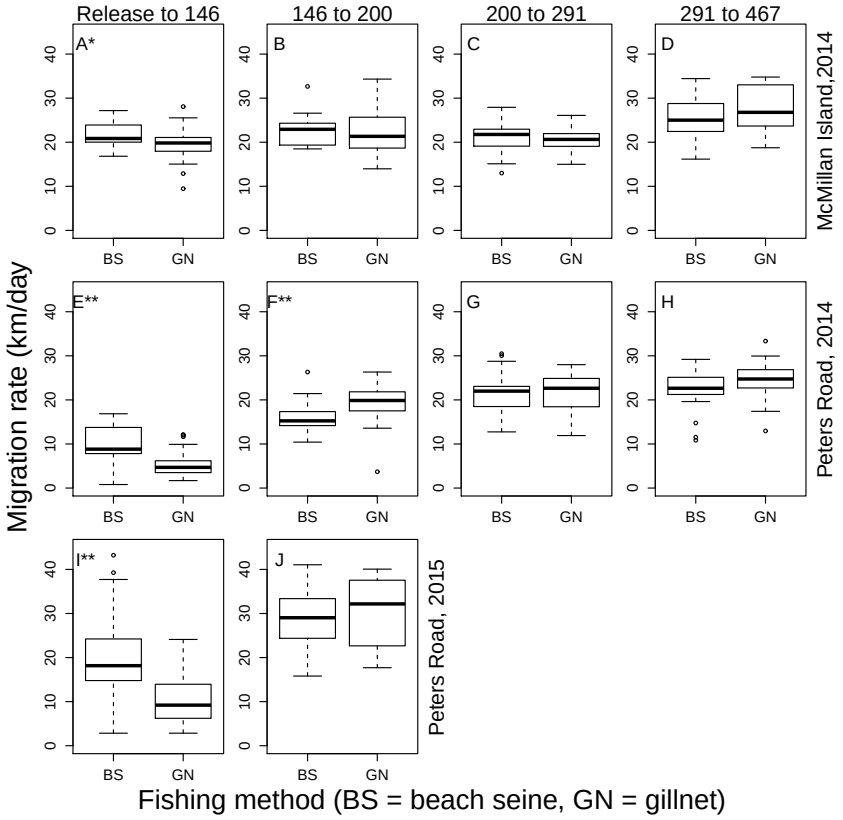

Figure 2.6: Boxplots comparing migration rates (km/day) between sockeye salmon captured by beach seine (BS) and gillnet (GN), radio tagged, and released in the Fraser River, BC. Capture location and year is indicated on the right side of each row of plots. Sample sizes are as follows: A = 17, 30 (BS, GN); B = 16, 23; C = 34, 34; D = 21, 16; E = 41, 38; F=36, 26; G = 58, 36; H = 39, 24; I = 171, 35; J = 82, 8. An asterisk next to the title letter of a panel indicates significance at $p < 0.05$ and two asterisks indicate $p \leq 0.001$. Comparisons were not available for migration beyond rkm 200 in 2015 due to low survival.

terminal fisheries).

Two findings in this study point towards the differences between beach seine and gillnet captured salmon that likely lead to elevated mortality for the latter. As evidenced by netscore, the physical damage caused by gillnets was almost always greater. Open wounds provide points of entry for infectious agents (SVENDSEN and BØGWALD 1997) and visible damage caused by contact with nets has been associated with mortality (BAKER and SCHINDLER 2009; OLSEN *et al.* 2012). The second finding indicating a difference between the two capture methods was the temporary migratory delay that occurred for gillnet caught fish. The stress response, anaerobic exercise, and asphyxiation are all experiences that cause an increase of lactate in fish blood and tissue (FARRELL *et al.* 2000; KOJIMA *et al.* 2004) and deep respiration accompanied by reduced movement is required for a return to homeostasis (HØGÅSEN 1998; LEE *et al.* 2003a). The gillnet capture experience may include burst swimming, constriction, asphyxiation resulting from the net sealing the operculum closed, and air exposure while removing fish from nets. From this list (assuming a net mesh appropriate for the target or bycatch species), the beach seine method employed herein (not dragging the beach seine on

to shore) likely only features burst swimming. I suggest that the delay of gillnet fish relative to beach seine fish was symptomatic of a need for additional recovery time and indicative of a more severe physiological impact (DONALDSON *et al.* 2010, 2011; JAIN *et al.* 1998) that could have long-term consequences in the form of increased consumption of stored energy and impaired immune defense (LUPES *et al.* 2006; MAULE *et al.* 1989).

An important caveat to the comparisons of FRIM between gillnet and beach seine capture is that the highest number of fish captured in a given beach seine set in this study (approx. 100) was low compared to the number of salmon (> 1000) in large sets where RABY *et al.* (2014) found oxygen levels to drop and asphyxiation occurred as a result. Interestingly, RABY *et al.* (2014) did not find catch size to be a significant predictor of survival to spawning grounds, although it was positively associated with an index of reflex impairment. The beach seine capture conducted in our study is likely more representative of a "best case scenario" where catch size was low and nets were kept in deeper water while fish were removed. However, the case is the same for gillnet capture; if fish densities were such that 1000 fish would be captured per beach seine, one would expect to catch many more fish in gillnet drifts, and fish would spend more time entangled in gillnets as others are disentangled. I therefore maintain that, although our results should not be applied to all scenarios involving gillnets and beach seines, in many circumstances gillnets will cause more FRIM than beach seines.

Although the survival for sockeye salmon captured and tagged during high water temperatures (2015) was lower than that of those tagged under cooler conditions (2014) and the difference in survival between fishing methods was magnified (see β estimates for fishing method), there are several caveats that prevent me from definitively ascribing these phenomena to temperature. First, I tagged the Late Shuswap population in 2014 and a mixture of summer-run populations in 2015 and previous studies have demonstrated that response to capture stressors may vary between populations (DONALDSON *et al.* 2010, 2012). Second, in-river fisheries pressure was considerably higher in 2015 compared to 2014, and fisheries openings occurred in close temporal and spatial proximity to our tagging dates. Regardless, many studies have confirmed the impact of temperature on adult Pacific salmon in the laboratory (ELIASON *et al.* 2011; GALE *et al.* 2011) and by using biotelemetery (GONIEA *et al.* 2006; MATHES *et al.* 2009). A study combining multiple years and populations of sockeye salmon in the Fraser River also identified this phenomenon (MARTINS *et al.* 2011). Furthermore, chronic stress has been shown to hinder wound healing (MATEUS *et al.* 2017), and the stressful elevated water temperatures experienced by the 2015 sockeye salmon therefore likely exacerbated the impact of gillnetting. Although not confirmed by a statistical comparison in this study, I suggest that high water temperatures contributed to the low survival and greater difference between the impacts of beach seine and gillnet observed in 2015.

Salmon skin thickens as Pacific salmon migrate upstream (ROBERTSON and WEXLER 1960), and scales are reabsorbed, more so for males than females (KACEM *et al.* 1998). As salmon approach spawning grounds, cortisol levels increase (BAKER and VYNNE 2014; ROBERTSON *et al.* 1961). These factors may lead to lower incidence of FRIM for fish captured close to spawning grounds since the integument is harder to rupture and stressors will cause minimal departure from baseline cortisol levels (RABY *et al.* 2013). Indeed, netscore at the furthest upstream capture location (Savona) was lowest for all fishing method and sex

combinations, although the difference was significant only for gillnet captured males. The summer-run sockeye salmon captured in 2015, which were the least mature fish based on their life history strategy (late-run Shuswap salmon mature more while milling in the estuary, while summer-run fish typically enter the Fraser River immediately (HINCH *et al.* 2012)), had the highest netscores for all combinations. In addition, the development of secondary sexual characteristics at Savona may have prevented males from becoming badly entangled in gillnets. I noticed that many males were snagged by their teeth, which were much larger at Savona than in the lower river, or were prevented from becoming entangled around their operculum by the development of their dorsal hump. Although the GLM analysis indicated that fishing method was not associated with survival to spawning grounds, there are two caveats to this result. The migration from Savona to spawning grounds was relatively short and characterized by slow moving water, which may have been an inadequate distance and rigor to invoke FRIM. Additionally, fishing by gillnet at Savona was conducted using a set net and entangled fish were brought to shore, disentangled and immediately placed in netpens after 5 min of struggle (whereas fish gillnetted in the lower river were often entangled for 5–10 min and were transported to netpens by boat). The capture experience at Savona might have been less stressful and damaging than drift gillnetting in the Fraser River, and the fact that gillnet fish arrived at spawning grounds in significantly less time than beach seined fish suggests that salmon captured by beach seine at this location required a longer recovery period. Although the finding that there was no difference in survival between the two capture treatments at Savona corroborates one of the few other studies that examined FRIM in close proximity to spawning grounds (RABY *et al.* 2013), I encourage more experiments to determine how broadly this phenomenon applies.

The top model for the first tagging location included body size as a significant explanatory variable, with larger fish more likely to survive than smaller fish. When a variety of fish sizes are corralled in a beach seine, smaller fish might be more likely to receive injury or be crushed than larger fish as has been observed in other net gears that corral fish (DAVIS *et al.* 2001; NEILSON *et al.* 1989). A study comparing the selectivity of different gillnet mesh sizes showed that 13.3 cm gillnets (used in this study) were most selective for Fraser River sockeye salmon with fork lengths of 58-60 cm (PETERSON 1954). It is likely that fish in this size range and below experienced greater damage since removal from gillnets would have been more difficult due to tighter entanglement around the operculum or anterior to the dorsal fin (median FL for population = 60 cm).

A design limitation of this study is that I only estimated survival to spawning areas and have no measures of actual spawning success (e.g., failed spawning is indicated by egg retention in females) for the experimental groups . BAKER and SCHINDLER (2009) found that while sockeye salmon with gillnet injuries arrived very close to the mouth of their spawning stream, 98, 92, 33, and 10% of fish with no, minor, moderate, and major injuries (respectively) entered the spawning stream. Of those that did enter, moderate and severely injured fish died rapidly, limiting spawning opportunities. These results indicate the importance of measuring spawning success when studying the impacts of FRIM, and I encourage the collection of these data in future studies.

In summary, I have provided evidence that gillnet capture in the lower Fraser River causes greater injury and subsequent mortality for sockeye salmon than beach seine capture. My findings of the interaction of gear type, injury, and location address a key information gap regarding the role of salmon maturity in

migration success and behavior (PATTERSON *et al.* 2017b). In some cases, prioritizing the use of one gear type over another may reduce FRIM to an acceptable level so that Pacific salmon fisheries can persist, and this approach will become more common as climate change proceeds. I caution that managers must give adequate consideration to the underlying context of capture (environmental and biological) for a given fishery, because this is sometimes more important than the gear type employed. Ultimately, my findings will contribute to improved science advice given to managers regarding FRIM and lead to improved accounting of fishing-related mortality (PATTERSON *et al.* 2017a).

Chapter 3

Visible gillnet injuries predict migration and spawning failure in adult sockeye salmon

3.1 Synopsis

Fish that survive fisheries interactions may subsequently die; a phenomenon generically termed fisheries-related incidental mortality (FRIM). Gillnets, which typically asphyxiate fish and visibly damage their integument, inflict higher rates of FRIM than other commonly used gears. To better define FRIM associated with gillnet encounters, an observational study coupled with biotelemetry measured migration survival and spawning success of a sockeye salmon population during the final 45 km of their freshwater spawning migration (in 2014, 2015, and 2016). The daily prevalence of gillnet injuries ranged from 0% to 80% of fish, resulting in annual prevalence of 21% to 29% for females and 13% to 22% for males (over three years). Fish with visible gillnet wounds had a 16% lower probability of completing their migration and female fish with gillnet wounds had an 18% lower probability of successfully spawning. As a result, the annual proportion of effective female spawners dying in the final 45 km of their migration due to gillnet injuries was estimated to range from 3.8% to 9.9% (500 to 1600 females). In addition, stray sockeye salmon from upriver populations commonly died at the tagging site, and visible gillnet injuries were observed in half of these fish in a year with high mortality. If salmon populations continue to decline as climate change progresses, fisheries managers will be under greater pressure to minimize FRIM.

3.2 Introduction

Commercial and subsistence fisheries provide significant economic, cultural, and food benefits around the world. For countries surrounding the northern Pacific Ocean, Pacific salmon (*Oncorhynchus* spp.) are a major component of these fisheries. In some regions, however, Pacific salmon returns have undergone significant declines (LICHATOWICH and LICHATOWICH 2001; NEHLSEN *et al.* 1991). Environmental factors such as climate change and anomolous climatic conditions (e.g. the "Pacific Blob" (BOND *et al.* 2015)) pose serious threats to the survival of cold-water dependent salmonids (BEAMISH and BOUILLON 1993; DALY *et al.* 2017; MARTINS *et al.* 2011). In addition to reductions in harvest size in response to dwindling fisheries, efforts have been made to minimize the negative side-effects of fishing. Of major concern are non-take fisheries interactions (NTFI), which encompass the capture and release of non-targeted fish (RABY *et al.* 2011), as well as unassisted disentanglement and escape of fish from fishing gear (BAKER and SCHINDLER 2009). Fish experiencing NTFI are subject to "fisheries-related incidental mortality" (hereafter "FRIM", PATTER-

SON *et al.* 2017a). The persistence of Pacific salmon fisheries will likely depend in part on the ability to minimize NTFI associated FRIM.

Following NTFI, salmon may experience associated "short-term" FRIM, within 24 hr of NTFI, or "delayed" FRIM, over the next several weeks (RABY *et al.* 2012; THOMPSON *et al.* 1971). Short-term FRIM is usually caused by asphyxiation (KOJIMA *et al.* 2004), pH disequilibrium following prolonged burst swimming (WOOD *et al.* 1983), or severe injury (CHOPIN and ARIMOTO 1995). Delayed FRIM may result from the immuno-suppressive aspects of the stress response to capture (PICKERING and POTTINGER 1989), pathogen infections due to disruption of the integument (SVENDSEN and BØGWALD 1997), and physiological imbalance caused by the loss of barrier function provided by the integument (MATEUS *et al.* 2017; OLSEN *et al.* 2012). The likelihood of NTFI resulting in FRIM may be dependent upon the physiological and environmental context (BROBBEL *et al.* 1996) under which NTFI occurs, and the type of fisheries gear in use (PATTERSON *et al.* 2017a).

Of the fisheries gear employed to capture upstream migrating sockeye salmon, *O. nerka*, (including gillnets, seines, fish wheels, rod and reel, weirs, and dipnets), gillnets typically cause the most physical damage (PATTERSON *et al.* 2017a). Researchers have linked gillnet NTFI to elevated stress (BAKER *et al.* 2013; TEFFER *et al.* 2017), delayed maturation (BAKER *et al.* 2013), delayed migration (NGUYEN *et al.* 2014; TEFFER *et al.* 2018), reduced longevity on spawning grounds (BAKER and SCHINDLER 2009), infection (BAKER and SCHINDLER 2009; TEFFER *et al.* 2018), and migration failure (DONALDSON *et al.* 2012; TEFFER *et al.* 2018). For planning the impacts of gillnet salmon fishing on non-target populations, Fisheries and Oceans Canada (DFO) utilizes an estimate that 60% of salmon that are released will experience FRIM (DFO 2017). There is no specified time period pertaining to this mortality estimate, but short-term mortality is assumed based on previous management plans. In some studies, the DFO 60% estimate has been consistent with experimental measures of migration survival for salmon released from gillnets (DONALDSON *et al.* 2010, Chapter 2). However, the endpoint for the DFO mortality estimate does not include the ability to spawn, which is a better proxy for lifetime fitness, nor does it include fish that escape from gear.

Most studies of FRIM involve either a simulation of fisheries gear (LUPES *et al.* 2006) or use of a "real-world" fisheries capture technique to collect fish (CANDY and QUINN 1999). FRIM is then determined by observing fish in a holding tank or using biotelemetry to track survival. When visible marks resulting from NTFI are present, a third strategy is to capture fish after they have already interacted with the fishery by using a lower impact capture method (BAKER and SCHINDLER 2009). Regardless of the technique for applying a NTFI treatment, most telemetry experiments on salmon only examine survival to a spawning area, even though multiple studies indicate that fisheries capture impacts reproductive development (BAKER *et al.* 2013; KUBOKAWA *et al.* 1999; SCHRECK 2010). In fact, there are no published biotelemetry studies that test the effects of fisheries NTFI on a direct measure of spawning success (for an indirect measure, see BAKER and SCHINDLER 2009).

In this study, a large, three-year biotelemetry experiment, accompanied by carcass evaluation, was conducted to investigate the impacts of gillnet NTFI on migration survival and spawning success for sockeye salmon during the last 45 km of a 360 km upstream migration. I hypothesized that sockeye salmon with visible gillnet injuries would be less likely to survive migration and have lower spawning success than those

without. To measure the occurrence of gillnet-associated FRIM among fish entering our study system, gillnet injuries were assessed on recently deceased fish collected at the tagging site. Fish with and without visible injuries were tagged to test for an association between gillnet NTFI and subsequent migration survival. To determine whether visible injuries were associated with reduced spawning success following successful migration, egg retention was assessed in female carcasses collected on spawning grounds.

3.3 Methods

3.3.1 Study area

Gates Creek sockeye salmon (GCss) spawn in Gates Creek, located at the southern end of Anderson Lake, in the Seton-Anderson watershed (Figure 3.1). The Seton River is located 312 km upstream from the mouth

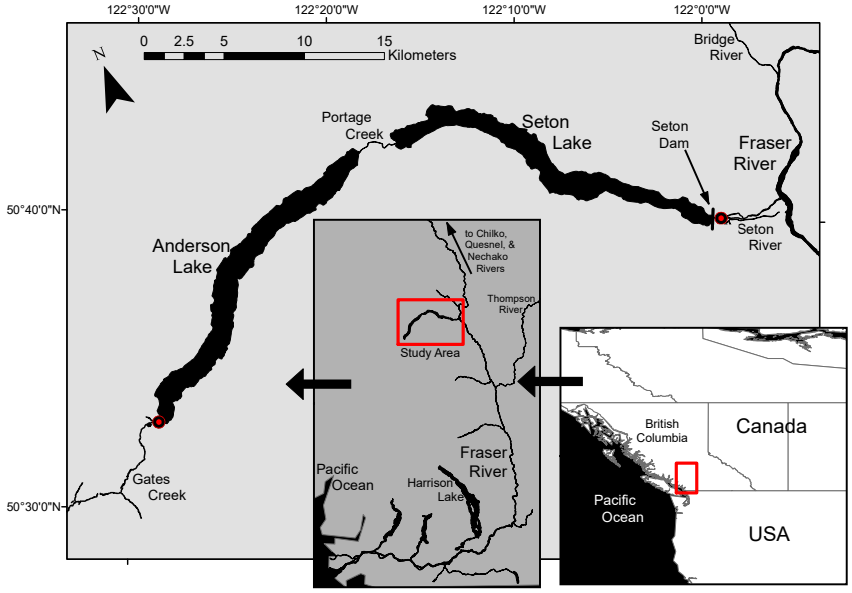

Figure 3.1: Map of the Seton-Anderson watershed in British Columbia, Canada. Red circles indicate PIT antennas at the Seton Dam (sockeye salmon were captured, tagged and released 200 m downstream of Seton Dam) and the mouth of Gates Creek.

of the Fraser River. After entering the Seton River, GCss swim 4 km to the Seton Dam, then migrate 45 km through Seton Lake, Portage Creek (where there is a small rod and reel fishery), and Anderson Lake to reach Gates Creek. GCss require approximately 10 days to migrate from the ocean to the Seton Dam (CROSSIN *et al.* 2009), and spend an average 10 days in Seton and Anderson Lakes before entering Gates Creek (MINKE-MARTIN *et al.* 2018). The peak Fraser River entry of GCss occurs in late-July, exposing this population to gillnet fisheries primarily targeting the larger "summer-run" populations migrating further upstream (entering the Chilcotin, Nechako, and Quesnel Rivers). As a consequence, many GCss passing through the system have sustained gillnet injuries during their migration (CASSELMAN *et al.* 2016), anywhere from the estuary to the Seton River confluence. Since most gillnet fisheries occurring during the GCss

migration target sockeye salmon, I expected that the vast majority of GCss experiencing NTFI have escaped nets on their own, as opposed to being released by fishers.

3.3.2 Fish collection, biopsy, and tagging

In the summers of 2014, 2015, and 2016, sockeye salmon were collected from a river-spanning fishing fence (200 m downstream of Seton Dam; N 50.6702°, W -121.9751°, Figure 3.1). Fence panels were installed in a downstream-facing "V" to direct fish into a trapbox at the upstream end of the fence. Captured fish were transferred via dip-net from the trapbox to in-river flow-through holding pens and held up to one hour prior to tagging. Fish with all levels of visible injury, so long as behavior of the fish did not indicate imminent mortality, were selected for tagging in this study (unlike in fish-passage studies conducted from the fence where only fish with minor or no injuries were selected: BURNETT *et al.* 2017; MINKE-MARTIN *et al.* 2018).

Fish were transferred from the holding pens into a V-shaped trough continuously supplied with fresh river water and restrained by hand. First, gross somatic energy (GSE) was measured using a fish FatMeter (Model FM 692, Distell, West Lothian, Scotland, UK) as described in CROSSIN and HINCH (2005). By comparing GSE measurements to population origin determined by genetic tests, CASSELMAN *et al.* (2014) found that a proportion of sockeye salmon captured below the Seton Dam did not originate from the Gates Creek population and these fish could be reliably identified via elevated GSE estimates. To attain migration and spawning success estimates unbiased by the inclusion of salmon from other populations, only fish with a FatMeter reading $\leq$2.7 (equivalent to a GSE of $\leq$7.2 MJ/kg) were tagged in this study. Sockeye salmon meeting this criteria were tagged with individually-coded 32 mm half-duplex (HDX) passive integrated transponder (PIT) tags (Oregon RFID, Portland, OR, USA) implanted subcutaneously in the dorsal sinus. For visual identification, a 305 mm spaghetti tag (Floy Tag & Mfg. Inc., Seattle, WA, USA) was inserted behind the dorsal fin and secured with an aluminum crimp. Lastly, each fish was visually assessed for injuries, then released on the upstream side of the fence.

Gillnet injuries were readily identifiable as linear wounds that ranged in severity from thin dark lines to large open wounds. Such injuries were typically located around the snout, on the dorsal side posterior to the head, and extending from the anterior tip of the dorsal fin to the base of the pelvic fins (Figure 3.2C). Researchers had first-hand experience identifying gillnet wounds from previous studies where sockeye salmon were experimentally inserted into gillnets (TEFFER *et al.* 2017) and studies where fish were captured by gillnet (BASS *et al.* 2018).

Sea lice injuries were visible as scarring, scale loss, or skin loss lateral or posterior to the dorsal fin, posterior to the adipose fin, superior to the anal fin, or on the ventral side of the fish (Figure 3.2E,F). Predator wounds were apparent as parallel scars commonly on the lateral and ventral side of the fish, running down the body, or as large portions of missing tissue, sometimes including the pelvic fins, on the ventral side. Hook wounds consisted of portions of flesh missing from the mandible, maxillary, or proximate area. Lamprey-like wounds were identified as circular openings approximately 2 cm in diameter, along the sides of the fish, and through the dermis. Injuries that did not meet any of the above criteria, including missing skin and scales, split or partially missing fins, bruises or abrasions, and deep wounds, were recorded as "unknown" in origin (Figure 3.2G). Multiple injury types were often present on a single fish.

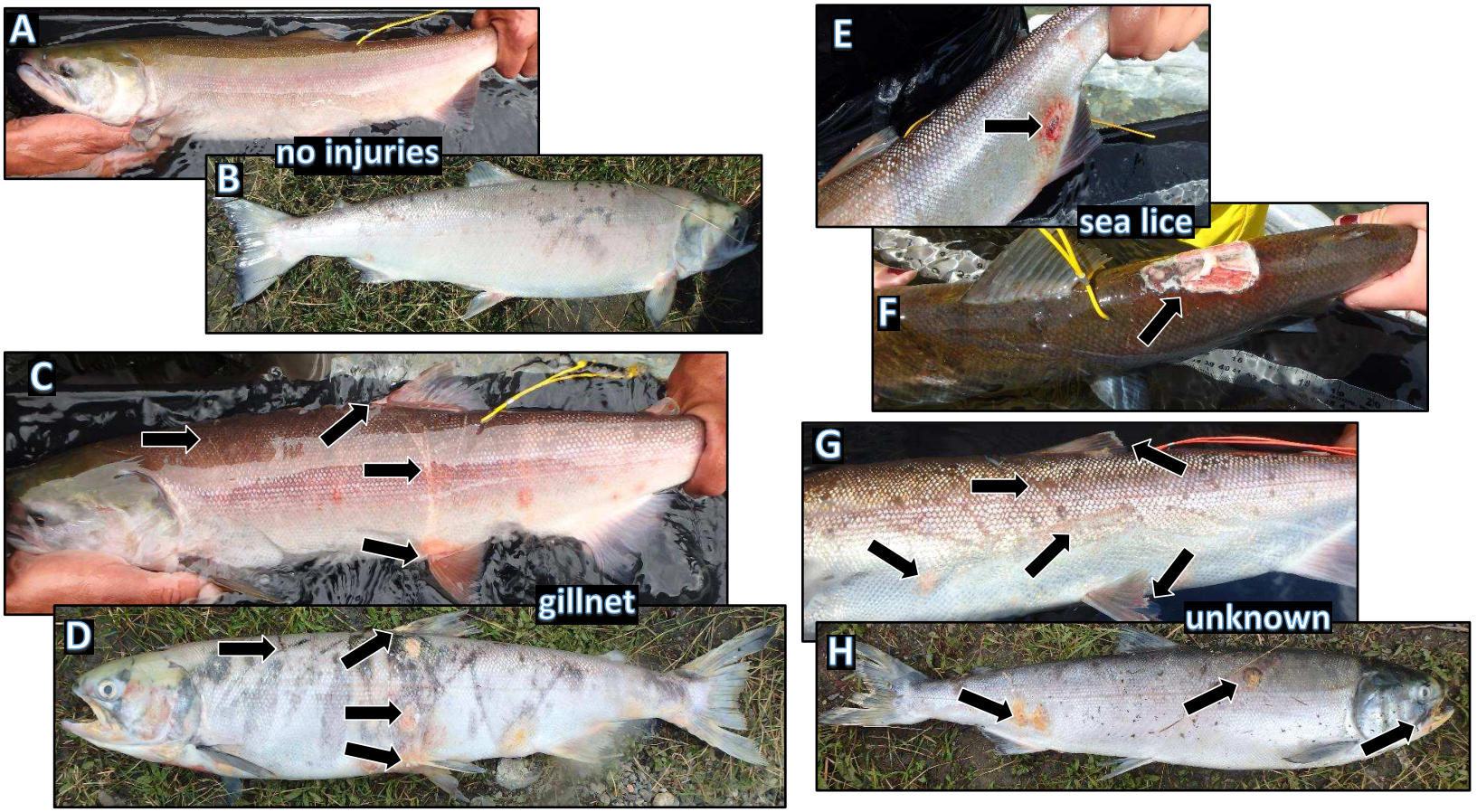

Figure 3.2: Sockeye salmon with the three most prevalent visible injury categories (C–H) and without injury (A,B) photographed during the tagging process (A,C,E,F,G) and after recovered dead (B,D,H) from the fishing fence, Seton River, British Columbia (August 8–17, 2015). Arrows in C and D indicate faint gillnet marks posterior to the head, damage to the insertion of the dorsal and pelvic fins, and linear wounds extending between dorsal and pelvic fins. Sea lice scars were commonly found at the base of the anal fin (E) and between the dorsal and adipose fins (F, an extreme example). Injuries with no obvious origin, including descaling, skin damage, and frayed and eroded fins (G and H) were categorized as “unknown”.

3.3.3 Recovery of mortalities from the fishing fence

Because Seton Dam is a migration obstacle that requires vigorous swimming to pass (BURNETT *et al.* 2014), a number of sockeye salmon expire immediately downstream. The majority of these dead salmon were washed downstream and collected on the fishing fence. On a daily basis (sometimes several times a day during periods of high mortality), researchers removed dead salmon from the fence, measured their fork length, and determined sex by dissection. Injuries were assessed as described above (Figure 3.2). An adipose fin clip was collected for population identification. Microsatellite analysis was conducted at the Pacific Biological Station (Nanaimo, B.C.), on a subset of fish collected each year, to determine population of origin by variation in the major histocompatability complex (BEACHAM *et al.* 2004).

3.3.4 Resistivity counter at Seton Dam

Salmon exiting the fishway at Seton Dam and entering Seton Lake were enumerated using electronic fish counters that use resistivity sensors to record passing fish (Logie 2100c, Aquantic Ltd., Scotland, UK). See BURNETT *et al.* (2017) for a detailed description of the resistivity counters and equations to estimate the abundance of sockeye salmon passing Seton Dam. In 2015, a large run of pink salmon, *O. gorbuscha*, migrated through the fishway starting in late August and species ratios determined by video validation of the resistivity counter data were applied to estimate GCss abundance (CASSELMAN *et al.* 2016).

3.3.5 PIT telemetry

PIT antennas were installed at the Seton Dam, at the mouth of Gates Creek, and at the entrance to the Gates Creek spawning channel. In this study, only detections at the Gates Creek receivers were required for determining migration survival. In lower Gates Creek, a 20 x 1 m pass-through antenna was constructed from 2.5 cm PVC pipe and stranded electrical wire connected to a tuning box and a single-antenna HDX PIT reader (Oregon RFID). At the entrance pool of the Gates Creek spawning channel, a 5 x 1 m pass-through antenna was installed. The combined detection efficiency for the Gates Creek PIT antennas was determined by calculating the proportion of physically recovered GCss (see below) that were detected by any antenna in Gates Creek. Combined detection efficiency of the Gates Creek installations was 76, 90, and 87% for 2014, 2015, and 2016, respectively.

3.3.6 Carcass recoveries and spawning success

Fish that successfully reached Gates Creek were able to spawn in either the Gates Creek artificial spawning channel, in lower Gates Creek (from downstream of the spawning channel to the mouth), or in upper Gates Creek (upstream of the spawning channel). The spawning channel was walked daily, and Gates Creek was walked at least once weekly, to recover carcasses identified via the external spaghetti tag or a hand-held PIT reader. Dead females were dissected and the percentage of eggs retained was scored in three categories. Females that had not deposited any eggs were categorized as complete retention, females with greater than $>$500 eggs remaining were categorized as partial retention, and females with $<$500 eggs remaining were categorized as completely spawned.

A small portion of tagged fish were captured in a rod and reel subsistence fishery at Portage Creek. The existence of this fishery was unknown to researchers in 2014. Voluntary reporting, canvassing, and streamside surveys were conducted in 2015 and 2016 to determine the identities of these fish. Any fish that were recovered from fisheries were removed from the analysis herein.

3.3.7 Statistical analyses

I calculated the daily prevalence of gillnet marks for GCss as the number of PIT tagged fish with gillnet marks divided by the number of fish PIT tagged on a given day. I calculated this estimate for each study year, along with the prevalence of other visible injuries.

Physical characteristics of PIT-tagged GCss, including fork length and GSE, were compared between males and females using Student's t-tests. The probability of occurrence of the three most prevalent types of visible injury (gillnet marks, sea lice scars, unknown injury) were compared between the sexes using Pearson's chi-squared tests. Chi-squared tests were also used to compare sex ratios and gillnet prevalence between the PIT-tagged and dead fence-collected fish.

To determine whether gillnet injuries were associated with migration survival and spawning success, I used a model averaging approach based on AIC selection of generalized linear models including covariates measured at the time of tagging (GRUEBER *et al.* 2011). Time-related covariates included year of tagging and tagging date (as day of year). Covariates associated with individual characteristics were sex, fork length and GSE. In addition to gillnet injury, I included the two other most prevalent types of injury (sea lice scars and unknown injury; Table 3.1) as binary variables (present = 1, absent =0). The same set of explanatory variables were used in models of migration survival and spawning success (excluding sex, only females were used in spawning success models). All statistical analyses were conducted in R version 3.4.0 (R CORE TEAM 2017).

I employed a generalized linear model (GLM) with a binomial response (family = binomial, link = logit) for all models. Arrival at spawning grounds was determined by either detection at the lower Gates Creek antenna, detection at the spawning channel antenna, tag recovery in Gates Creek or the spawning channel, or carcass recovery of tagged individuals. Binary responses for spawning success among females were designated as "0" for complete and partial egg retention, and "1" for completely spawned. Note that this parameterization assumes that there is no fitness benefit for a female salmon to stop spawning after releasing only half of its eggs, and therefore partial retention should be considered incomplete spawning due to prespawn mortality. Furthermore, preliminary analysis using an ordinal regression model with the three different retention categories showed no difference between complete and partial retention in their relation to the explanatory variables.

Both full models were tested for multi-collinearity using the "vif" function from the "car" package (FOX and WEISBERG 2011), and the variance inflation factor never exceeded 2. I standardized all explanatory variables using the "standardize" function from the "arm" package, so that the relative effect could be compared between explanatory variables (GELMAN and SU 2016). Using this function, continuous variables were standardized to a mean of 0 and a standard deviation of 0.5. Standardizing the continuous variables precluded the need for data transformation. Binary variables were scaled to a mean of 0 with a difference of

1 between their two categories. I used the "dredge" function from the "MuMin" package (BARTON 2016) to compare all possible variable combinations and rank them based on AIC (BURNHAM and ANDERSON 2003). Variable coefficients were averaged based on the 95% confidence model set, as determined by AIC weight. I used the zero average method (aka the "full model set") (GRUEBER *et al.* 2011) where variables were assigned a zero when not included in a model. Ninety-five percent confidence intervals (CI) were calculated for the model coefficients. Variables were considered to have an important association with the response when their 95% CI did not overlap with zero.

To interpret logistic regression coefficients, we presented the odds ratio as percentages with 95% CI, as well as the average-of-marginal-effects method for a more intuitive interpretation (FERNIHOUGH 2011). Although variance explained in ordinary least squared regression models is indicated by r^2, this metric does not exist for a binomial GLM. I therefore estimated McFadden's pseudo-r^2, where 0.2–0.4 is indicate of an extremely good model fit (LOUVIERE *et al.* 2000).

I used the following equation to estimate, for each study year, the percent of GCss effective female spawners lost from the population as a result of gillnet injury during their migration from the fishing fence to Gates Creek:

$$EF_L = (SU_U \cdot SP_U - SU_M \cdot SP_M) \cdot P_M \tag{3.1}$$

Where EF_L is the proportion of effective female spawners lost, SU is the proportion surviving migration, SP is the proportion spawning successfully, and P is prevalence (proportion) of gillnet injuries at the fishing fence. Subscripts M and U indicate gillnet marked and unmarked fish, respectively. Equation 3.1 assumes that fish without gillnet marks provide a baseline survival estimate for the GCss population and the difference between this and the survival estimate for gillnet marked fish can be attributed to the impact of gillnet NTFI. To determine the 95% CI for EF_L, I bootstrapped (1000 samples) the standard error for each parameter in Equation 3.1 using the "boot" package (DAVISON and HINKLEY 1997) and used the following equation to calculate the standard error:

$$\frac{\delta EF_L}{EF_L} = \sqrt{\left(\frac{\left(\sqrt{\left(\frac{\delta SU_U}{SU_U}\right)^2 + \left(\frac{\delta SP_U}{SP_U}\right)^2} \cdot EF_U\right)^2 + \left(\sqrt{\left(\frac{\delta SU_M}{SU_M}\right)^2 + \left(\frac{\delta SP_M}{SP_M}\right)^2} \cdot EF_M\right)^2}{EF_U - EF_M}\right)^2 + \left(\frac{\delta P_M}{P_M}\right)^2} \tag{3.2}$$

Where δ is the standard error for each parameter and EF_M and EF_U are simply the products of SU and SP for fish with and without gillnet marks, respectively. δEF_L was multiplied by 1.96 and added to and subtracted from EF_L to calculate the upper and lower CI.

3.4 Results

3.4.1 Physical condition of PIT-tagged sockeye salmon

Peak passage at Seton Dam varied considerably across the study years, occurring as early as August 5 in 2016 and as late as August 23 in 2014 (Figure 3.3). Researchers captured, tagged, and released 497, 455,

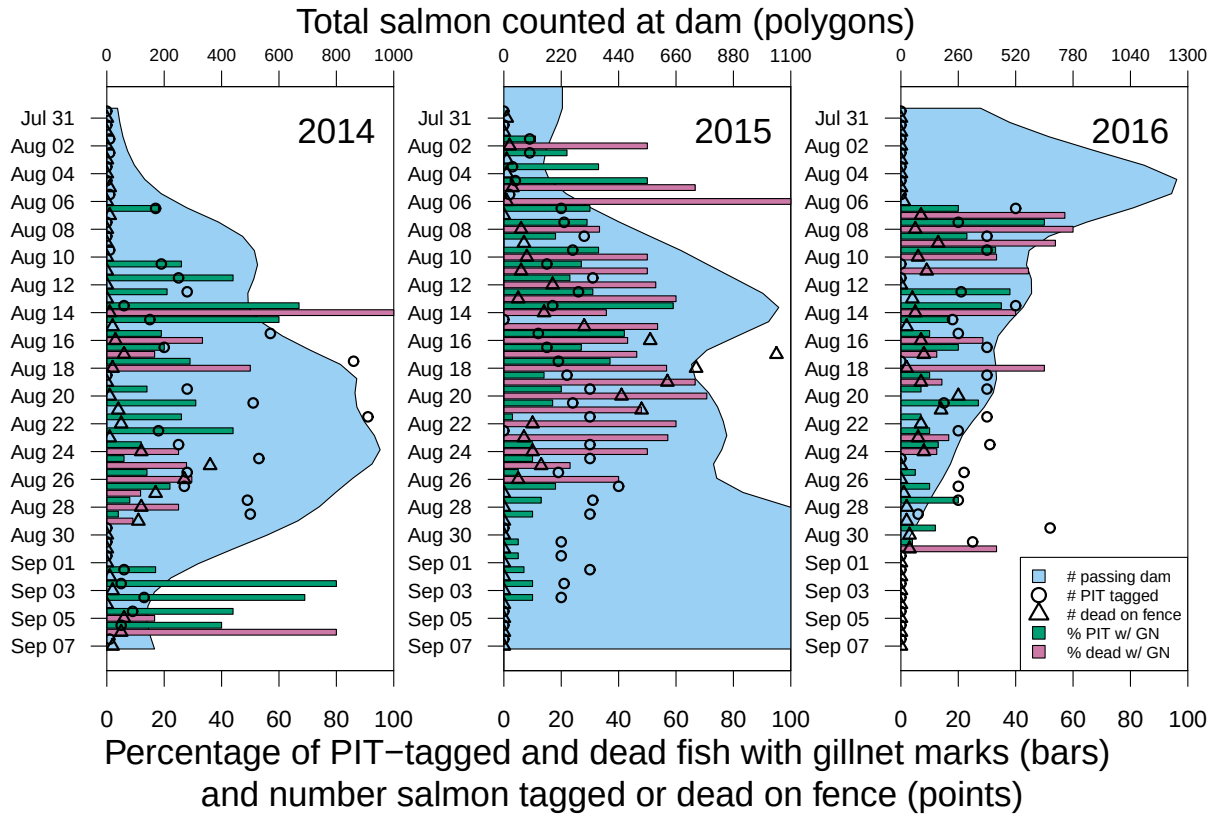

Figure 3.3: Barplots of gillnet (GN) injury prevalence in PIT-tagged and dead collected sockeye salmon over three years in the Seton River, British Columbia. The number of fish detected passing Seton Dam (blue background polygon), gillnet prevalence (bars), and sample size (points) are represented. In 2015, the pink salmon migration overlapped with the later portion of the sockeye salmon migration (first pink salmon observed near fence on August 18th) and thus the daily number of salmon appears to increase in late August, but this is a mix of the two species.

and 433 sockeye salmon at the fishing fence in 2014, 2015, and 2016, respectively. Females were always more abundant than males in the tagged population (Table 3.1). Based on all years combined, males were larger than females ($p<0.001$) but females had higher GSE ($p<0.001$). GSE was significantly lower in 2015 than in 2014 and 2016 ($p<0.001$). Daily gillnet injury prevalence varied from 0 to 80% throughout the tagging periods but generally peaked in early- to mid-August, except in 2014 when there was a peak towards the end of the migration (Figure 3.3).

Gillnet injuries, sea lice scars, and unknown injuries were the most prevalent categories of visible injury (Table 3.1). Across the three study years, annual gillnet injury prevalence was 21–29% for females and

Table 3.1: Summary statistics (± standard deviation) for sockeye salmon captured, PIT-tagged, and released at a fishing fence in the Seton River, British Columbia (2014 –2016). FL = fork length (cm), GSE = gross somatic energy (MJ/kg). Visible injuries are presented as percentages of the total number of female or male fish. Multiple injury types can be present in a single fish. GN = gillnet marks, SL = sea lice scars, HO = hook wound, PR = predator wound, LA = lamprey wound, UNK = injury of unknown origin, NONE = no injury.

				GSE	Injury (%)						
Year	Sex	N	FL (cm)	(MJ/kg)	GN	SL	HO	PR	LA	UNK	NONE
2014	F	302	58.7 ±3.5	5.9 ±0.6	29	21	4	3	0	19	41
	M	195	60.6 ±4.8	5.8 ±0.6	22	19	8	3	0	19	45
2015	F	234	57.1 ±2.7	5.7 ±0.6	21	46	1	7	1	48	13
	M	221	59.1 ±3.1	5.4 ±0.6	17	37	1	9	1	35	28
2016	F	232	56.8 ±2.4	5.9 ±0.6	24	43	0	7	8	47	13
	M	201	60.2 ±2.6	5.9 ±0.6	13	30	0	9	5	41	30
All	F	770	57.7 ±3.1	5.8 ±0.6	24	36	2	5	1	37	24
years	M	637	59.5 ±3.7	5.7 ±0.6	18	31	3	7	1	30	33

13–22% for males (Table 3.1). Twenty-eight percent of GCss had no injuries, 56% had a single injury (from the previously mentioned categories), and 16% had two or more. When data from the three study years were pooled, both gillnet injuries and sea lice scars occurred in a greater percentage of females than males ($p<0.001$).

3.4.2 Migration survival

Migration survival from the fishing fence to Gates Creek for the PIT-tagged fish was consistently between 77 and 78% for the three study years (Table 3.2). I found no significant effect of year or tagging date in a GLM with arrival at Gates Creek as the response variable (Figure 3.4). Of the individual characteristics, both fork length and GSE were associated with survival, but sex was not significant in any models, even though it was included in a model competing with the top model (Table 3.3, Figure 3.4). For each standard deviation increase in fork length (3.5 cm), GCss had 21% (95% CI = 4–45%) increased odds of arriving at Gates Creek. For each standard deviation increase in GSE (1.4 MJ/kg), GCss had 40% (95% CI = 30–50%) decreased odds of arriving at Gates Creek. Expressed as average marginal effects, a standard deviation increase in fork length and GSE results in a 3% increase and 7% decrease in probability of arrival at Gates Creek, respectively (Figure 3.5). All three of the most prevalent visible injuries (gillnet marks, sea lice scars, and unknown injury) were negatively associated with migration survival (Figure 3.4). Fish with gillnet marks had 62% (95% CI = 48–72%) lower odds of arrival at Gates Creek than those without gillnet marks; equivalent to an average marginal effect of a 16% decrease in probability of survival (Figure 3.5). Fish with sea lice scars and unknown injuries had 43% (95% CI = 22–58%) and 42% (95% CI = 19–58%) lower odds of successful migration than those without, respectively; equivalent to an 8% lower probability of survival

Table 3.2: Migration survival and spawning success of PIT-tagged Gates Creek sockeye salmon released from a fishing fence in the Seton River, British Columbia (2014–2016). N_{surv} = population size used for estimating migration survival and determining associated variables. N_{spawn} = population size used for estimating spawning success and modeling associated variables. The percent of fish captured in a small food fishery in Portage Creek is presented (not reported in 2014).

Year	Gillnet marks	Sex	N_{surv}	% Surviving to Gates Ck	% Caught in fishery	N_{spawn}	Complete retention (%)	Partial retention (%)	Complete spawned (%)
2014	absent	female	214	85	–	118	15	9	76
		male	152	85	–	–	–	–	–
	present	female	88	59	–	42	36	14	50
		male	43	60	–	–	–	–	–
	Total		497	78	–	160	21	10	69
2015	absent	female	186	76	4	64	25	15	60
		male	184	83	3	–	–	–	–
	present	female	48	67	2	25	56	4	40
		male	37	70	0	–	–	–	–
	Total		455	78	3	89	34	12	54
2016	absent	female	177	74	2	69	17	9	74
		male	175	86	1	–	–	–	–
	present	female	55	58	4	19	47	5	47
		male	26	69	12	–	–	–	–
	Total		433	77	2	88	24	8	68
All years	absent	female	577	79	2	251	19	10	71
		male	511	85	2	–	–	–	–
	present	female	191	61	2	86	44	9	47
		male	106	66	3	–	–	–	–
	Total		1385	78	2	333	25	10	65

for each (Figure 3.5). McFadden's pseudo r^2 for the top model was 0.07.

3.4.3 Spawning success

Spawning success for GCss females was 65% on average, and lowest in 2015 at 54% (Table 3.2). Although year was included in all top GLM models, and 2015 was significantly lower in the top models, the CI for 2015 overlapped with zero (Figure 3.4). Females in 2015 had 51% (95% CI = -8–78%) lower odds of successful spawning than those in 2014. The marginal effect of 2015 was a decrease of 18% in the probability of successful spawning (Figure 3.6). Females with gillnet marks and higher GSE were more likely to retain half or more of their eggs at death (Figure 3.4). Females with gillnet marks had 59% (95% CI = 30–76%) lower odds of successful spawning than those without. This equates to a marginal effect of a decrease of 18% in the probability of successful spawning (Figure 3.6). For each standard deviation increase in GSE (1.3 MJ/kg), females had 41% (95% CI = 20–56%) lower odds of successful spawning. The marginal effect of a standard deviation increase in GSE was a 12% decrease in the probability of successful spawning (Figure 3.6). McFadden's pseudo r^2 for the top model was 0.10.

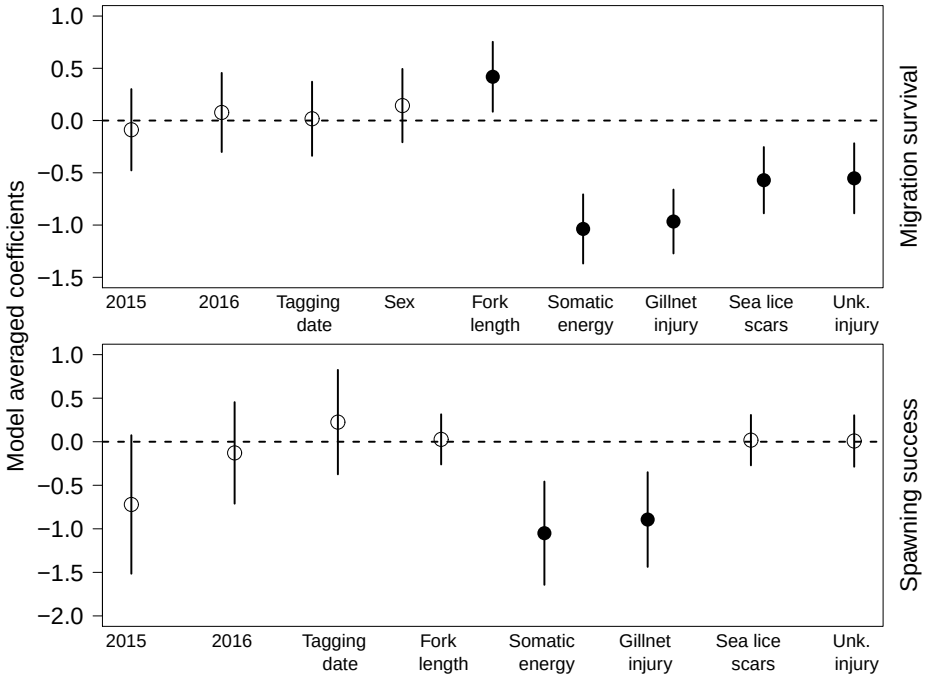

Figure 3.4: Model-averaged, standardized coefficients from GLMs of migration survival and spawning success for Gates Creek sockeye salmon PIT-tagged in the Seton River, British Columbia. Dark circles represent variables that are significantly different from zero (represented by the horizontal dashed line). Vertical lines represent the extent of the 95% C.I. for each explanatory variable. Coefficients are standardized so that effect sizes are comparable among variables.

3.4.4 Population level estimates of gillnet associated mortality

From 2014 to 2016, 54, 42, and 52% of the PIT-tagged female GCss population arriving at the fishing fence were estimated to have been effective spawners (% surviving migration multiplied by % completely spawned, Table 3.2). Based on Equations 3.1 and 3.2, I estimate that delayed mortality from gillnet injuries removed roughly 9.9% (95% CI = 5.8–14.1%), 3.8% (0.3–7.4%), and 6.4% (2.1–10.8%) (2014, 2015, and 2016, respectively) of the effective female spawners from the GCss population between the fish fence and Gates Creek. Based on counts of sockeye salmon from the resistivity counters at Seton Dam and the percent of females in the sampled population, this would equate to 1642 (95% CI = 962–2322) female sockeye salmon in 2014, 508 (40–989) in 2015, and 648 (213–1084) in 2016.

3.4.5 Mortality below the Seton Dam

Researchers collected 155, 504, and 142 dead sockeye salmon downstream of Seton Dam (washed up on fishing fence) in 2014, 2015, and 2016, respectively (Table 3.4). The ratio of females to males was similar between the dead fish collected on the fence and the PIT-tagged fish, except in 2016 when females were more common in the dead group ($p \leq 0.001$). Prevalence of gillnet injuries (Table 3.4) was similar between the dead and PIT-tagged fish in 2014 and 2016 (21 vs 27% for 2014 and 23 vs 19% for 2016), but was

Table 3.3: Top five GLMs based on AIC selection for migration survival and spawning success of Gates Creek sockeye salmon PIT-tagged in the Seton River, British Columbia, 2014-2016. FL = fork length and GSE = gross somatic energy.

Response	Model structure	logLik	AIC	ΔAIC	weight
Migration survival	FL + gillnet inj. + sea lice + unk inj. + GSE	-682.8	1377.6	0	0.36
	FL + gillnet inj. + sea lice + unk inj. + GSE + sex	-682.5	1379.1	1.5	0.17
	FL + gillnet inj. + sea lice + unk inj. + GSE + tagging date	-682.8	1379.6	2.0	0.13
	FL + gillnet inj. + sea lice + unk inj. + GSE + year	-682.4	1380.9	3.3	0.07
	FL + gillnet inj. + sea lice + unk inj. + GSE + tagging date + sex	-682.5	1381.1	3.5	0.06
Spawning success (females)	gillnet inj. + GSE + year	-193.9	398.0	0	0.17
	gillnet inj. + GSE + year + tagging date	-193.0	398.2	0.2	0.15
	gillnet inj. + GSE + year + sea lice	-193.8	400.0	2.0	0.06
	gillnet inj. + GSE + year + FL	-193.9	400.0	2.0	0.06
	gillnet inj. + GSE + year + unk. inj.	-193.9	400.0	2.0	0.06

Table 3.4: Summary statistics (± standard deviation) for dead sockeye salmon collected after washing up on a fishing fence in the Seton River, British Columbia (2014 –2016). FL = fork length (cm). Visible injuries are presented as percentages of the total number of female or male fish. Multiple injury types can be present in a single fish. GN = gillnet marks, SL = sea lice scars, HO = hook wound, PR = predator wound, UNK = injury of unknown origin, NONE = no injury.

				Injury (%)					
Year	Sex	N	FL (cm)	GN	SL	HO	PR	UNK	NONE
2014	F	93	57.7 ±3.3	24	5	4	1	54	12
	M	62	58.6 ±7.4	23	2	6	0	65	5
2015	F	284	58.7 ±4.5	49	27	<1	1	49	1
	M	220	59.4 ±6.3	55	27	<1	1	39	2
2016	F	103	60.4 ±3.4	20	63	1	4	47	2
	M	39	62.5 ±3.2	26	54	0	5	56	0

significantly higher in the dead population in 2015 (51 vs 19%, $p \leq 0.001$). Peaks in gillnet mark prevalence for the dead population appeared to lag 2–4 days behind peaks in gillnet mark prevalence in the PIT-tagged population (Figure 3.3).

The population of origin for a subset of the dead fish was determined for all but one individual (Table 3.5). Although the Seton River is part of the migration route for only GCss, the majority of dead fish were not from the GCss population (79, 90, 80% in the three years, respectively). The most abundant population complex in the dead group was composed of sockeye salmon migrating through the Chilcotin River, a further

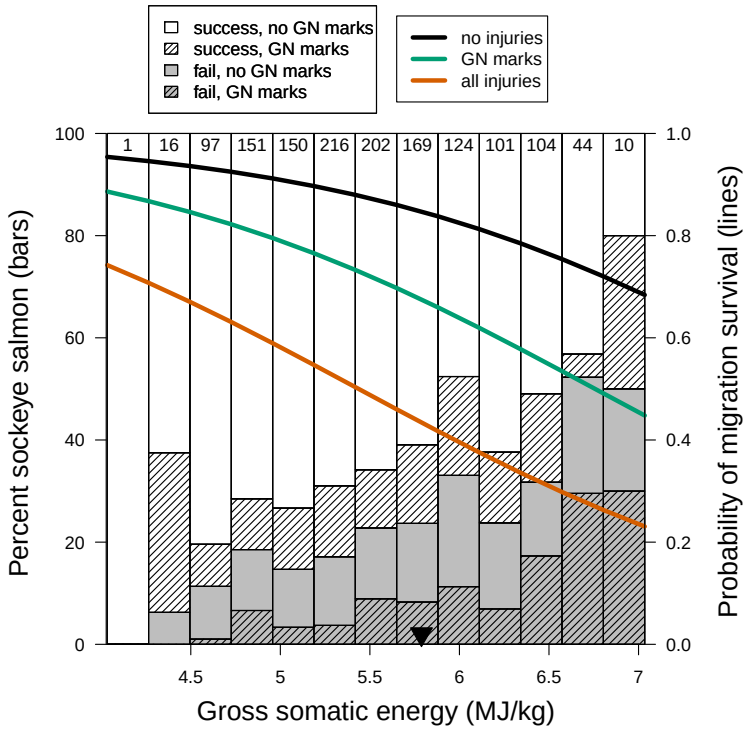

Figure 3.5: Probability curves of migration survival (from top GLM) for Gates Creek sockeye salmon PIT-tagged in the Seton River, British Columbia, 2014-2016. Curves show the difference in survival between fish with no injuries, gillnet injuries, and gillnet, sea lice, and unknown injuries, across a range of GSE. The background indicates the percentage of fish with and without gillnet marks surviving across a range of GSE. The population median GSE is indicated by the black triangle. Sample size for each bin is given at the top of the plot.

146 km upstream of the Seton River confluence (Table 3.5). At the extreme distances from the Seton River

Table 3.5: Population of origin (counts) for sockeye salmon found dead on a fishing fence in the Seton River, British Columbia (2014–2016).

Fraser River tributary	Spawning location	2014	2015	2016
Harrison River	Harrison River	1	0	0
Thompson River	Little River	2	0	0
	Fennel Creek	0	1	0
Seton River	Gates Creek	13	5	10
Chilcotin River	Chilko River	21	24	35
	Chilko Lake south	7	7	1
Quesnel River	Horsefly River	4	0	0
Nechacko River	Stellako River	9	9	0
	Paula, Felix Creeks	1	0	0
	Tachie River	2	0	0
	Middle River	1	0	0
	Nadina River	0	5	3
Bowron River	Bowron River	0	0	1

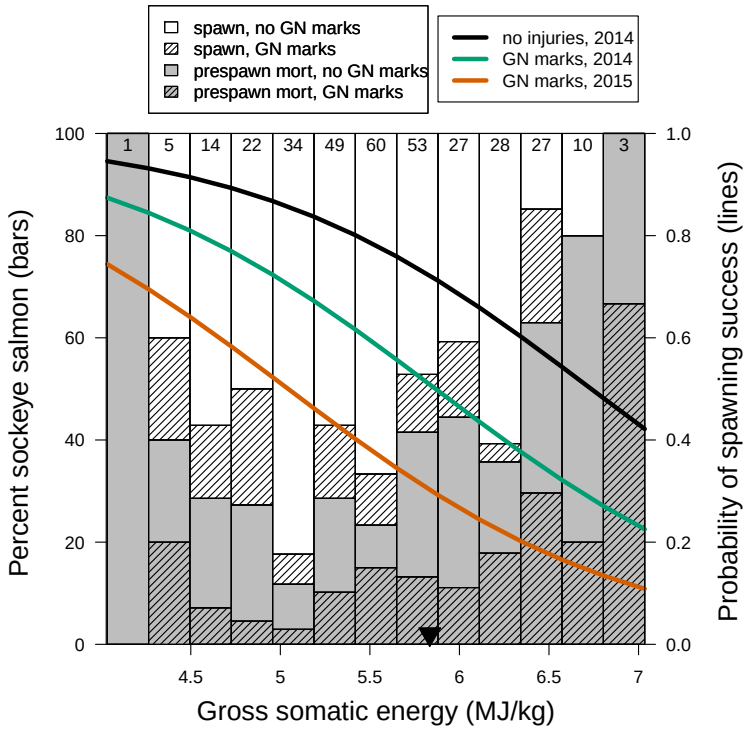

Figure 3.6: Probability curves of spawning success (from top GLM) for female Gates Creek sockeye salmon PIT-tagged in the Seton River, British Columbia, 2014-2016. Curves show the difference in survival between fish tagged in 2014 with no injuries, those tagged in 2014 with gillnet injuries, and those tagged in 2015 with gillnet injuries. The background indicates the percentage of fish with and without gillnet marks successfully spawning across a range of GSE. The population median GSE is indicated by the black triangle. Sample size for each bin is given at the top of the plot.

was one individual from the Harrison River (rkm 101 in the Fraser River) and one individual from the Bowron River (rkm 885).

3.5 Discussion

By intercepting sockeye salmon midway along their migration using a low-impact capture technique, applying PIT telemetry, and assessing egg retention on spawning grounds, I confirmed my hypotheses that gillnet injuries acquired prior to entering the Seton River are associated with reduced probability of migration survival and spawning success. For the GCss population that has survived the Fraser River and arrived at Seton Dam, this equated to the loss of 3.8–9.9% of effective female spawners (508–1642 fish) occurring between Seton Dam and Gates Creek. To my knowledge, this is the first study of wild fish to show an association between fisheries related injuries and a direct measure of spawning success, in this case egg retention. In addition, the unique opportunity to collect recently deceased sockeye salmon mid-migration revealed gillnet injuries on 21–51% of dead fish, and sockeye salmon from multiple Fraser River populations dying below the Seton Dam. Together, these results reveal that gillnet NTFI has a consistent, significant impact on Fraser

River sockeye salmon.

I found a higher prevalence of gillnet marks in female compared to male salmon I examined. This distinction could be explained by males showing less marking following a gillnet encounter, higher rates of retention of males due to greater entanglement, or males experiencing elevated FRIM prior to arrival at the Seton River. In Chapter 2, I found that fisheries gear generally caused greater visible injuries to female sockeye salmon compared to males. This phenomenon could lead to a failure to detect gillnet injury on males arriving at the fishing fence. Researchers have shown that gillnets tend to select larger sockeye salmon, (BAKER *et al.* 2011; PETERSON 1954) and males tend to be larger than females. Therefore, gillnets may more effectively trap males while females are more likely to disentangle. Although Chapter 2 showed no support for the hypothesis that FRIM is elevated for males, gillnet mark prevalence was slightly higher for dead males compared to females collected at the fence, suggesting that males with gillnet marks were dying earlier. An important distinction between the two studies is that in Chapter 2, fish were manually released from nets, while in this study, they likely escaped on their own. I am unable to conclude why gillnet mark prevalence was consistently higher in females in this study, but I believe it warrants further investigation.

Annual gillnet injury prevalence in the Seton River ranged from 19–27%, which is within the range observed by BAKER *et al.* (2014) in a multi-year study of Alaskan sockeye salmon (6–44%). However, as previous evidence shows that short-term mortality is high following gillnet capture (Chapter 2), which likely occurs several days downstream of the Seton River, my estimates of gillnet injury prevalence are probably much lower than the percentage of GCss experiencing NTFI. Based upon the DFO FRIM estimate for gillnets (60%, DFO 2017), a baseline estimate of 70% survival for Fraser River sockeye salmon from river entry to spawning grounds (CROSSIN *et al.* 2009), and the numbers of male and female GCss arriving at Gates Creek with and without gillnet marks, I estimate that on average 45% of female and 28% of male GCss experienced gillnet NTFI in the Fraser River. While these (likely conservative) estimates seem simplified, I was unable to find studies that provided better estimates of the rate of NTFI (for gillnets or any other gear) for the Fraser River or beyond. As studies providing FRIM estimates for multiple gear types in multiple contexts accumulate (PATTERSON *et al.* 2017a), inadequate knowledge of the rates of NTFI becomes a limiting factor in improving management to minimize FRIM. Since our results indicate that a considerable number of salmon experience gillnet NTFI, I suggest that studies more robustly quantifying NTFI for in-river fisheries would provide valuable information for fisheries managers working to conserve Pacific salmon.

Other variables representing damage to skin, sea lice scars and unknown injury, were negatively associated with migration survival. Sea lice injuries have been indirectly linked to prespawn mortality of sockeye salmon (JOHNSON *et al.* 1996). Virtually all sockeye salmon are expected to encounter sea lice during their coastal migration (BEAMISH *et al.* 2005) but given the prevalence of scars at the fence, nearly half of the fish must be able to heal their wounds after sea lice were shed upon freshwater entry, or perhaps those with visible wounds experienced greater infestation. A failure to heal wounds or vulnerability to greater infestation may be indicative of a stress-induced reduction in immune function (MACKINNON 1998), which could lead to elevated mortality during migration. Alternatively, others have identified osmoregulatory failure due to the loss of the skin's barrier function as a proximate cause of mortality for fish with missing skin (MATEUS *et al.* 2017; OLSEN *et al.* 2012), and this would be relevant to both fish with sea lice scars (SACKVILLE

et al. 2011) and unknown injuries in this study. Regardless of the mechanisms of mortality, the association between visible injuries and migration failure indicates that damage to the integument can have significant consequences for salmonids migrating in freshwater.

The most important covariate in this study aside from visible gillnet injuries, gross somatic energy, was consistently and negatively associated with both migration survival and spawning success. Endogenous energy reserves that salmon store prior to entering freshwater are dedicated to routine maintenance, migration, the development of gonads, and spawning activities (GILHOUSEN 1980). Although one might expect depressed energy reserves to be associated with failed migrations, higher GSE late in the migration may indicate a failure to mature and convert stored energy into secondary sexual characteristics and gonads, potentially from exposure to stress (BAKER *et al.* 2013). In a holding study of adult migrating sockeye salmon, both males and females exposed to experimentally elevated water temperature presented higher GSE than those at cooler temperatures and GSE was negatively correlated with gonad mass, indicating that maturation was delayed (Amy Teffer, unpublished data). Together, these findings suggest that above average GSE, particularly later in the migration, may be indicative of previous exposure to stressors and future migration failure and/or prespawn mortality.

Of the three years included in this study, 2015 is noteworthy due to the abundance of dead fish collected from the fence, significantly greater occurrence of gillnet marks on these dead fish, below average prevalence of gillnet marks on PIT-tagged fish, significantly lower GSE in PIT-tagged fish, and significantly lower spawning success. I suspect that these factors were caused by a combination of extremely low river levels, elevated water temperature, and an in-river fishery opening that occurred during a high water temperature period in early August. Sockeye salmon that were captured by gillnet (in the Fraser River, 181 km downstream of the Seton River confluence) and then radio-tagged during this period experienced very poor survival, with $<$10% of 87 tagged fish surviving to the Seton River (Chapter 2). Both GSE and gillnet prevalence were likely reduced in the PIT-tagged population due to the mortality of high GSE and gillnet marked fish being shifted downstream as a result of more extreme conditions (thus higher mortality at Seton Dam). While migration survival was consistent with other years, lower spawning success was likely due to stressors experienced earlier in the migration (MINKE-MARTIN *et al.* 2018). Had my study been simply based on the migration survival of PIT-tagged fish (a common endpoint for many biotelemetry studies), and not included observations of mortalities at the fence and spawning success, the negative combined effects of suboptimal environmental conditions and open fisheries in 2015 would not have been apparent. These results emphasize the potential limitations of telemetry studies that do not account for survival prior to tagging and prespawn mortality.

From my survey of dead salmon, it was clear that the majority of sockeye salmon dying below the Seton Dam were not from the GCss population. Although these data do not permit conclusions regarding the factors causing sockeye salmon from other populations to enter the Seton River, I suspect that in addition to elevated temperatures in the Fraser River (BETT *et al.* 2017), gillnet NTFI may play a role. Because salmon exposed to a capture stressor and held at high temperatures die more rapidly than those at optimal temperatures (TEFFER *et al.* 2018), the stress from NTFI and its associated injuries may prompt salmon to rest in cooler tributaries of the Fraser River. Indeed, this is a commonly observed phenomenon in tributaries

up- and downstream of the Seton River, where salmon with visible injuries have been observed holding (pers. obs. Jamie Scroggie, DFO catch monitoring). In the year with the greatest mortality at the Seton Dam, 2015, gillnet injury was significantly more prevalent in the dying fish than the tagged population. Straying salmon from large donor populations may dilute the evolved traits characteristic to a smaller population (BETT *et al.* 2017), but if stress or injury contributes to straying, non-natal strays would be less likely to contribute to the spawning population. Fisheries-related injury has not been linked to straying to date (BETT *et al.* 2017), and thus its role warrants further research.

Gillnet injury and the covariates used in GLMs for this study only represent a subset of possible factors affecting migration survival and spawning success of GCss, potentially explaining the low McFadden's r^2 values for the top models. Previous work studying GCss intercepted in the Seton River have demonstrated that plasma glucose concentration (MINKE-MARTIN *et al.* 2018), anaerobic swimming through fishway attraction flows (BURNETT *et al.* 2014), and flow release strategy at Seton Dam (BURNETT *et al.* 2017) were associated with survival to Gates Creek. Furthermore, date of arrival at Gates Creek (BURNETT *et al.* 2017), thermal experience, and Seton Dam discharge (MINKE-MARTIN *et al.* 2018) were associated with spawning success. PIT-tagging with minimal biopsy (DNA sample) precludes the measurement of most of these variables, but also provides data with less potential for handling-related bias and thus more reliable estimates of survival. Although gillnet injuries may account for a small proportion of variance in my models and are likely not the most important factor determining migration survival and spawning success, their impact was consistently significant across years, indicating their importance.

As climate change progresses and Pacific salmon populations face increased environmental stressors, freshwater fishers will experience increased pressure to minimize FRIM. In an example of climate change impacting fisheries, Fraser River salmon fishing openings are subject to in-season reductions based on elevated water temperatures (DFO 2017; MACDONALD *et al.* 2010). This study adds to the current literature on the association of gillnet NTFI and FRIM by showing a direct impact of visible injuries on migration survival and spawning success. In gillnet fisheries where FRIM can be reduced, potential solutions include reducing fishery open periods, altering fishing techniques (e.g. shorter net soak times), and exploring alternate gears (e.g., multi- vs mono-filament nets, smaller mesh size, changing hang ratios, or transitioning away from gillnets). My conservative estimate of effective female spawners lost to gillnet FRIM is one of the first meaningful attempts to quantify the mortality of salmon that escape gillnets in the Fraser River, a hitherto important component of characterizing FRIM across the entire freshwater migration that has been challenging to ascertain for Fraser River salmon populations (PATTERSON *et al.* 2017b).

Chapter 4

Fisheries capture and infectious agents are associated with travel rate and survival of Chinook salmon during spawning migration

4.1 Synopsis

Following interactions with fisheries gear, fishes may experience delayed mortality or display modified behavior. The physiological status of fish at the time of capture, including the presence of infectious agents, can also influence survival outcomes. To explore the relationship between capture, infectious agents and fate, I simulated gillnet capture on adult Chinook salmon returning to a hatchery, used quantitative PCR to quantify infectious agents in non-lethal gill biopsies, and determined longevity, migratory fate, and migration rate using radio telemetry. A parallel holding experiment investigated the relationship between infectious agent load and longevity. Males were 5.1 times more likely to arrive at spawning grounds compared to females and also migrated faster. Gillnetted fish took 1.4 times longer to migrate than biopsy-only fish, but there was no difference in longevity or migratory success among treatment groups. Longevity for fish infected with *Cryptobia salmositica*, a freshwater parasite, was similar between the holding (median = 7.0 days) and telemetry (7.4 days) experiments, but significantly lower than that of uninfected fish (17.4 days in telemetry experiment). The impact of *C. salmositica* on adult Chinook salmon in this study was demonstrated at the molecular (genes), physiological (plasma variables), and organism (migratory success) levels. These results demonstrate an instance where sex and infection were better predictors of migratory fate than an experimentally applied capture experience.

4.2 Introduction

Wherever fisheries exist, the process of fishing can have detrimental impacts on birds, mammals, non-target fish species, and even target fish species that interact with gear but are not landed (ALVERSON *et al.* 1994; BAKER and SCHINDLER 2009). While release or escape may provide an opportunity for survival, fish interacting with fisheries gear are likely to undergo an energy intensive and immunosuppressive stress response, receive external and internal damage, and become vulnerable to predators during or following capture (CHOPIN and ARIMOTO 1995; UHLMANN and BROADHURST 2015). This experience can lead to mortality immediately following the capture experience (THOMPSON *et al.* 1971), delayed mortality occurring days to weeks later (DONALDSON *et al.* 2011), or sub-lethal effects that alter behavior (e.g. delayed or slow migration) (BERNARD *et al.* 1999) and ultimately lead to a reduction in fitness (DICKERSON *et al.* 2005). Although recovery from capture stressors is possible, semelparous fish undergoing freshwater spawning migrations, such as Pacific salmon (*Oncorhynchus spp.*), are particularly vulnerable since they have a fixed energy budget (feeding ceases prior to freshwater entry) and such capture events often occur in the presence of high water temperature, predators, and infectious agents (RABY *et al.* 2015).

Due to their relatively low cost, ease of deployment, and effectiveness in a variety of environments, gillnets are frequently employed to capture Pacific salmon during their freshwater spawning migrations. Gillnets are made of fine nylon thread with mesh sizes corresponding to the girth of a target fish. Fish have difficulty detecting these nets in poor visibility and become enmeshed around the head and gills, anterior to the dorsal fin, around fins, or even by teeth and jaws. Entanglement in gillnets may cause asphyxiation, resulting in accumulation of blood potassium and lactate, as well as acidosis culminating in cardiac failure (KOJIMA *et al.* 2004). Suffocation may also occur from air exposure while fish are being disentangled from nets out of water. Contact with nets can remove mucus, scales, and skin, resulting in large open wounds (DAVIS 2002). Such integument damage may provide points of entry for infectious agents (SVENDSEN and BØGWALD 1997). Entangled fish sometimes escape by struggling, but potentially incur serious injury in the process (BAKER and SCHINDLER 2009; THOMPSON *et al.* 1971). For Pacific salmon, gillnet non-retention has been associated with acute and latent mortality (THOMPSON *et al.* 1971; VANDER HAEGEN *et al.* 2004), physiological stress indicated by altered blood chemistry (KOJIMA *et al.* 2004), the presence of infection (BAKER and SCHINDLER 2009), and delayed maturation (BAKER *et al.* 2013).

As a consequence of stress-induced immunosuppression following a capture event, fish with minor infections may subsequently experience an elevated infection (LUPES *et al.* 2006; PICKERING and POTTINGER 1989). Alternatively, injuries to the integument could render uninfected fish vulnerable to infectious agents in the environment (SVENDSEN and BØGWALD 1997). Mature Pacific salmon are naturally exposed to a large variety of infectious agents when they return to freshwater that encompasses viruses, bacteria, protozoa, myxozoa, and fungi (KENT 2011; MILLER *et al.* 2014). Most, if not all, adult salmon entering freshwater are infected by multiple infectious agents (MILLER *et al.* 2014, Appendices A and B) but the identification of infectious agents capable of affecting migratory success on a population level rarely occurs since dying fish are difficult to recover (BAKKE and HARRIS 1998). Although infection is not often included as a central factor in Pacific salmon spawning migration studies due to the difficulty of obtaining non-lethal samples, many authors have speculated that it is an important mechanism of mortality (BAKER and SCHINDLER 2009;

KEEFER *et al.* 2010; RABY *et al.* 2015). Studies of infectious agents occurring during wild adult salmon migrations have shown that infection can cause significant mortality on spawning grounds (BRADFORD *et al.* 2010b; TRAXLER *et al.* 1997) and in earlier sections of migrations (KOCAN *et al.* 2004; MILLER *et al.* 2014).

While both the impacts of fisheries capture stress and infectious agents have been studied, few researchers have investigated the intersection of the two (but see TEFFER *et al.* (2017)). The continued decline of wild Pacific salmon and the increased risk to salmon populations as climate warms have prompted researchers and policy makers to call for more studies focused on the cumulative impact of multiple stressors as opposed to just one in isolation (COHEN 2012; PATTERSON *et al.* 2017a). Recent advancements in quantitative polymerase chain reaction (qPCR) technology have made it possible to rapidly and non-lethally test a large number of individual salmon for a wide breadth of infectious agents (MILLER *et al.* 2016, 2014). By applying this technology to studies where biotelemetry is used to determine the fate of salmon experiencing simulated or true fisheries capture, it is possible to simultaneously investigate the effects of both stressors, and any potential interactions.

In this study I examined the impacts of both infectious agents and fisheries capture on the migration rate and migratory success of Chinook salmon, *O. tshawytscha*. Holding and telemetry experiments were conducted using the same source population of Chinook salmon. The holding experiment identified infectious agents associated with survival. In the telemetry study, salmon were exposed to a simulated gillnet capture, biopsied, and then released to migrate to spawning grounds. The simulated capture treatments included a group that was air exposed to mimic intentional release by a fisher, a group released from the gillnet underwater to imitate underwater escape, and a group that was only biopsied, then released. The collection of gill biopsies at the beginning of both experiments allowed the determination of pathogen presence and load, via qPCR. Both measurement of salmon immune and stress genes and analysis of blood plasma physiological indices were used to evaluate the impacts of infectious agents and other factors (e.g. sex, transport group).

4.3 Methods

4.3.1 Experimental design

A schematic is provided in the supporting materials to give an overview of the experimental and analytical design (Figure E.1). Adult Chinook salmon were collected from the attraction channel at the Chilliwack River Hatchery (Chilliwack, British Columbia, Canada) for both the holding and telemetry experiments. In both experiments, fish were biopsied and tagged (discussed below) in an identical fashion. For the holding experiment, fish were returned to the holding channel after tagging. The holding experiment was conducted to (1) identify infectious agents associated with survival, (2) determine whether there was a survival impact of gastric tagging, (3) determine whether gastric tag loss occurred, and (4) assess the effect of transport on blood plasma variables and host gene expression.

For the telemetry study, fish were trucked downstream to a release location (river km [rkm] 6.5, Figure 4.1) where they were exposed to a simulated gillnet treatment, biopsied for gill and blood samples, radio tagged, and then released into the river to conduct an upstream migration back to the hatchery, monitored

using fixed radio receivers. The telemetry experiment was conducted to (1) investigate the impact of gill-

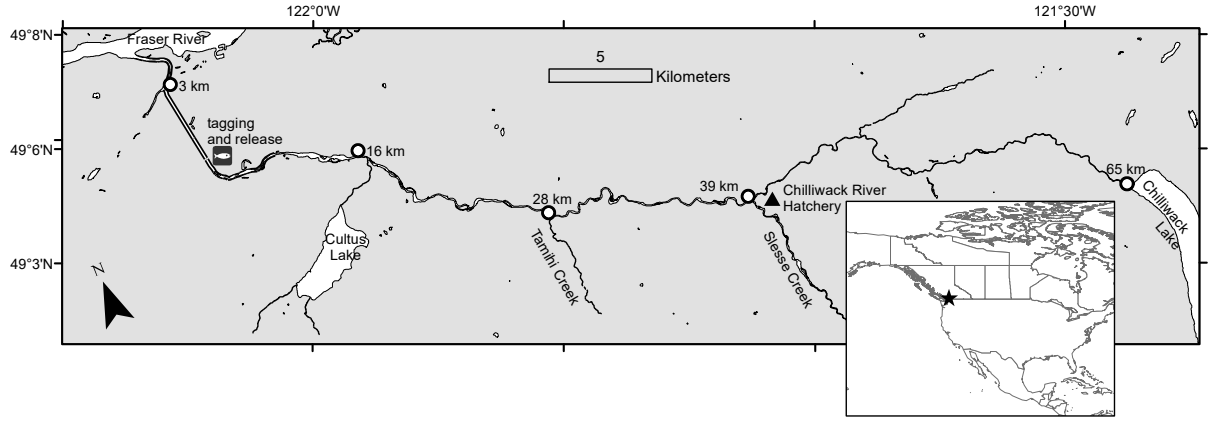

Figure 4.1: Map of 2013 study area, the Chilliwack River, British Columbia, Canada. Circles represent stationary radio receivers. Fish were acquired at the Chilliwack Hatchery (indicated by the black triangle) and trucked downstream to the release location.

net capture and infectious agents on migratory rate and success, (2) determine whether migratory rate and success differed between fish escaping gear underwater and those air exposed and then released, and (3) use blood plasma variables and immune gene transcription to identify mechanisms associated with survival. For all objectives, I tested the null hypothesis. For example, there would be no infectious agents associated with survival and there would be no difference in migratory rate or success among the different treatment groups.

4.3.2 Fish collection, biopsy, and tagging

Chinook salmon were chosen for this study since they are captured in gillnets from California to Alaska. Moreover, in the Fraser River watershed, the largest Canadian producer of Chinook salmon (DFO 1999), 14 of 17 assessed Chinook salmon Conservation Units in the Fraser River watershed have declined by 30% or more over the last three generations (RIDDELL *et al.* 2013). While the population used in this study were not technically "wild" since they were sourced from a hatchery population, I expect that they were exposed to the same environmental conditions, infectious agents, and fisheries capture experiences as wild bred fish.

Summer run adult Chinook salmon (average body length male: 67.1±6.2 cm and female: 76.1± 7.3 cm; average body depth male: 14.6±1.4 cm and female: 15.6±1.9 cm) were collected at the Chilliwack River Hatchery, located 40 river kilometers (rkm) upstream from the Chilliwack River mouth (which is 85 rkm upstream from the Fraser River mouth), from August 14th to 19th 2013 (Figure 4.1). From 1985-1989, this population was created from the transplantation of Upper Fraser populations including Quesnel River, Bowron River, Slim Creek, Finn Creek and Chilko River (Robert Stanton, Chilliwack Hatchery, pers. comm.). Migrating adults begin to arrive in early July and fish become sexually mature in early September. University of British Columbia (UBC) researchers collected fish under Fisheries and Oceans Canada (DFO) permits XR 201 2013 and XR 355 2013. This work was performed according to the UBC animal care and use permit, A12-0250.

Fish arriving at the hatchery voluntarily held in a channel immediately adjacent to the hatchery raceway. Since individuals were not tracked prior to either experiment, their residence time at the hatchery was unknown. Temperature in the holding channel during the study period was 14.5°C on average and ranged from 12.8 to 16.6°C. At the time of tagging, fish were not considered completely mature. Hatchery staff first allowed the fish into the raceway for gamete collection on August 27th. To obtain fish from the channel, researchers corralled them into the lower end of the fish ladder accessing the raceway and then dip-netted them out. Fish for the holding experiment were transferred directly from the channel to a tagging trough. Fish for the telemetry experiment were transferred into a 1500 L oxygenated transport tank for a 30 min drive to the tagging and release site (rkm 6.5, Figure 4.1). No more than 20 fish were held in the transport tank at one time. Dissolved oxygen was monitored to maintain proper concentration (8 - 11 mg/L) during transport and as fish were removed from the tank for tagging.

For the holding experiment, a total of 52 fish were tagged and biopsied, half received gastrically implanted radio tags and half received external visual ID tags (see below). Fish were returned to the attraction channel immediately after this process. Although longer holding would have been preferred, the hatchery staff conducted a spawning take (collection of gametes from fish in the raceways) 10 days after the first day of tagging which resulted in the end of the experiment. Recaptured fish were assessed for tag loss at this time.

A total of 108 fish (36 in each treatment group) were included in the telemetry experiment. After arrival at the downstream release site, fish were removed from the truck one at at time and transferred to either a 1000 L tank of fresh river water (gillnet treatments) or a tagging trough (biopsy only) supplied with a constant flow of river water. To simulate a brief gillnet entanglement followed by escape, I enmeshed each fish in a panel of gillnet (20 cm mesh strung on a dip-net hoop) for 20 s, and then gently removed them from the net underwater. Research in Bristol Bay, Alaska indicated that escape of sockeye salmon (*O. nerka*) from gillnets was a common occurrence, with 11-29% of fish displaying gillnet marks on spawning grounds over a three year study (BAKER and SCHINDLER 2009). To simulate a gillnet capture followed by release by a fisher (and associated air exposure as the fisher disentangles the fish out of water), I performed an identical entanglement, disentangled the fish directly into a dip-net in air, and then suspended the fish so that total air exposure time was one minute. A 20 s gillnet entanglement followed by 1 min air exposure has been demonstrated as sufficient for influencing survival in sockeye salmon (TEFFER *et al.* 2017).

The biopsy and tagging process was identical for all fish, regardless of the experimental manipulation. Fish were not anesthetized because there was the possibility that they could be recaptured and consumed (anesthetics allowed for use on fish in Canada [metomidate, tricaine methanesulfonate] cannot be used on potential food fish). One researcher held a fish in the trough while the other performed the biopsy and tagging procedure. Blood was drawn from the caudal peduncle using a heparinized vacutainer and immediately placed on ice. A small gill sample (2-3 mm of 3-4 gill filament tips) was removed with sterilized end clippers and placed in 1.5 mL of RNAlater® (Life Technologies, Carlsbad, CA). Gill tissue is a point of entry and infection for many infectious agents, includes small amounts of blood which contain circulating infectious agents and host RNA, and allows for low-impact non-lethal sampling. A Pisces 5® radio tag (43 mm length × 16 mm diameter, 15.2 g in air, 5 s burst rate; Sigma Eight Inc, Newmarket, ON) was placed into

the stomach, immediately behind the esophageal sphincter with the wire antenna hanging out of the fish's mouth. Fork length was measured and sex was determined by observing secondary sexual characteristics. An elastic visual identification tag (aka "spaghetti" tag, Northwest Marine Technology, Shaw Island, WA) was looped through the musculature posterior to the dorsal fin for the purpose of recovering fish at the hatchery and by anglers.

Within one hour of sampling, blood samples were centrifuged for 7 min at 7000 g and plasma was stored at -80° C. Hematocrit and leukocrit were calculated as the percentage of packed red blood cells and leukocytes in whole blood, measured after centrifuging in capillary tubes for 2 min. Plasma osmolality, sodium, potassium, chloride, glucose, and lactate were measured in duplicate or triplicate using the procedures outlined in FARRELL *et al.* (2000). Testosterone and 17β-estradiol samples were extracted in ethyl ether according to manufacturer's protocols. Plasma cortisol, testosterone, and 17β-estradiol were measured using commercial ELISA kits (Neogen Corporation, Lansing, MI), and run in duplicate at appropriate dilutions. Gill samples in RNAlater® were preserved according to the manufacturer's recommendations: after 24 hours at 4°C they were stored at -20°C for 1-2 months and then -80°C until extraction. Sex assignments were confirmed by viewing scatterplots of testosterone and 17β-estradiol (females are clearly higher for both variables).

4.3.3 Radio Telemetry

Six fixed radio telemetry receivers (Orion®, Sigma Eight Inc), each equipped with a 3 element Yagi antenna, were positioned along the Chilliwack River (Figure 4.1). At the hatchery, one receiver was placed immediately upstream of the hatchery to detect fish moving further upstream, and another was placed immediately downstream of the attraction channel to detect fish entering and leaving. At installation, range was tested using a radio tag to ensure coverage of the river channel. Stationary receiver efficiency was calculated for the 4 middle receivers ($>95\%$) based on detections of tagged fish at adjacent upstream and downstream receivers, and therefore this could not be estimated for the furthest downstream or upstream receivers. Mobile tracking was performed along the entire length of the river on Aug 20-23, 26, 30, and Sept 4 using a Lotek SRX 600 (Newmarket, Ontario, Canada) with a truck mounted 5 element Yagi antenna. Mobile tracking data were used to determine maximum upstream detection location, to calculate antenna efficiency, and to visualize detection data to aid in interpretation of movement patterns.

By evaluating plots of all fixed telemetry receiver and mobile tracking detections, I was able to assign survival times for each fish. I assumed that rapid downstream detections (equating to a rough approximation of river velocity) indicated moribund or deceased individuals. These rapid downstream movements were easily recognizable in plots and were not followed by subsequent upstream movements. Survival times were assigned to the first detection in the series of downstream detections. To my knowledge, only two fish were captured in the Chilliwack River recreational fishery and these were not included in the analysis.

4.3.4 Molecular Methods

Gill samples were screened quantitatively for the presence of 15 infectious agents (Table E.1) known to infect Fraser River Chinook salmon (Appendix A), using HT-qPCR on the Fluidigm BioMark™ microfluidics

platform. Simultaneously, 35 salmon genes, primarily immune and stress related, were assayed (Table E.2). MILLER *et al.* (2016) analytically validated the BioMark platform for research applications with salmon, with assays for 45 potential salmon pathogens that use the same TaqMan probes as other single assay platforms.

Assays were designed for mRNA, which enables the inclusion of some RNA viruses, focuses on infectious agents in an active state, and allows measurement of host gene expression (MILLER *et al.* 2016, 2014). Host stress and immune gene assays (Table E.2) were chosen to represent components of innate and acquired immunity as well as genes found to be correlated with survival in previous studies. An important caveat is that gene expression does not directly relate to gene translation. However, previous studies using these techniques have shown relationships between gene expression and survival or physiological parameters. Genes labeled "MRS" in Table E.2 were components of a "mortality related signature" found to be predictive of survival for upstream migrating adult sockeye salmon (MILLER *et al.* 2011). Other genes included here were associated with survival for juvenile sockeye salmon (JEFFRIES *et al.* 2014a) and a stress response in adult pink, *Oncorhynchus gorbuscha*, and sockeye salmon experimentally exposed to elevated temperatures (JEFFRIES *et al.* 2014b).

Detailed laboratory methods are described in Appendix A and TEFFER *et al.* (2017). In short, gill tissue was homogenized and RNA was extracted from aqueous phase aliquots using an automated liquid handling instrument. Normalized RNA ($31.25\,\mathrm{ng}/\mu\mathrm{L}$) was reverse transcribed to cDNA. Because per sample volume used by the BioMark is small, cDNA was pre-amplified (15 cycles) before qPCR on the BioMark.

All assays were run in duplicate. A serial dilution of artificial positive constructs (APC clones) of all infectious agent assays was run as six samples on the dynamic array. This serial dilution allowed for the calculation of infectious agent assay efficiency and infectious agent RNA copy number. The APC clones contain an additional probe (labeled with NED fluoresence) that allows for the detection of vector contamination (see MILLER *et al.* (2016) for further detail). To determine assay efficiency for host genes, I created a 5 sample serial dilution of pooled, pre-amplified samples. The pooled sample was diluted in DNA suspension buffer to create the serial dilution. Assays with a low or high amplification factor (< 1.8 or > 2.2; equivalent to $<$80% or $>$120% efficiency) or low R2 ($<$0.98) were removed from further analysis (C3, C7, CIRP, EIF4E, HSC70, KCTD, MCSF, SHOP21, TF, see Table E.2).

Cycle threshold was determined using the BioMark Real-Time PCR analysis software. Reaction curves for each positive sample-assay combination were visually evaluated for abnormal curve shapes, close correspondence between replicates, and presence of APC contamination as indicated by fluoresence of the NED probe. Using custom functions in R statistical software (R CORE TEAM 2015), I calculated efficiency for each infectious agent assay, omitted results where only one duplicate was positive for a sample-assay combination, removed APC contaminated samples, and averaged duplicates. For host genes, I performed the same steps but kept results where only one duplicate was positive and did not apply APC contamination screening.

For infectious agents, the number of RNA copies per sample was calculated using the standard curve method (LARIONOV *et al.* 2005). Host genes were normalized using the delta delta Ct method (SCHMITTGEN and LIVAK 2008). I normalized relative to the geometric mean of the reference genes and the population average Ct for a given gene.

4.3.5 Statistical Analyses

Infectious agent presence, load, and RIB

Infectious agent prevalence was calculated as the percent of the population with a positive detection for a given infectious agent. To determine whether an infectious agent load was associated with sex, transport, or tagging day I created a separate multiple linear regression model for each infectious agent. The log transformed RNA copy number of a given infectious agent was the response variable and the factors in the preceding sentence were the explanatory variables. For each model, plots of residuals were observed to ensure that the assumptions of normality and homoscedasticity were met. *P*-values were evaluated with the same criteria described below in "Physiological and transcriptomic variables".

I calculated relative infectious burden (RIB), as a means of collapsing the infectious agent data to a single metric so that a parameter representing overall infection (determined by qPCR) could be included in each model below. Relative infectious agent burden was calculated as:

$$\sum_{i=1}^{m}(\frac{L_i}{Lmax_i})$$

where for a given individual, the load (RNA in gill sample) of the *ith* infectious agent (L_i) is divided by the maximum load within the population testing positive for the *ith* infectious agent ($Lmax_i$) and this is summed across all infectious agents (m) infecting the given individual. This metric is therefore relative within the study population. When included in models below, this parameter was always log transformed due to its long-tailed distribution. The infectious agents rickettsia-like organism (RLO) and viral erythrocytic necrosis (VEN) were not included in this calculation due to only one and two positive samples, respectively.

Accelerated Failure Time Modeling

Because the primary response variables were survival and migration times, including censored observations (individuals that are lost from the experiment but whose time of death is unknown), I utilized time to event (aka survival) analysis using the "survival" package in R (THERNEAU 2015). Accelerated Failure Time (AFT) models were used instead of the commonly used Cox proportional hazards regression because exploratory analyses revealed that the data did not meet the proportional hazards assumption (SWINDELL 2009). Because including all potential infectious agents would over-fit each model, I first conducted single variable survival models for each infectious agent that was present in 20% of the population or greater. Infectious agents that were significant in single AFT models at α= 0.05 were included in the final model. Sex was included in all final models because multiple studies of migrating Pacific salmon have shown that females have lower migratory success than males (JEFFRIES *et al.* 2012a; MARTINS *et al.* 2012; TEFFER *et al.* 2017) and data exploration indicated the same for this study. To represent overall infection state, RIB was also included in all models.

To meet the assumption of the appropriate error distribution for all AFTs, distributions were selected via Akaike's Information Criterion (AIC) after each model was fit using the loglogistic, lognormal, logistic,

exponential, Weibull, and gaussian distributions (SWINDELL 2009). The two distributions with the lowest AIC values were visually assessed by plotting the negative log of the Cox-Snell residuals against time. The distribution demonstrating better agreement with a line through the origin with a slope of 1 was selected. To determine whether significant categorical variables (sex, treatment, infectious agent presence) had a multiplicative effect that was consistent across time, a major assumption of AFT models, quantile-quantile (QQ) plots were created where the survival time quantiles of one group was plotted against survival time quantiles of the other and a straight line indicates fulfillment of the assumption (SWINDELL 2009).

Holding Experiment

A single AFT model was created to determine whether any infectious agents were associated with survival (aka longevity) for the holding Chinook salmon and also whether gastric tagging was associated with survival. *Cryptobia salmositica* presence was included in the model based upon the single variable exploratory analyses. The final model included the following explanatory variables: tag type (spaghetti vs gastric radio), sex, RIB, and *C. salmositica* presence. In this dataset, individuals that left the attraction channel (determined via radio detection) or were euthanized when the hatchery staff conducted a spawning take were censored (71% of population was censored). The exponential distribution was selected for this model.

Telemetry Experiment

For the telemetry experiment, to test whether gillnet capture and infectious agents were associated with migratory success, I created an AFT model with survival times determined by detections from the fixed antenna array. Individuals without survival times were assigned a censored survival time at their last detection (69% censored). The final model included simulated gillnet treatment (three categories: gillnet, gillnet and air exposure, biopsy), sex, RIB, and *C. salmositica* presence. The exponential distribution was selected for this model. I also fit multiple models with interactions between gillnet treatment, sex, and *C. salmositica* presence but this did not result in any improved models.

Due to the high percentage of censored observations, I also conducted a logistic regression analysis where detection or tag recovery above river km 28 (Figure 4.1) was considered migratory success. This location is considered to be the beginning of spawning habitat for Chinook salmon in the Chilliwack river (pers. comm. Robert Stanton, Chilliwack Hatchery) and I observed Chinook on redds several km upstream. To determine whether infectious agents should be included in this analysis we conducted chi-square tests for the presence of an infectious agent against migratory success and simple linear regressions between the maximum river km where an individual was ever detected (by fixed or mobile telemetry) and the load of each infectious agents. The final model included gillnet treatment, sex, RIB, *C. salmositica* presence, and log *Flavobacterium psychrophilum* load. Including interaction terms as above did not improve this model. To meet the assumption of multicollinearity, I estimated the variance inflation factor to be sure that it was below 4 for all variables (O'BRIEN 2007). A Hosmer Lemeshow goodness of fit test indicated that the model fit was appropriate.

To determine whether there were associations between migration rate and treatment group and/or infectious agents, I created an AFT model where an arrival event was a detection at the river km 28 receiver and

time to event was calculated as the difference between the first detection at river km 28 and release. Fish that were detected at rkm 16 and not subsequently at rkm 28 were censored at their last detection at rkm 16 (6% were censored). Fish never detected at rkm 16 or 28 were not included in this analysis. Gillnet treatment, sex, and RIB were included as explanatory variables in this model and the Weibull distribution was selected as most appropriate.

Physiological and Transcriptomic comparisons

To explore the potential mechanisms underlying survival I created multiple linear regression models with each blood plasma variable and host gene (hereafter physiological parameters) as the response variables. For each model, infectious agent load (for each of the infectious agents that were at least 20% prevalent), sex, transport, and tagging date were the explanatory variables. Sex was included due to substantial evidence in the literature (JEFFRIES *et al.* 2012a; MARTINS *et al.* 2012; TEFFER *et al.* 2017) and corroborating exploratory analyses in this study indicating its association with survival. Transport was included because the capture and confinement process was likely to evoke a stress response. I also included the date of tagging as an explanatory variable because I observed that some host genes appeared to change over the five day period. The associations between physiological parameters and both sex and transport discussed in the results were from the model including RIB as an explanatory variable since this incorporated the entire population (individuals from both experiments, n = 149). For each model, plots of residuals were observed to ensure that the assumptions of normality and homoscedasticity were fulfilled. Due to a lack of normality, plasma cortisol, testosterone, and 17β-estradiol were log-transformed. Infectious agent loads and RIB (as above) were all log transformed. For all of these tests, I did not penalize *p* values for multiple hypothesis testing. However, while we highlighted results in tables that had *p* values below the α = 0.01 level, I only discuss results that were significant at the α = 0.001 level. At this α we would expect false discovery to occur less than one percent of the time for 300 statistical tests (for example).

To visualize the results from these models pertaining to infectious agents, I created a heatmap (using the "heatmap3" package (ZHAO *et al.* 2015)) representing Pearson correlation coefficients for comparisons between infectious agents and physiological parameters. Comparisons that were significant at the α = 0.001 level in the above multiple linear regression models were highlighted on this heatmap. To compare overall patterns of gene expression to infectious agent presence and migratory fate, I created a heatmap ("heatmap3") that used hierarchical clustering to group individual fish from the telemetry experiment based on gene expression. For each individual fish on the heatmap, categories for RIB, *C. salmositica* load, sex, and migratory fate were annotated.

4.4 Results

4.4.1 Infectious agent presence

Of the 15 infectious agents assayed in 151 Chinook salmon, 14 were detected in gill samples (Piscine orthoreovirus was not detected, Figure 4.2). The number of infectious agents detected in a single individual ranged from 2 to 8, with a median of 4. Relative infectious agent burden (RIB) ranged from 0.002 to 2.26,

with a median of 0.13 (before log transformation). Six infectious agent species including, '*Candidatus* Bran-

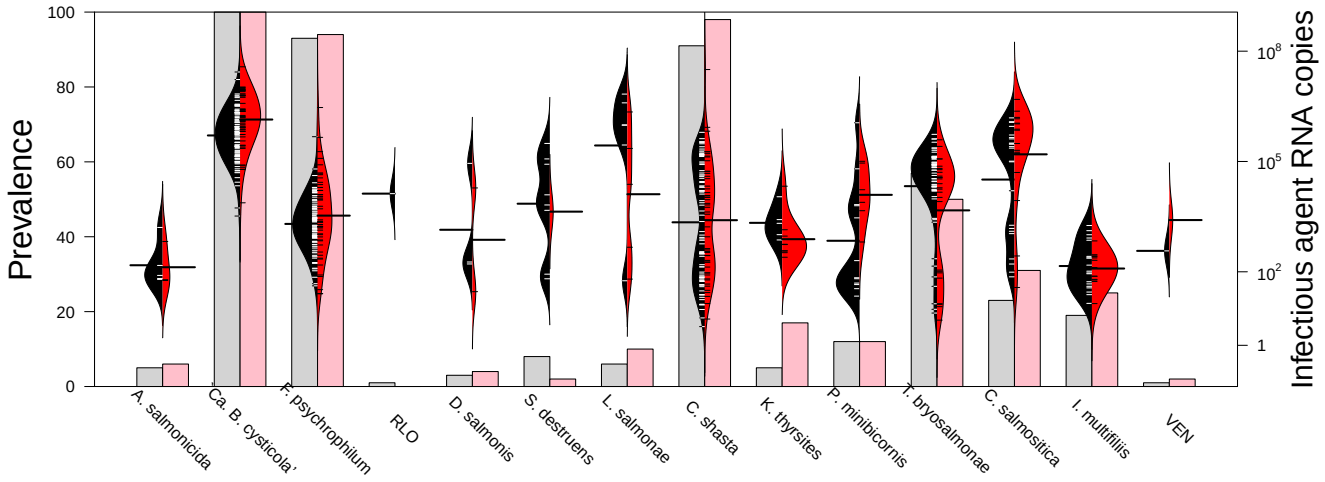

Figure 4.2: Barchart of infectious agent prevalence overlaid with beanplot of infectious agent load. Gray bars and black bean halves represent males (n=99) and pink and red represent females (n=52). Bean lobes are smoothed histograms with small ticks indicating individuals and large ticks representing group means.

chiomonas cysticola', *Flavobacterium psychrophilum*, *Ceratonova shasta*, *Tetracapsuloides bryosalmonae*, *Cryptobia salmositica*, and *Ichthyophthirius multifiliis* had a >20% prevalence, which provided sufficient statistical power for further analyses.

The '*Ca.* B. cysticola' log load was significantly higher in females (mean ± 95% C.I.: 14.1 ± 3.2 log copies RNA) compared to males (13.2 ± 3.0 log copies RNA) (multiple linear regression, p = <0.001, Figure 4.2). The log-transformed load of *Flavobacterium psycrophilum* was significantly lower in transported fish (7.3 ± 4.0 log copies RNA) compared to fish sampled at the hatchery (8.8 ± 3.0 log copies RNA) (p = < 0.001). No infectious agents varied significantly with date of tagging.

4.4.2 Differences in physiological parameters between experimental groups and sexes

The fish transported downriver for the telemetry study showed significantly elevated levels of hematocrit and plasma osmolality, and decreased levels of leukocrit relative to non-transported fish (Tables 4.1 & E.3). Genes that showed higher transcription in the transported group included ATP5G3, B2M, CD4, CD83, IL-15, JUN, MHCII-B, and RIG-I (Table E.3). Females had the expected higher levels of cortisol, testosterone, and 17β-estradiol but also had elevated expression of hepcidin (HEP) and Interleukin-11 (IL-11) compared to males (Table E.3).

4.4.3 Hatchery holding experiment

Survival histories were determined for 35 of 52 Chinook salmon biopsied and returned to the channel, based on discovery of carcasses, detections of radio-tagged individuals leaving the attraction channel, and collection of survivors at the end of the experiment. Median survival time was greater than the length of the holding experiment (10 days) and therefore could not be estimated. *Cryptobia salmositica* presence was the

Table 4.1: Mean ± standard deviation for each physiological parameter in blood biopsies from adult Chinook salmon, Chilliwack River, BC. Means were calculated for each sex at each release location (river releases experienced a 1 hour transport prior to sampling). Sample size is given in parantheses for each group.

	Hatchery		River	
	Female (13)	Male (34)	Female (39)	Male (63)
hematocrit (%)	38.3 ±4.3	38.9 ±8.8	45.4 ±9.6	50.1 ±8.3
leucocrit (%)	1.6 ±1.0	1.7 ±1.0	1.3 ±1.1	1.1 ±0.8
glucose (mmol/L)	3.9 ±1.7	3.7 ±1.2	3.3 ±1.2	4.2 ±1.0
lactate (mmol/L)	4.5 ±3.1	5.8 ±3.3	7.3 ±3.8	5.8 ±3.1
chloride (mmol/L)	130.1±7.0	131.5±7.5	129.1±5.8	129.5±4.2
osmolality (mOsm/kg)	301.8±7.6	307.6±16.4	313.3±15.0	316.6±10.6
sodium (mmol/L)	153 ±5.0	157.4±10.2	152.7±7.1	155.8±6.4
potassium (mmol/L)	2.0 ±0.9	2.4 ±0.9	2.8 ±1.5	3.8 ±2.5
cortisol (ng/mL)	635.3 ±815.9	223.7 ±170.3	395.7 ±419.8	203.0 ±229.9
17β-estradiol (ng/mL)	8.1 ±10.5	0.3 ±0.1	12.4 ±9.8	0.4 ±0.1
testosterone (ng/mL)	106.8 ±82.8	27.1 ±25.2	99.1 ±81.3	28.5 ±18.1

only variable in the AFT model that was associated with survival for the held Chinook salmon (Table 5.4). The model indicated that fish without *C. salmositica* survived 4.3 times (95% C.I. = 1.0 – 18.2) longer than

Table 4.2: Model results for survival analysis and logistic regression models applied to Chinook salmon in both holding and telemetry experiments, Chilliwack River, BC, 2013. Dashes indicate that explanatory variable was not included in a given model. *P* values for explanatory variables significant at $p < 0.05$ are indicated in boldface.

				Explanatory Variables						
Response Variable	Model type (distribution)	N obs	Model Parameter	Gillnet	Gillnet + Air	Tag type (radio)	Sex (F)	RIB	*C. sal* presence	*F. psych* load
Holding Exp.	Accelerated	35	β	–	–	0.59	-0.75	-0.37	-1.46	–
Survival time	Failure Time	(10 events)	SE			0.67	0.85	0.28	0.74	
	(Exponential)		*p*			0.38	0.37	0.19	**0.05**	
Telemetry Exp.	Accelerated	102	β	0.44	0.47	–	-0.60	-0.15	-0.85	–
Survival time	Failure Time	(32 events)	SE	0.44	0.45		0.38	0.14	0.40	
	(Exponential)		*p*	0.32	0.30		0.11	0.28	**0.03**	
Telemetry Exp.	Logistic	92	β	0.58	-0.20	–	-1.62	0.06	-1.59	0.04
Arrival at rkm 28	Regression		SE	0.63	0.62		0.52	0.12	0.60	0.12
(arrival = 1)			*p*	0.35	0.74		**0.002**	0.74	**0.01**	0.76
Telemetry Exp.	Accelerated	78	β	0.35	0.37	–	0.26	0.01	–	–
Migration time to	Failure Time	(73 events)	SE	0.14	0.14		0.12	0.04		
rkm 28	(Weibull)		*p*	**0.01**	**0.01**		**0.03**	0.81		

those testing positive. By fitting the model, median survival time for a male fish with a spaghetti tag, an average RIB, and *C. salmositica* was 7.0 days but the same value for a similar fish testing negative could not be estimated since so few of these fish died before the termination of the holding experiment (Figure 4.3A). There was no radio tag loss among the hatchery held fish and tag type was not significantly associated with survival in the AFT model (Table 5.4).

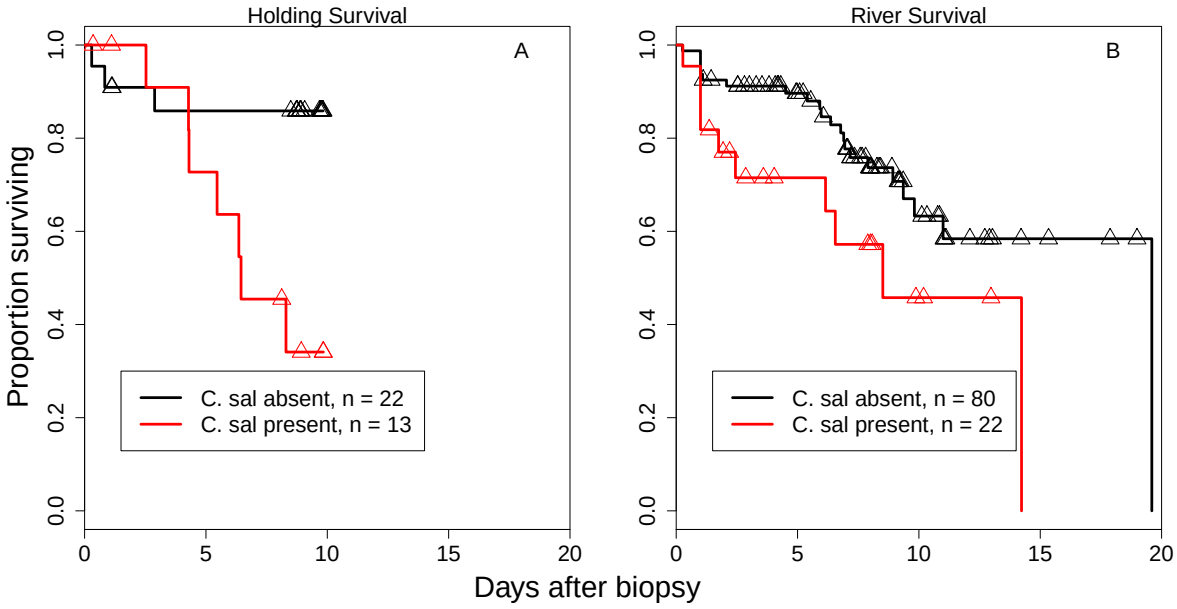

Figure 4.3: Kaplan-meier curves comparing survival of Chinook salmon with and without *Cryptobia salmositica* infection. Panel A is the 10 day holding experiment at the Chilliwack River Hatchery, BC and Panel B is the telemetry experiment in the Chilliwack River. Triangles represent censored individuals.

4.4.4 River telemetry experiment

Following simulated gillnet treatments and biopsy, 15% of the tagged fish died without being subsequently detected at receivers upstream or downstream of the release site and 10% were detected at the receiver located 3 km downstream of the release site and not detected upstream thereafter (Figure 4.4A). Although 12% were detected by mobile tracking upstream of the hatchery (four fish from each treatment group, max rkm = 55.5), no fish were detected at the Chilliwack Lake receiver (rkm 60, Figure 4.1).

Median survival time for Chinook salmon released with radio tags was 14.2 days. The AFT model indicated that fish testing negative for *C. salmositica* survived 2.3 times longer (95% C.I. = 1.1–4.9) than those testing positive (Table 5.4). According to the model, a male, biopsy treatment fish with average RIB and testing positive for *C. salmositica* would survive for a median of 7.4 days and the same fish without *C. salmositica* would survive for a median 17.4 days (Figure 4.3).

Logistic regression, with arrival at the beginning of spawning habitat as the dependent variable, revealed that sex and *C. salmositica* presence were associated with the likelihood of arrival (Table 5.4). Males were 5.1 times more likely to arrive at spawning grounds compared to females (95% CI: 1.9–14.7) and fish without *C. salmositica* were 4.9 times more likely to arrive than those testing positive (95% CI: 1.6–16.8). Not a single female salmon that tested positive for *C. salmositica* (n = 10) arrived at the beginning of spawning habitat while exactly half of females that tested negative for *C. salmositica* (n = 28) arrived. The respective proportions for males were 58 and 75%.

Similar proportions of the gillnet treatment groups (72% of gillnet, 69% of gillnet + air, and 67% of biopsy) were detected at the beginning of spawning habitat (Figure 4.4A). However, an AFT model showed

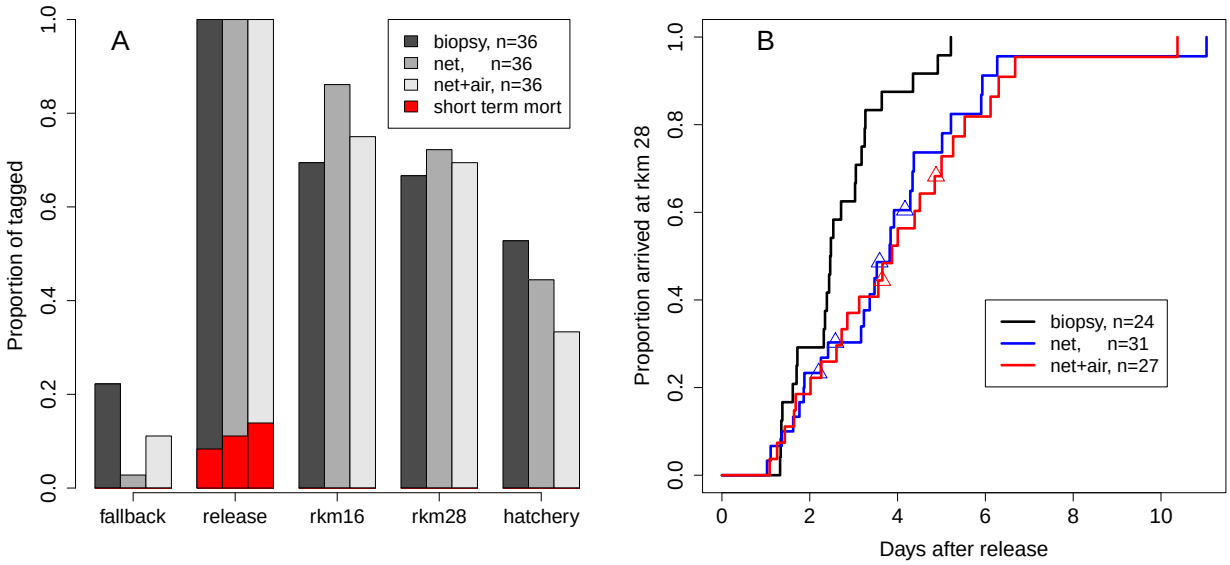

Figure 4.4: A) Barchart of migratory fate split by treatment group for Chinook salmon released in the Chilliwack River, BC. Short term mortalities include fish that died close to the release site within 24 hrs of tagging. Fallback includes fish that were detected at the rkm 3 receiver and not upstream subsequently. B) A cummulative incidence curve of time to arrive at the beginning of spawning habitat (river km 28) split by treatment. Triangles represent censored individuals.

that the time required to arrive at this location (migration rate) was significantly different between treatment groups and sexes (Figure 4.4B, Table 5.4). Biopsied individuals migrated significantly faster than both gillnetted and gillnetted and air exposed fish. The two gillnet treatments were not significantly different from one another and the model indicated that it took both gillnetted groups 1.4 times longer (95% C.I. = 1.1–1.9) than biopsy fish to arrive at the beginning of spawning habitat. Female Chinook salmon migrated significantly slower than males, requiring 1.3 times longer (95% C.I. = 1.0–1.6) to arrive. According to the model, the median time required to migrate from release to spawning habitat (a distance of 22 km) for a male biopsied fish, a female biopsied fish, a male gillnetted fish, and a female gillnetted fish was 2.4, 3.0, 3.4, and 4.5 days, respectively.

4.4.5 Gene expression, plasma variables, and infectious agents

A comparison of the load of all infectious agents and RIB to gene expression and plasma variables showed that *C. salmositica* was significantly correlated with 4 plasma variables and the expression of 3 genes (Figure 4.5, Table E.4). The genes HEP, IGMS (Immunoglobulin M), and MMP13 (matrix metallopeptidase 13) all showed a similar highly significant, positive correlation with *C. salmositica* load and were highly expressed in *C. salmositica* positive fish (Figure 4.6, Table E.4). Hematocrit, glucose, and potassium were negatively associated with *C. salmositica* load and presence (Table E.4). Although the bacteria '*Candidatus* Branchiomonas cysticola' did not meet the criteria to be included in any of the models of survival and migration rate, it was significantly, positively associated with the MX, IL-1R, and IL-11 genes and nega-

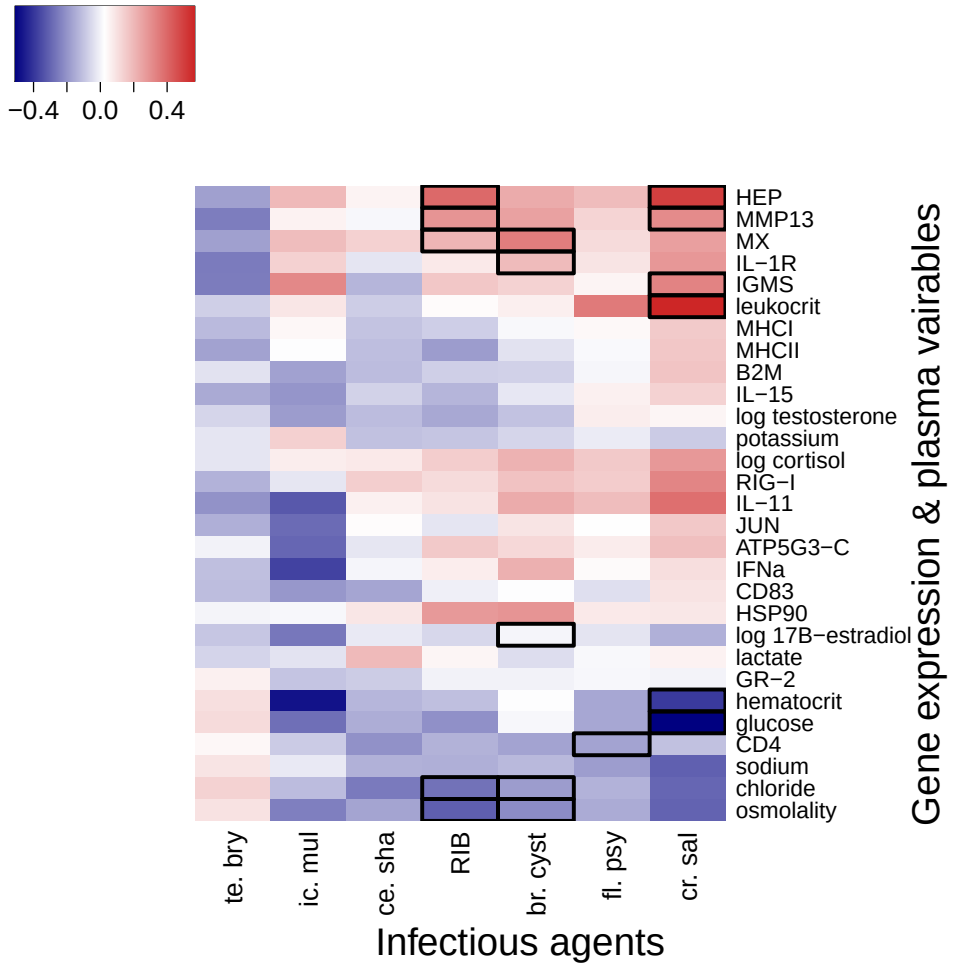

Figure 4.5: Heatmap of correlations between infectious agent load and physiological parameters (host gene expression and blood plasma indices) in adult Chinook salmon (n = 149), Chilliwack River, BC. Comparisons with black borders were significant in a multiple linear regression at $p < 0.001$ (see Table E.4). te. bry = *Tetracapsuloides bryosalmonae*, ce. sha = *Ceratonova shasta*, ic. mul = *Ichthyopthirius multifiliis*, br. cyst = '*Candidatus* Branchiomonas cysticola', RIB = relative infectious agent burden, fl.psy = *Flavobacterium psychrophilum*, cr. sal = *Cryptobia salmositica*. Full names for genes can be found in Table E.2.

tively associated with plasma sodium, chloride, and osmolality (Figure 4.5). While not significant (except in the case of IL-1R), the direction of these correlations appeared similar for *Ichthyophthirius multifiliis*. RIB was significantly, positively correlated with the expression of HEP and MMP13, and *Flavobacterium psychrophilum* was negatively associated with the expression of CD4.

A heatmap of all of the Chinook salmon in the telemetry experiment clustered by gene expression (Figure 4.6) showed tight clustering of individuals with high *C. salmositica* loads (cluster C), while low load individuals were widely distributed among those testing negative for *C. salmositica*. The high load group was disproportionately females that did not arrive on spawning grounds and was characterized by relatively high expression of HSP90, HEP, MMP13, and IGMS. Cluster A in Figure 4.6, which represented a group with 56% migratory success was characterized by a near universal up-regulation of GR-2, MHCI, CD83,

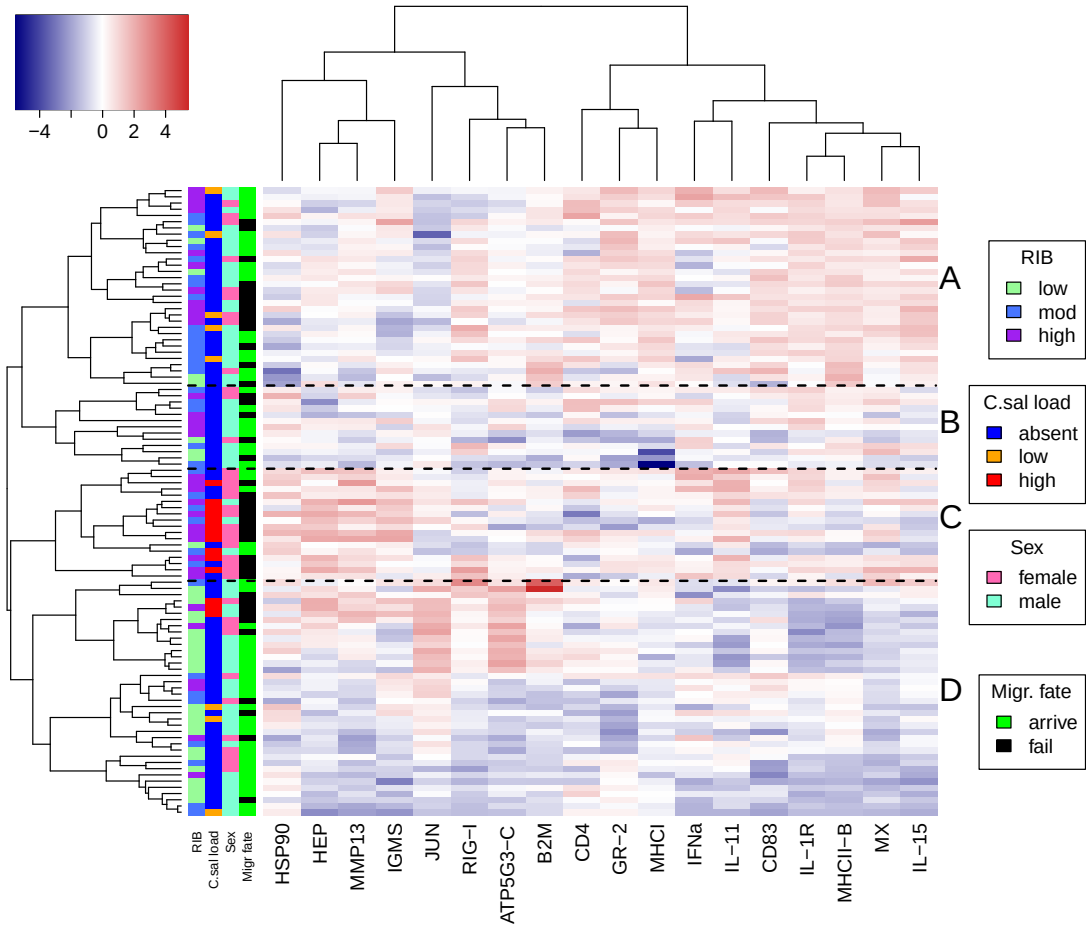

Figure 4.6: Heatmap plotting expression of 18 stress and immune genes for each Chinook salmon (n=101) radio-tagged and released into the Chilliwack River, BC. Relative expression was scaled by gene by the "heatmap3" plotting function. Color bars on left side indicate RIB (low = <0.04, mod = >0.04 & <0.30, high = >0.30), the load of *Cryptobia salmositica* (low = <22,000 copies *C. salmositica* RNA per gill sample, high = >22,000), sex, and migratory fate determined by radio telemetry. Dashed horizontal lines indicate the division between four clusters (A,B,C,D) on the secondary branches of the dendrogram clustering individual fish (row side).

IL-1R, MHCII-B, MX, and IL-15. Clusters B and D, which had higher incidence of migratory success (62 and 74%, respectively) generally presented more neutral or lower expression of all evaluated genes.

4.5 Discussion

Both the condition of a fish at capture and the capture experience itself can alter the physiology, behavior, and survival of Pacific salmon released or escaping from fisheries gear. My simulation of a gillnet fisheries capture and measurements of infectious agent load revealed that Chinook salmon infected with a blood-borne protozoan, *Cryptobia salmositica*, had a reduced likelihood of arriving on spawning grounds. Although gillnetted fish did not experience lower migratory success or shorter survival than biopsy fish, they did take

significantly longer to reach spawning grounds. While failing to arrive on spawning grounds results in zero lifetime fitness, delayed migration for a semelparous fish results in late arrival on spawning grounds and can impact spawning success and offspring fitness (DICKERSON *et al.* 2005; SEAMONS *et al.* 2004). Females were less likely to successfully migrate and migrated more slowly than males. Taken together, these findings indicate that while even a brief fisheries encounter at a late stage in the migration can have a measurable impact on migration rate, aspects of the biological context underlying a fisheries capture (fish condition and sex) may be more important predictors of survival.

The process of dipnetting, transporting, handling, and biopsying likely resulted in a stress response for all fish tagged in this study (PICKERING *et al.* 1982). Transported fish displayed significant changes to blood plasma physiology reminiscent of a fisheries capture experience (FARRELL *et al.* 2001). Genes associated with the cell-mediated immune response were up-regulated within the hour following capture, transport, and confinement. Similarly, WISEMAN *et al.* (2007) demonstrated up-regulation of immune genes (including MHCII) in the liver of rainbow trout, *Oncorhynchus mykiss*, just one hour after a handling stressor. Transcription factor jun-B (JUN) was up-regulated in transported fish in our study, as in previous studies where capture and handling stressors were applied (DONALDSON *et al.* 2014; MOMODA *et al.* 2007). Load of *F. psychrophilum* was significantly lower in transported fish, potentially a result of increased lysozyme activity in response to the capture and confinement stress (DEMERS and BAYNE 1997). These results demonstrate rapid transcriptomic changes associated with handling stressors in the field, and researchers would be prudent to optimize the time between the application of a stressor (such as handling and tagging) and collection of biopsies (intended for analysis by qPCR) according to the objectives of their study. In the river release study, it appears that the stress of handling and transport contributed to increased mortality (immediate mortality). However, median survival times were similar for *C. salmositica* positive telemetry experiment fish (7.4 days) and *C. salmositica* positive holding experiment fish (7.0 days). Furthermore, the handling and transport stress was uniform across all river released fish so differences associated with treatment, sex, and infectious agents are not artifacts of transport.

While all fish in this study likely required some degree of recovery following the aforementioned transport and handling stress, the gillnet treatment groups migrated slower, suggesting a need for additional recovery time compared to biopsy-only fish. In cases where earlier arriving fish have greater reproductive success, delayed arrival on spawning grounds can have important implications (DICKERSON *et al.* 2005). By virtue of migrating slowly, netted fish may be more vulnerable to additional encounters with fishery gear and spend more time exposed to higher water temperature prior to entering cooler natal streams. I was unable to identify the mechanisms linking gillnet treatment to migration rate because I only biopsied fish immediately following gillnet treatments which did not allow adequate time for associated physiological changes to occur. A future study with similar treatments but including repeated sampling post-treatment (as in DONALDSON *et al.* (2014)) could provide a mechanistic understanding of the impacts of gillnet capture on Chinook salmon.

There was no significant difference between the treatment groups for survival post-release or migratory success. This may be due to the mild nature of the simulated gillnet treatments; a 20 s entanglement is short relative to what fish may experience in a more realistic "capture-and-release" scenario but it could

be an accurate representation of some "entangle-and-escape" situations. TEFFER *et al.* (2017) found that an experimentally applied 20 s gillnet entanglement caused a significant difference in survival (relative to controls) for sockeye salmon captured early in their migration and held in tanks for five weeks. In contrast, RABY *et al.* (2013) found that mature chum (*O. keta*) and pink (*O. gorbuscha*) salmon on spawning grounds were highly resilient to multiple simulated stressors, including a 3 min gillnet entanglement. The difference in impact between these studies may be dependent upon the context of physical and physiological development along the migration. Pacific salmon collected from the final stage of their migration have thickened skin (ROBERTSON and WEXLER 1960) and elevated cortisol (BAKER and VYNNE 2014; ROBERTSON *et al.* 1961). These adaptations, potentially evolved to protect fish on spawning grounds, could render salmon physiologically refractory to fisheries capture towards the end of their migration. Given this possibility, I discourage the application of our findings to populations of Pacific salmon encountering fisheries at early periods of their migration. Future field studies that compare the impacts of fisheries encounter at multiple points along the migration are warranted.

Sex was a significant predictor of migratory success and slower migration in this study and there was one infectious agent ('*Ca.* B. cysticola'), three blood plasma variables (17β-estradiol, cortisol, testosterone), and two genes (IL-11, HEP) that varied significantly between sexes. In previous holding and telemetry studies finding higher mortality for female compared to male Pacific salmon, authors attributed the phenomenon to the greater reproductive investment, reduced immune function, and lower tolerance to stress of female fish (JEFFRIES *et al.* 2012a; MARTINS *et al.* 2012; TEFFER *et al.* 2017). As expected from previous studies (FAGERLUND 1967; HRUSKA *et al.* 2010), 17β-estradiol, testosterone, and cortisol in my study were significantly higher in females than males. Hepcidin and interleukin 11, which were both expressed higher in females, have previously been positively associated with bacterial infection (SHIKE *et al.* 2002; WANG *et al.* 2005) but neither gene was associated with bacteria load in this study. The load of '*Ca.* B. cysticola' was significantly higher in females but I have seen no evidence in my work to suggest that '*Ca.* B. cysticola' is associated with mortality, and other researchers recently suggested the same with Atlantic salmon (GUNNARSSON *et al.* 2017). While identifying the underlying cause of lower survival in females is beyond the scope of this study, my results contribute to the continued effort to understand this important phenomenon.

Cryptobia salmositica was the only infectious agent that appeared to be associated with survival. The primary vector for *C. salmositica* is the Pacific salmon leech, *Piscicola salmositica*, which I frequently saw on salmon collected at the hatchery. I suspect that the hatchery attraction channel provided favorable conditions for the leech: low water velocity, gravel substrate, and high fish densities (BECKER and KATZ 1965). *C. salmositica* is known to cause anemia in fish (LOWE-JINDE 1986; WOO 1979), and this was evident in this study as a negative correlation between hematocrit and *C. salmositica* load and presence, similar to the results of LAIDLEY *et al.* (1988) and LOWE-JINDE (1986). Consistent with LAIDLEY *et al.* (1988), I also found a negative correlation between *C. salmositica* load and plasma glucose. This could be due to *C. salmositica* consuming glucose (LI and WOO 1991) or conversely it is indicative of chronic stress (LAIDLEY *et al.* 1988). Contrary to LOWE-JINDE (1986), I found that leucocrit (% white blood cell) was positively associated with *C. salmositica* load. However, associations between leucocrit and infectious agent load have not been well-established (WEDEMEYER *et al.* 1983). Overall, the association of several plasma

variables with *C. salmositica* corroborated what has been observed in a previous study (LAIDLEY *et al.* 1988) and was indicative of infection-mediated disease.

I found a positive correlation between *C. salmositica* load and the expression of three immune genes (HEP, MMP13, IGMS) in gills. Hepcidin plays an important role in sequestering iron, a nutrient required for many pathogenic microbes to replicate and cause disease (SKAAR 2010), so that it is not available to pathogens (DRAKESMITH and PRENTICE 2012). *C. salmositica* lyses erythrocytes in the bloodstream, likely causing an extracellular release of iron, a potential explanation for the increased expression of hepcidin. Elevated levels of hepcidin occur in fish challenged with bacteria (MARTIN *et al.* 2006; SHIKE *et al.* 2002) and in this study there was also a correlation between RIB and hepcidin. The positive association between *C. salmositica* and hepcidin is not without precedent as ferroportin, an iron exporting protein whose function is modulated by hepcidin, has been associated with iron regulation in mice parasitized by a blood-borne protozoan, *Trypanosoma brucei* (STIJLEMANS *et al.* 2008). Matrix metalloproteinases, including MMP13, play a role in the restructuring of damaged tissue and their expression is increased in any disease that is associated with inflammation (PARKS *et al.* 2004). Through histological investigation of rainbow trout infected with *C. salmositica*, BAHMANROKH and WOO (2001) found that lesions became evident two weeks post-infection in liver, spleen, and gill tissue and that inflammation occurred simultaneously and increased in severity with parasitemia over time. Elevated transcription of IGMS, the immunoglobulin M antibody, suggests that *C. salmositica* infected Chinook salmon in this study experienced increased antibody production. WOO (2007) demonstrated that adult Pacific salmon contract *C. salmositica* shortly after returning to freshwater and that correspondingly, complement-activating antibody production is detectable two weeks after infection. In a challenge study where *C. salmositica* was injected into Atlantic salmon (*Salmo salar*), ARDELLI and WOO (2002) observed an increase in antibody production and decrease in the cell-mediated response. While correlations were non-significant at my α level, several components of the cell-mediated response appeared in heat maps to be down-regulated in individuals with high *C. salmositica* load and high IGMS expression. After considering the expression of HEP, MMP13, and IGMS in the context of previous literature, I suspect that elevated transcription of these genes is an immune response to the presence of and damage caused by *C. salmositica*.

The impact of *C. salmositica* on adult Chinook salmon in this study was demonstrated at the molecular (genes), physiological (plasma variables), and organism (migratory success) levels. However, I do not expect this infectious agent to be a common problem for upstream migrating adult Chinook salmon prior to arrival in natal streams. The infectious agent is encountered primarily in spawning habitat (BECKER and KATZ 1965) and the fact that it played an important role in upstream migration may be an artifact of my experimental design (moving fish from "spawning grounds" and requiring them to again migrate upstream). It could however, play an important role in shaping spawning ground demographics and reproductive capacity (WOO 2003). There are some accounts of *C. salmositica* associated with epizootics in Chinook salmon aquaculture and prespawn mortality (death following arrival on spawning grounds but preceding spawning) in wild fish (WOO 2003). Most importantly, my study provides an example of how one infectious agent species can play a significant role in determining migratory success, and how this phenomenon can be tested.

Because an infectious agent was a stronger predictor of longevity and survival to spawning than simu-

lated fisheries capture and release (or escape), this study illustrates the importance of the biological context underlying fisheries capture (RABY *et al.* 2015). Due to rapidly advancing high-throughput molecular technologies, like those I employed in this study, it may soon be possible to monitor infectious agent prevalence or the expression of select genes on a real-time basis to inform in-season management decisions, similar to how real-time monitoring of Fraser River sockeye salmon stock components and water temperature is currently incorporated (MACDONALD *et al.* 2010). Meanwhile, conducting field studies where multiple capture techniques are applied to different stages of maturity (varied using capture location) and infectious agents are measured (as in this study) is a logical next step to better understanding the intersection of fisheries capture, infectious agents, and mortality in salmon migrations.

Chapter 5

Severity of visible injuries is associated with survival, immune function, and thermal selection in stratified lakes for sockeye salmon late in their migration

5.1 Synopsis

The integrity of skin, which serves as a barrier to infectious agents and an aniso-osmolar environment, is vital to the survival of all fish species. Pacific salmon, which conduct challenging migrations featuring varying salinities and exposure to a multitude of infectious agents, are particularly vulnerable to skin damage, which is often caused by interactions with in-river fisheries. I intercepted sockeye salmon after they successfully passed through an in-river gillnet fishery, and categorized their skin injuries according to severity. Collection of non-lethal biopsies of gill tissue enabled the determination of their loads of infectious agents and immune gene expression by qPCR. Radio tags with thermal loggers were implanted in fish to determine migration survival and temperature selection in two stratified lakes as fish approached spawning grounds. Fish with the most severe injuries had a 63% lower probability of migration survival compared to those with no injury, and displayed high expression of genes associated with an acute phase inflammatory response. Visible wounds from sea lice (family: Caligidae) were also associated with lower migration survival and reduced spawning success. Fish with more severe injuries were also more likely to utilize cooler water in a stratified lake, and this strategy was associated with lower spawning success in females. These results indicate that fishing gears that minimize injury will also minimize delayed mortality.

5.2 Introduction

The proper function of skin is essential for the survival of all fish (ELLIOTT 2011). Acting as an osmotic barrier, skin prevents the diffusion of ions from fish musculoskeletal tissue into the surrounding water in freshwater and the reverse process in saltwater (GLOVER *et al.* 2013). By providing a protective layer, skin acts as the first layer of the immune system, and in most fish is coated with antimicrobial mucus that actively destroys bacteria and other infectious agents (microscopic organisms including viruses, bacteria, fungi, protozoans, etc.) (ÁNGELES ESTEBAN 2012). While wounds in fish skin may heal rapidly under proper conditions (QUILHAC and SIRE 1999), damage to the integument, especially large-scale damage,

leaves fish vulnerable in an aniso-osmolar and infectious-agent-rich environment.

While there are many natural sources of damage to fish skin, including predators, intra-specific competition, spawning behavior, enivronmental conditions, macroparasites (e.g. family Caligidae), infectious agents, and swimming obstacles, fisheries are a common anthropogenic source (DAVIS 2002). Fish may interact with gear and volitionally escape, or they may be intentionally released, as is sometimes mandated for a certain species in a commercial fishery or practiced by a catch and release recreational fisher. Herein, we use the term "non-take fisheries interaction" (NTFI) to encompass any instance where a fish interacts with fisheries gear but is subsequently released alive. NTFI events can range in intensity from the relatively invisible removal of mucus from a fish contacting a landing net to the removal of large areas of skin from a fish entangled in the mesh of a commercial net and struggling to escape.

Since diadromous migrations bring abundant populations of fish within close proximity of human populations, they often give rise to large-scale fisheries where a high frequency of NTFI is expected to occur. Fish conducting anadromous migrations, including Pacific salmon (*Oncorhynchus spp.*), present a special case for skin injuries. First, in addition to the sources of injury mentioned in the previous paragraph, these fish face a great range of environmental and physical conditions through which their skin must provide protection including large variation in salinities and water temperatures. Second, migrating adults entering freshwater environments are exposed to a broad diversity and high density of freshwater infectious agents (MILLER *et al.* 2014, Appendices A and B). Third, fish conducting anadromous migrations often rely on stored energy (referred to here as gross somatic energy, GSE), which implies limited resources for wound healing, an energy-intensive process (ARCHIE 2013). Although there is great variability among populations, Pacific salmon tend to mature during their adult spawning migrations so that their skin undergoes a transformation from thin and fragile to thick and tough as they move upstream and scales are absorbed (KACEM *et al.* 1998; ROBERTSON and WEXLER 1960). Visible damage resulting from NTFI has been associated with fishing-related incidental mortality (FRIM) for multiple stocks of Pacific salmon, with gillnets being identified as a commonly used, and particularly damaging gear type (BAKER and SCHINDLER 2009; THOMPSON *et al.* 1971, Chapter 2). Furthermore, researchers have demonstrated that gillnet NTFI imposes size and sex-biased selection on salmon through FRIM (BAKER *et al.* 2011), and also delays maturation (BAKER *et al.* 2013).

The close relationship between skin damage and fish mortality has been demonstrated in laboratory and field studies where researchers linked damage to skin with osmoregulatory failure (MATEUS *et al.* 2017; OLSEN *et al.* 2012) or the lack of defense against infectious agents (BADER *et al.* 2006; BAKER and SCHINDLER 2009; SVENDSEN and BØGWALD 1997; THOMPSON *et al.* 1971). Damage to the integument is followed by upregulation of genes associated with the acute phase response (APR), a mobilization of pro-inflammatory cytokines that guides synthesis of tissue-repair and antimicrobial proteins (BAYNE and GERWICK 2001; GONZALEZ *et al.* 2007; KRASNOV *et al.* 2012). Although the APR primarily involves release of acute phase proteins (APP) from the liver, its effects on the organism are systemic and therefore can be measured in fish blood (BAYNE and GERWICK 2001), even within small, non-lethal collections of gill tissue. The APR can serve as an indicator of life-threatening injury or infection, and to my knowledge it has never been documented in, or linked to mortality in, wild fish *in situ*.

During freshwater migrations, salmon utilize cold water refugia in response to phsyiological condition

(ROSCOE *et al.* 2010), to escape elevated temperatures (GONIEA *et al.* 2006), and while holding prior to spawning (NEWELL and QUINN 2005). It has been suggested, but not conclusively demonstrated, that sockeye salmon (*O. nerka*) utilize colder water layers in stratified lakes to hinder the development of pathogenic infections (BRADFORD *et al.* 2010b; MATHES *et al.* 2009). Recently, JENSEN *et al.* (2015) demonstrated that experimentally inflicted wounds on Atlantic salmon heal significantly faster at 12°C than at 4°C, although no related temperature preference by fish in such a situation has been demonstrated to date. If factors like infectious agent load and injury can influence the propensity of migrating salmon to access cold water, one might expect injuries from gillnet NTFI to influence the thermal experience of salmon as they hold or transit natal lakes.

In Chapter 3, I demonstrated that sockeye salmon with wounds caused by gillnet NTFI (and other visible skin injuries) experienced reduced migration survival and spawning success. In this study I observed skin injuries on sockeye salmon in the same location, the Seton-Anderson Watershed, and categorized them according to severity. I then compared these injuries to basic physical characteristics, infectious agent load, immune gene expression, migration survival, spawning success, and temperature selection in two thermally stratified lakes. I hypothesized that, compared to uninjured fish, severely injured fish would: 1) experience elevated migration failure and prespawn mortality, 2) be disproportionately large and male, 3) present elevated GSE, 4) harbor higher infectious agent loads, 5) express upregulation of immune genes, and 6) select cooler strata when transiting lakes, compared to uninjured fish.

5.3 Methods

5.3.1 Study area

Gates Creek sockeye salmon (hereafter GCss) spawn in Gates Creek, located at the southern end of Anderson Lake, in the Seton-Anderson watershed (Figure 5.1). Characteristics of GCss and their migration are described in Section 3.3.1. GCss have been in freshwater approximately 10 days prior to their arrival in the Seton River.

5.3.2 Fish collection, biopsy, and tagging

From August 15th to 24th, 2015, GCss were collected from a river-spanning fishing fence (200 m downstream of Seton Dam; N 50.6702°, W -121.9751°, Figure 5.1). The fishing fence and its operation are described in Section 3.3.2. Because the intent of this study was to investigate gillnet injuries, which are generally present in 25% of GCss at the fishing fence (Chapter 3), fish with noticeable gillnet marks were selected from the trapbox. For each fish selected for a visible injury, I selected another fish with no injury, to maintain a balanced design throughout the tagging period, although gillnet injuries were rare in the last two sampling days.

Fish were transferred from the holding pens into a V-shaped trough (continuously supplied with fresh river water) and restrained by hand. First, gross somatic energy (hereafter GSE) was measured using a fish FatMeter (Model FM 692, Distell, West Lothian, Scotland, UK) as described in CROSSIN and HINCH (2005). A 6 mm tissue punch from the adipose fin was taken from each fish to determine population origin by

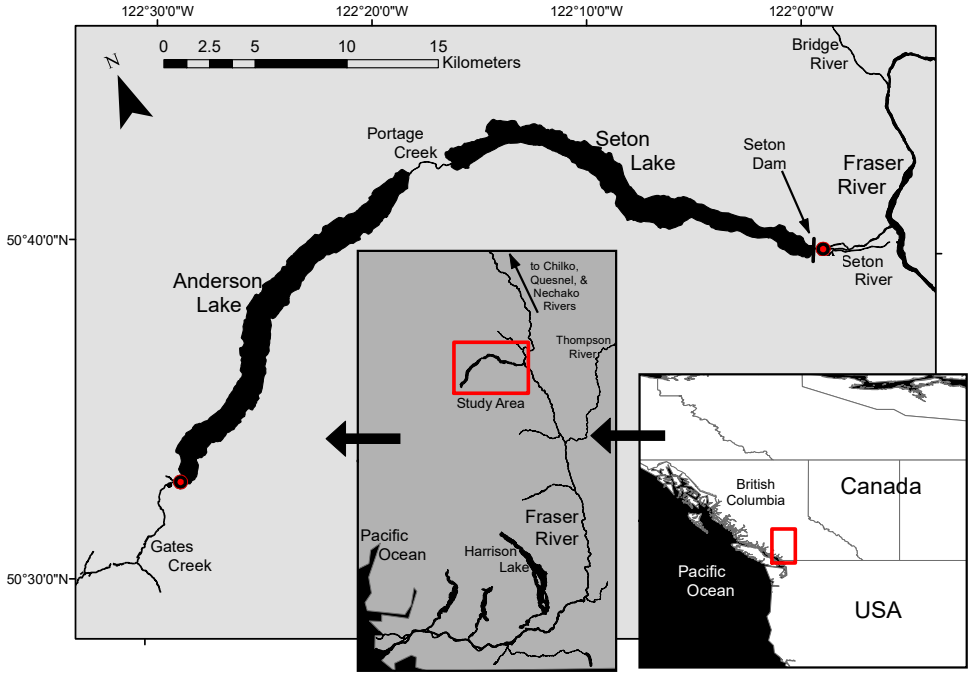

Figure 5.1: Map of the Seton-Anderson watershed in British Columbia, Canada. Red circles indicate PIT antennas at the Seton Dam (sockeye salmon were captured, tagged and released 200 m downstream of Seton Dam) and the mouth of Gates Creek.

variation in the major histocompatability complex (BEACHAM *et al.* 2004). While only GCss spawn in the Seton-Anderson Watershed at this time of year, straying sockeye salmon are frequently encountered at the fishing fence, many with gillnet injuries (Chapter 3). A small gill sample (2-3 mm of 3-4 gill filament tips) was removed with sterilized end clippers and placed in 1.5 mL of RNAlater ® (Life Technologies, Carlsbad, CA). Gill tissue is a point of entry and infection for many infectious agents, includes small amounts of blood which contain circulating infectious agents and salmon immune gene RNA, and allows for low-impact non-lethal sampling (COOKE *et al.* 2005).

Each fish was tagged with individually-coded 32 mm half-duplex (HDX) passive integrated transponder (PIT) tags (Oregon RFID, Portland, OR, USA) implanted subcutaneously in the dorsal sinus. A Pisces 5® radio tag (43 mm length $\times$ 16 mm diameter, 15.2 g in air, 5 s burst rate; Sigma Eight Inc, Newmarket, ON) was guided through the mouth and into the stomach, to rest immediately behind the esophageal sphincter with the wire antenna hanging out of the fish's mouth. Every radio tag had an Ibutton® thermochron model DS1921Z logger (6 mm height $\times$ 16 mm diameter, 3.3 g in air, 30 min recording; Maxim Integrated, San Jose, CA) affixed to record the water temperature experienced by each fish. Acquisition of these data require recovery of the ibutton. For visual identification, a 305 mm spaghetti tag (Floy Tag & Mfg. Inc., Seattle, WA, USA) was inserted behind the dorsal fin and secured with an aluminum crimp. Lastly, each fish was visually assessed for injuries, then released on the upstream side of the fence.

Gillnet injuries were readily identifiable as linear wounds that ranged in severity from thin dark lines

to large open wounds (Figure 5.2). Such injuries were typically located around the snout, on the dorsal side posterior to the head, and extending from the anterior tip of the dorsal fin to the base of the pelvic fins. Researchers had first-hand experience identifying gillnet wounds from previous studies where sockeye salmon were experimentally inserted into gillnets (TEFFER *et al.* 2017) and studies where fish were captured by gillnet (Chapter 2). Gillnet injuries were categorized similarly to BAKER and SCHINDLER (2009) on a 4 step scale (hereafter "netscore"), where 0 = no visible damage, 1 = minimal net marking, 2 = multiple net marks, no exposed flesh, and 3 = extensive net marks from head to dorsal fin, exposed flesh (Figure 5.2). Sea

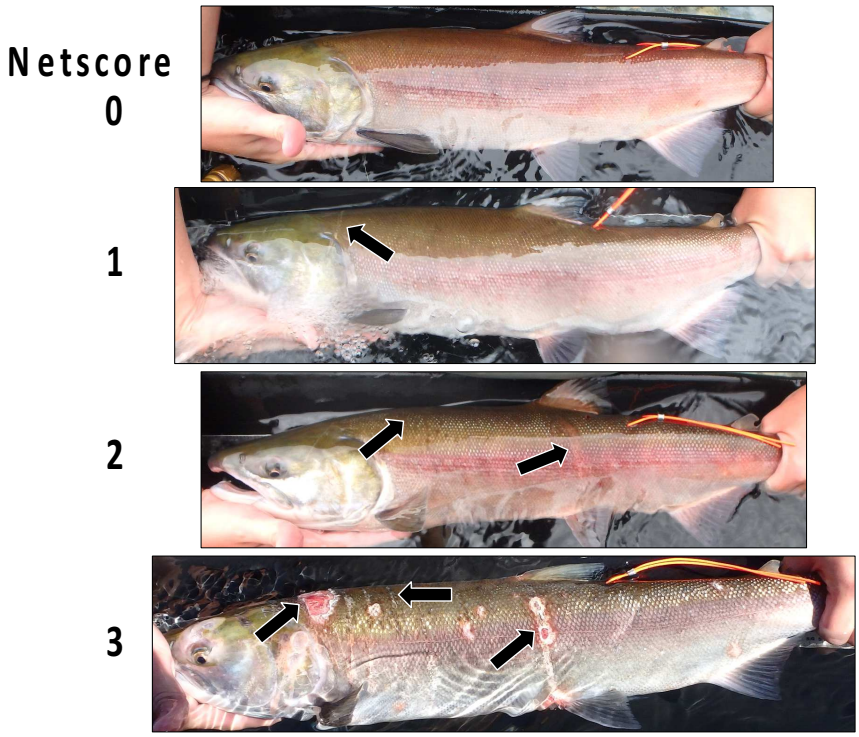

Figure 5.2: Gates Creek sockeye salmon demonstrating the four levels of netscore, Seton River, British Columbia, 2015. Arrows indicate locations where fish became entangled in gillnets and received visible injury.

lice injuries were visible as scarring, scale loss, or skin loss lateral or posterior to the dorsal fin, posterior to the adipose fin, superior to the anal fin, or on the ventral side of the fish (Figure 3.2). Sea lice injuries were scored on an ordinal scale as an estimated percentage of body surface area (maximum = 30%). A single observer estimated netscore and sea lice injury on every fish in the study.

5.3.3 PIT and Radio Telemetry

PIT antennas were installed at Seton Dam, at the mouth of Gates Creek, and at the entrance to the Gates Creek spawning channel. Details of PIT installations are available in Section 3.3.5. Fixed radio telemetry receivers (Orion®, Sigma Eight Inc.), each equipped with either a 3 or 4 element Yagi antenna, were positioned at strategic locations throughout the Seton-Anderson Watershed for concurrent studies (Figure 5.1). The three receivers used in this study were positioned between the two lakes at the mouth of Portage Creek, in

Lower Gates Creek near the PIT antenna, and at the Gates Creek spawning channel (antenna efficiency of all antennas $\geq$ 90%). Radio detections were filtered for each individual fish so that detections at a given receiver separated by more than 2 minutes (likely false positives) or less than 5 seconds (pulse rate of radio tags) were removed. The detection history for each fish was plotted against receiver location so that false detections could be identified and removed.

5.3.4 Thermal experience in Seton and Anderson Lakes

Previous studies in the Seton-Anderson Watershed have demonstrated that females select specific temperature strata as they migrate through the two lakes in relation to sexual maturity and energy levels (ROSCOE *et al.* 2010), and that there is an association between thermal experience and spawning success (MINKE-MARTIN *et al.* 2018). As mentioned above, ibutton thermocrons affixed to radio tags recorded the thermal experience of GCss every 30 min as they traveled from release to Gates Creek. After retrieving and downloading ibuttons, temperature data recorded outside the lakes were removed by determining the lake entry and exit times using PIT and/or radio telemetry data from Seton Dam and Gates Creek. The final radio detection at Portage Creek was used to separate detections between Seton and Anderson Lakes, which implies that temperature measurements recorded in Portage Creek were analyzed as part of the Anderson Lake data. Tags from fish that were recovered from the Portage Creek fishery only have temperature data for Seton Lake.

5.3.5 Carcass recoveries and spawning success

The methods for carcass and tag recovery, as well as determination of spawning success was identical to that described in Section 3.3.6. A small percentage of tagged fish were captured in a rod and reel subsistence fishery at Portage Creek, and tags and ibuttons were recovered from some of these fish.

5.3.6 Molecular Methods

Gill samples were screened quantitatively for the presence of 17 infectious agents (Table 5.1) known or suspected to infect Fraser River sockeye salmon, using HT-qPCR on the Fluidigm BioMark^{TM} microfluidics platform. Using the same technology, I measured the expression of 43 sockeye salmon genes in gill tissue (Table 5.2). The platform, populated by assays to 45 potential salmon pathogens, was analytically validated for research applications with salmon (MILLER *et al.* 2016) and utilizes the same TaqMan probe assays as other single assay platforms. Assays were designed for RNA, which enables the inclusion of some RNA viruses, focuses on infectious agents in an active state, and allows measurement of host gene expression (MILLER *et al.* 2016, 2014). Salmon gene assays were chosen to represent components of innate immunity, cell-mediated immunity, the response to viral disease development (VDD) (MILLER *et al.* 2017), and osmoregulation. The methods for tissue homogenization, RNA extraction, amplification, and quantitative PCR were identical to those described in Section 4.3.4.

Table 5.1: Infectious agent assayed in gill tissue of sockeye salmon collected from the Seton River, British Columbia, 2015. Positive detections within the BioMark limit of detection (LOD) are denoted "Y", agents not detected in any samples within the LOD are denoted "N". Assay specific cutoffs for LOD were applied, and can be found in Appendix C.

taxa group	Scientific Name	Positives within LOD
bacteria	'*Candidatus* Branchiomonas cysticola'	Y
	Flavobacterium psychrophilum	Y
	Gill chlamydia	N
	Piscichlamydia salmonis	N
	Rickettsia-like organism	N
	Yersinia ruckeri	N
mesomycetozoea	*Dermocystidium salmonis*	Y
myxozoa	*Ceratonova shasta*	Y
	Parvicapsula minibicornis	Y
	Parvicapsula pseudobranchicola	N
	Tetracapsuloides bryosalmonae	N
protozoa	*Ichthyophthirius multifiliis*	Y
microsporidia	*Loma salmonae*	Y
	Paranucleospora theridion	Y
virus	Infectious hematopoietic necrosis virus	N
	Pacific salmon parvovirus	N
	Piscine reovirus	N

Table 5.2: Genes amplified in sockeye salmon gill tissue collected from the Seton River, British Columbia, 2015. Gene abbreviation, name, group (relevant to this study), and primer and probe sequences are provided. For group, VDD = viral disease development (MILLER *et al.* 2017), CMR = cell-mediated response, APR = acute phase response, osmo = osmoregulatory, ref = reference genes.

Abbreviation	Gene Name	Group	PCR Efficiency (%)	Forward	Reverse	Probe
52RO	52 kDa Ro protein 2	VDD	103	TGCACTATTGCCCAGTAACCAT	TGCAAGAGGAGATGCCAACA	AGTAGGATTCACAGAGAGTT
78d16.1	S100 calcium binding protein	ref	109	GTCAAGACTGGAGGCTCAGAG	GATCAAGCCCCAGAAGTGTTTG	AAGGTGATTCCCTCGCCGTCCGA
C1Qc	complement component 1q	APR	111	CGCCGGTGAGTGGAATCTA	CTTCTCCATCATGTGGTGTGCTA	ACCTCCAAACATAGAAGAG
C3	complement component 3	APR	105	ATTGGCCTGTCCAAAACACA	AGCTTCAGATCAAGGAAGAAGTTC	TGGAATCTGTGTGTCTGAACCCC
C7	complement component 7	APR	113	ACCTCTGTCCAGCTCTGTGTC	GATGCTGACCACATCAAACTGC	AACTACCAGACAGTGCTG
CA054694	mitochondrial ribosomal protein VAR1	VDD	100	CCACCTGAGGTACTGAAGATAAGACA	TTAAGTCCTCCTTCCTCATCTGGTA	TCTACCAGGCCTTAAAG
CD4	cluster of differentiation 4	CMR	105	CATTAGCCTGGGTGGTCAAT	CCCTTTCTTTGACAGGGAGA	CAGAAGAGAGAGCTGGATGTCTCCG
CD8a	cluster of differentiation 8a	CMR	104	ACACCAATGACCACAACCATAGAG	GGGTCCACCTTTCCCACTTT	ACCAGCTCTACAACTGCCAAGTCGTGC
COIL-P84	coiled-coil domain-containing protein 84	ref	103	GCTCATTTGAGGAGAAGGAGGATG	CTGGCGATGCTGTTCCTGAG	TTATCAAGCAGCAAGCC
CXCR4	fusin	CMR	110	GGAGATCACATTGAGCAACATCA	GCTGCTGGCTGCCATACTG	TCCACGAAGATCCCCA
DEXH	ATP-dependent RNA helicase 58	VDD	103	TGGAGAAGAAGGGTGTGACAGA	CGCAGGTGGAGAGCACACT	AGGAACAGACTGCTGGC
GAL3	Galectin-3-binding protein precursor	VDD	112	TTGTAGCGCCTGTTGTAATCATATC	TACACTGCTGAGGCCATGGA	CTTGGCGTGGTGGC
HEP	hepcidin antimicrobial protein	APR	120	GAGGAGGTTGGAAGCATTGA	TGACGCTTGAACCTGAAATG	AGTCCAGTTGGGGAACATCAACAG
HERC6	Probable E3 ubiquitin protein ligase	VDD	115	AGGGACAACTTGGTAGACAGAAGAA	TGACGCACACACAGCTACAGAGT	CAGTGGTCTCTGTGGCT
IFI44A	IFN-induced protein 44-1	VDD	116	CGGAGTCCAGAGCAGCCTACT	TCCAGTGGTCTCCCCATCTC	CGCTGGTCCTGTGTGA
IFIT5	IFN-induced protein with tetratricopeptide repeats 5	VDD	103	CCGTCAATGAGTCCCTACACATT	CACAGGCCAATTTGGTGATG	CTGTCTCCAAACTCCCA
IgMs	immunoglobulin	CMR	106	CTTGGCTTGTTGACGATGAG	GGCTAGTGGTGTTGAATTGG	TGGAGAGAACGAGCAGTTCAGCA
IL-11	interleukin 11	APR	113	GCAATCTCTTGCCTCCACTC	TTGTCACGTGCTCCAGTTTC	TCGCGGAGTGTGAAAGGCAGA
IL-15	interleukin 15	CMR	114	TTGGATTTTGCCCTAACTGC	CTGCGCTCCAATAAACGAAT	CGAACAACGCTGATGACAGGTTTTT
IL-1B	interleukin 1 beta	APR	99	AGGACAAGGACCTGCTCAACT	CCGACTCCAACTCCAACACTA	TTGCTGGAGAGTGCTGTGGAAGAA
IL -8	interleukin 8	APR	90	GAGCGGTCAGGAGATTTGTC	TTGGCCAGCATCTTCTCAAT	ATGTCAGCGCTCCGTGGGT
IRF1	IFN regulatory factor 1	CMR	93	CAAACCGCAAGAGTTCCTCATT	AGTTTGGTTGTGTTTTTGCATGTAG	CTGGCGCAGCAGATA
MHCI	major histocompatability complex 1	CMR	113	GCGACAGGTTTCTACCCCAGT	TGTCAGGTGGGAGCTTTTCTG	TGGTGTCCTGGCAGAAAGACGG
MHCII-B	major histocomp. complex 2 beta	CMR	106	TGCCATGCTGATGTGCAG	GTCCCTCAGCCAGGTCACT	CGCCTATGACTTCTACCCCAAACAAAT
MMP13	matrix metalloproteinase-13	APR	113	GCCAGCGGAGCAGGAA	AGTCACCTGGAGGCCAAAGA	TCAGCGAGATGCAAAG
MMP25	matrix metalloproteinase-25	APR	114	TGCAGTCTTTTCCCCTTGGAT	TCCACATGTACCCACACCTACAC	AGGATTGGCTGGAAGGT
MRPL40	39S ribosomal protein L40, mitochondrial precursor	ref	114	CCCAGTATGAGGCACCTGAAGG	GTTAATGCTGCCACCCTCTCAC	ACAACAACATCACCA
Mx	IFN-induced GTP-binding protein	CMR	92	AGATGATGCTGCACCTCAAGTC	CTGCAGCTGGGAAGCAAAC	ATTCCCATGGTGATCCGCTACCTGG
NKA	NKA α subunit a3	osmo	102	GGAGACCAGCAGAGGAACAG	CCCTACCAGCCCTCTGAGT	AAGACCCAGCCTGAAATG
NKAa1-a	NKA α subunit a	osmo	109	TGGAATCAAGGTTATCATGGTCACT	CCCACACCCTTGGCAATG	ATCATCCCATCACTGCGA
NKAa1-b	NKA α subunit b	osmo	106	GCCTGGTGAAGAATCTTGAAGCT	GAGTCAGGGTTCCGGTCTTG	CCTCCACCATTTGCTCA
NFX	zinc finger NFX1-type	VDD	87	CCACTTGCCAGAGCATGGT	CGTAACTGCCCAGAGTGCAAT	TGCTCCACCGATCG

PCBL	precerebellin	APR	104	TGGTGTTGCTTTGCTGTT GT	GCCACTTTTGGTTTGCT CTC	ATGGTTGAGACTCAGAC GGAGAGTG
RSAD	radical S-adenosyl methionine domain containing protein 2	VDD	107	GGGAAATTAGTCCAATA CTGCAAAC	GCCATTGCTGACAATAC TGACACT	CGACCTCCAGCTCC
SAA	serum amalyoid	APR	116	GGGAGATGATTCAGGGT TCCA	TTACGTCCCCAGTGGTT AGC	TCGAGGACACGAGGAC TCAGCA
SRK2	tyrosine protein kinase FRK	VDD	89	CCAACGAGAAGTTCACC ATCAA	TCATGATCTCATACAGC AAGATTCC	TGTGACGTGTGGTCCT
STAT1	signal transducer and activator of transcription 1-alpha/beta	VDD	99	TGTCACCGTCTCAGACA GATCTG	TGTTGGTCTCTGTAAGG CAACGT	AGTTGCTGAAAACCGG
TCRα	t-cell receptor α	CMR	105	ACAGCTTGCCTGGCTAC AGA	TGTCCCCTTTCACTCTG GTG	CAGCGCACACAAGGCT AATTCG
TCRβ	t-cell recepter β	CMR	84	TCACCAGCAGACTGAGA GTCC	AAGCTGACAATGCAGGT GAATC	CCAATGAATGGCACAA ACCAGAGAA
TF	transferrin	APR	116	TTCACTGCTGGAAAATG TGG	GCTGCACTGAACTGCAT CAT	TGGTCCCTGTCATGGTG GAGCA
TNF	tumor necrosis factor	APR	120	CCCACCATACATTGAAG CAGATT	GGATTGTATTCACCCTC TAAATGGA	CCGGCAATGCAAAA
VHSV-P10	*Oncorhynchus mykiss* VHSV-induced protein 10	VDD	79	GCAAACTGAGAAAACC ATCAAGAA	CCGTCAGCTCCCTCTGC AT	TGTGGAGAAGTTGCAGG C
VHSVIP4	VHSV-induced protein 1	VDD	93	GCTCTCGTAAAGCCCCA CATC	GGGCGACTGCTCTCTGA TCT	AAACTGCACGTCGCGC
ZAP7	Tyrosine-protein kinase ZAP-70	CMR	106	TCACCTCCGGACCTTTC ATT	CCATGTGGGAAGCCTTT TCTT	TCTTGTATGGTTTTCCTC C

5.3.7 Statistical Analyses

All statistical analyses were conducted in R statistical software (R CORE TEAM 2017). To test the hypothesis that higher netscores are associated with elevated mortality, I created a binomial generalized linear model (GLM) with arrival at Gates Creek (determined by detection by radio or PIT telemetry, or carcass recovery) as the response variable (arrival = "1") and netscore as the explanatory variable of interest. Since previous work in this system indicated the importance of body size, GSE, and the presence of sea lice wounds in association with migration survival (Chapter 3), I included these variables as covariates in the model. I used a similar approach to test the hypothesis that females with higher netscore would have reduced spawning success, including the same covariates from the migration survival model. For this binomial GLM, both complete and partial egg retention were considered to be spawning failure (spawn = "0") and complete egg release was considered spawning success. Interpretation of coefficients was reported as percent change to the odds ratio and the average of marginal effects (FERNIHOUGH 2011).

To test the hypotheses that as gillnet injury severity increases, GCss would be more likely to be male I used the Freeman-Haltman extension of Fisher's test to test a 2 x 3 contingency table (sex x netscore). Netscore 0 individuals were excluded because the systematic sampling design (purposefully selecting injured fish and then non-injured fish to balance) would bias probabilities. Regardless, the percentage of male and female GCss with netscore 0 was nearly equal. To test the hypothesis that larger fish would have greater injuries, I used the "polr()" function from the "MASS" package (VENABLES and RIPLEY 2002) to conduct ordinal regression with netscore as the response variable and fork length as the predictor. To test the hypothesis that fish with more severe gillnet injuries would have lower levels of GSE, I conducted a two-way Analysis of Variance (ANOVA) with GSE as the response variable and sex and netscore as the predictor variables. Sex was included because GSE tends to be higher for females (Chapter 3). Tukey's test was applied to determine whether contrasts were significant.

Infectious agent presence in GCss was presented in terms of prevalence (percentage infected) and load as RNA copy number (log-transformed in all analyses). For each individual, the total number of infectious agents present, referred to as "infectious agent richness", was calculated and compared between netscores by one-way ANOVA. For each infectious agent, the difference between sexes in terms of prevalence was tested by Fisher's exact test, and in terms of load by T tests. I tested whether prevalence was significantly different between netscore groups using the Freeman-Haltman Fisher's test as described above. I tested the hypothesis that infectious agent load would increase with gillnet wound severity using a one-way ANOVA with infectious agent load as the response variable followed by Tukey's test.

To reduce the dimensionality of the gene expression data I conducted a principal components analysis (PCA) using the "prcomp()" function (with scaling) in R. Investigation of the first five principal components (PCs) revealed that PC1 showed the greatest variation with netscore and survival, so further analyses were only conducted using PC1. To test the hypothesis that immune genes would be upregulated as netscore increased, I conducted a one-way ANOVA with PC1 as the response variable followed by Tukey's test. So that the relationship between individual gene expression and netscore could be presented, the fold change relative to the netscore 0 group for each gene's expression at each netscore was also calculated. Confidence intervals (95%) were also provided for fold change values to demonstrate when injury categories did and did not overlap in terms of gene expression. To limit the influence of outliers, the geometric mean (as opposed to arithmetic) was used to calculate fold change and confidence intervals.

I explored multiple approaches for parameterizing the temperature data, including median, mode, and accumulated thermal units (ATU), but decided that averaging the temperature measurements in each lake provided the best representation of what GCss experienced and was most comparable between individuals (residence time in lakes varied substantially, so ATU could not be used). I modeled average temperature for each lake using multiple linear regression with netscore as the primary variable of interest and body size, GSE, and sea lice wounds as covariates. During data exploration I noticed that males and females had obvious differences in temperature selection, so sex was also included as a covariate.

To account for variance due to tagging date, I created mixed effects models for migration survival, spawning success, and thermal experience in Seton and Anderson Lakes with tagging date as the random effect ("glmer()" and "lmer()" from the "lme4" package (BATES *et al.* 2015)). By comparing AIC values (for GLMs), standard errors, and significance of fixed effects between fixed and mixed effects models, I determined that mixed effects models only provided an improved fit for thermal experience in Anderson Lake. For this model, I estimated p values by comparing the t-value to a normal distribution. A pseudo-r-squared value for this model was estimated using the "r.squaredGLMM()" function in "MuMin" package (BARTON 2016), and represents the variance accounted for by the fixed and random effects.

5.4 Results

5.4.1 Migration survival and spawning success

Ten of 183 sockeye salmon tagged at the fishing fence were determined by DNA analysis to not have originated at Gates Creek and were removed from analysis. Eighteen GCss (10% of tagged) were captured in a

subsistence fishery in Portage Creek (Table 5.3). Among the remaining GCss (n = 155), netscore 3 fish had

Table 5.3: Fate and thermal experience (± standard deviation) of Gates Creek sockeye salmon tagged in the Seton River, British Columbia, 2015. N_{fate} = the number of fish that were caught in the Portage Creek fishery, survived migration, and successfully spawned, N_{Set} = the number of fish with temperature data for Seton Lake, N_{And} = the same for Anderson Lake. The percentage of migration survivors was calculated after deducting fisheries captures. The percentage of spawning success was calculated based on the number of fish with a known spawning fate (< 50% of females arriving at Gates Creek).

			N_{fate} (%)						
Netscore	N	Sex	Fishery	Migration survivor	Spawning success	N_{Set}	Seton mean temp°C	N_{And}	Anderson mean temp°C
0	F	57	4 (7)	43 (81)	19 (61)	27	13.6 ±2.5	24	11.9 ±2.0
	M	49	8 (16)	35 (85)	–	27	15.2 ±1.6	20	13.6 ±1.6
1	F	17	3 (18)	12 (86)	3 (50)	7	14.1 ±1.2	5	11.9 ±0.7
	M	9	1 (11)	8 (100)	–	6	15.2 ±1.6	5	13.0 ±1.3
2	F	12	0 (0)	9 (75)	4 (33)	3	12.7 ±3.4	3	11.5 ±3.2
	M	8	1 (13)	6 (86)	–	4	12.2 ±1.7	4	13.5 ±4.4
3	F	7	0 (0)	2 (29)	1 (50)	1	11.1 ± NA	1	9.4 ± NA
	M	13	1 (8)	1 (8)	–	1	10.2 ± NA	0	NA

the lowest migration survival with only 3 fish (16%) arriving in Gates Creek (Figure 5.3). A GLM including netscore, fork length, GSE, and sea lice injuries indicated that netscore and sea lice injuries were negatively associated with survival (Table 5.4). Compared to fish with no net marks, netscore 3 fish had 96% lower odds of surviving to Gates Creek, but netscore 1 and 2 were not significantly less likely to survive than fish with no net marks (Table 5.4). For each standard deviation increase in sea lice scars (6.6%), there was a 30% decrease in the odds of surviving to Gates Creek (Table 5.4). The average marginal effect (AME) for netscore 3 was a 63% decrease in the probability of migration survival and for a standard deviation increase in sea lice scars, a 6% decrease in survival. While fish with higher netscore had slightly lower spawning success, there was no significant association between netscore and female GCss spawning success (Table 5.4). However, sea lice injuries were significantly, negatively associated with spawning success. For each standard deviation increase in sea lice injuries (6.6%), there was a 41% decrease in the odds of successful spawning. This equated to an AME of a 17% decrease in the probability of successful spawning for each standard deviation increase in sea lice injury.

5.4.2 Physical characteristics and gillnet injury

Since individuals were systematically selected to balance the number of gillnet marked and unmarked fish, the prevalence of the different levels of netscore do not reflect their occurrence in the population. The ratio of males to females was greatest for the highest netscore group (1.7 for netscore 3, 0.8 for netscore 0), however there was no significant association between sex and injury severity. Both fork length and GSE were not significantly different among the netscore groups (Table 5.5). However, there was a trend of increasing GSE

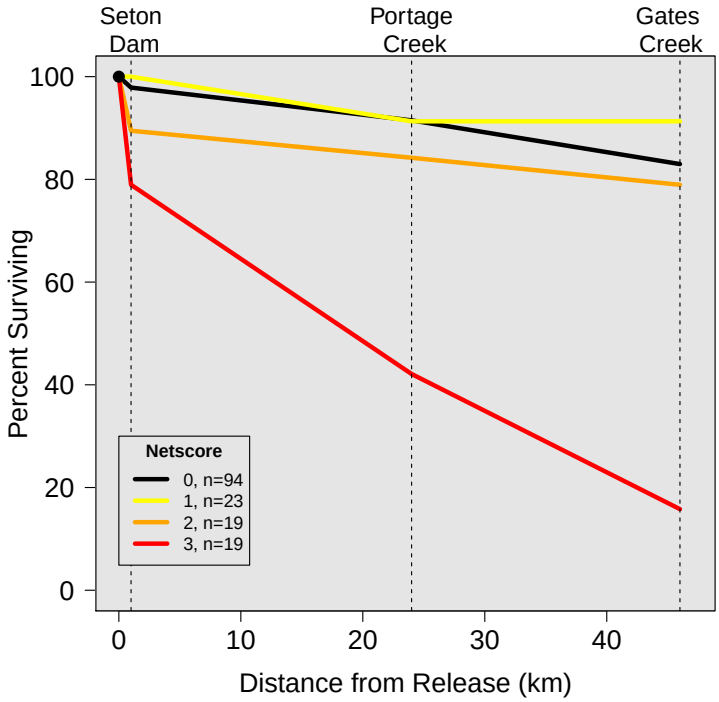

Figure 5.3: Migration survival of Gates Creek sockeye salmon radio tagged in the Seton River, British Columbia, 2015. Individuals are separated by gillnet injury severity (sample size provided in figure legend). Names at top indicate PIT and radio telemetry monitoring stations.

as netscore increased, with the greatest difference in groups between netscore 0 and netscore 3 fish (p = 0.13). A t-test indicated that sea lice injuries were significantly greater in females (mean = 8.0%) compared to males (mean = 5.1%, p = 0.002).

5.4.3 Infectious agents

Of the 17 infectious agent taxa assayed, eight were detected in the gill tissue of sockeye salmon collected at the fishing fence (Figure 5.4). There were no significant differences in infectious agent prevalence or load between sexes. There was no significant variation in infectious agent richness with netscore, and no apparent trend (Table 5.5). Plots of the three most prevalent infectious agents, *'Ca. B. cysticola'*, *C. shasta*, and *P. minibicornis*, indicated that there was little variation in infectious agent prevalence and load between netscore categories (Figure 5.5). The load of *C. shasta* appeared to decline across the netscore categories (Figure 5.5), but for the largest contrast, between netscore 1 and netscore 3, there was not a significant difference according to Tukey's test (p = 0.14).

5.4.4 Gene expression

The first three principal components (PCs) of a principal components analysis including 41 immune genes accounted for 49% of the variance in gene expression. Of these three PCs, only PC1 showed significant

Table 5.4: Regression model results for Gates Creek sockeye salmon migrating from the Seton River to Gates Creek, British Columbia, 2015. FL = fork length, GSE = gross somatic energy, and SLS = sea lice scars. Sex was not included in migration survival and spawning success models. R-squared values for logistic regression models are McFadden's r-squared, for linear regression is adjusted r-squared, and for the mixed model is pseudo r-squared. *P* values for explanatory variables significant at $p < 0.05$ are indicated in boldface.

				Explanatory Variables							
Response Variable	Model type	N obs	Model Parameter	Netscore 1	Netscore 2	Netscore 3	Sex	FL	GSE	SLS	r-sq.
Migration survival	Logistic regression	155	β	0.93	0.02	-3.13	–	0.41	-0.40	-0.93	0.26
			SE	0.80	0.67	0.70	–	0.44	0.47	0.43	
			p	0.25	0.97	**<0.001**	–	0.36	0.40	**0.03**	
Spawning success	Logistic regression	45	β	-0.09	-1.32	-0.35	–	0.93	-0.33	-1.70	0.18
			SE	1.04	1.06	1.49	–	0.79	0.72	0.77	
			p	0.93	0.21	0.82	–	0.24	0.65	**0.03**	
Mean Temperature (Seton Lake)	Linear regression	77	β	0.41	-2.02	-3.74	1.57	-1.10	-0.01	-1.13	0.25
			SE	0.61	0.79	1.51	0.51	0.53	0.49	0.48	
			p	0.51	**0.01**	**0.02**	**0.003**	**0.04**	0.99	**0.02**	
Mean Temperature (Anderson Lake)	Linear mixed model	63	β	0.04	0.20	-2.92	1.51	-0.19	0.14	-0.56	0.25
			SE	0.75	0.85	2.10	0.62	0.60	0.58	0.53	
			p	0.95	0.81	0.16	**0.01**	0.75	0.80	0.29	

Table 5.5: Summary statistics ($\pm$ standard deviation) at tagging for Gates Creek sockeye salmon tagged in the Seton River, British Columbia, 2015. GSE = gross somatic energy, SLI = sea lice injuries, N_{IAR} = the number of fish included in estimations of infectious agent richness.

Netscore	Sex	N	Fork length (cm)	GSE (MJ/kg)	SLI (%)	N_{IAR}	IA richness
0	F	57	57.0 $\pm$2.6	5.6 $\pm$0.5	7.2 $\pm$6.1	20	3.1 $\pm$1.1
	M	49	58.6 $\pm$3.6	5.5 $\pm$0.5	4.3 $\pm$4.2	10	3.1 $\pm$1.4
1	F	17	56.4 $\pm$1.4	5.6 $\pm$0.6	7.6 $\pm$7.7	15	2.9 $\pm$1.4
	M	9	58.3 $\pm$2.2	5.7 $\pm$0.7	3.3 $\pm$3.5	8	2.8 $\pm$1.4
2	F	12	57.0 $\pm$2.2	5.9 $\pm$0.6	9.2 $\pm$10.4	12	3.2 $\pm$1.0
	M	8	57.9 $\pm$2.8	5.4 $\pm$0.4	8.8 $\pm$7.4	7	3.3 $\pm$0.8
3	F	7	57.1 $\pm$1.8	5.8 $\pm$0.8	13.6 $\pm$7.5	6	2.7 $\pm$0.5
	M	13	58.0 $\pm$2.1	5.8 $\pm$0.6	6.9 $\pm$6.6	13	2.8 $\pm$0.8

variation across netscores. Fish with netscore 3 had significantly higher values of PC1 than all other netscore categories (Figure 5.6B,C). Genes associated with viral disease development and osmoregulation were neu-

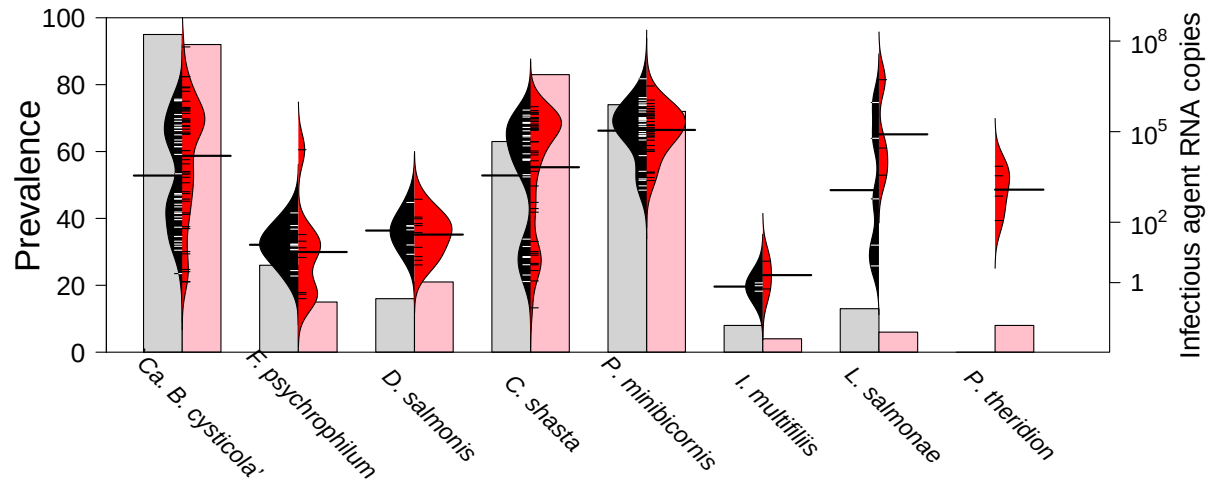

Figure 5.4: Prevalence and load (RNA copy number) of infectious agents detected in sockeye salmon gill sampled in the Seton River, British Columbia, 2015. Bars represent prevalence and beanplots represent load (small hash marks are individuals and heavy horizontal line is group mean) for males (gray bars, black beans) and females (pink bars, red beans).

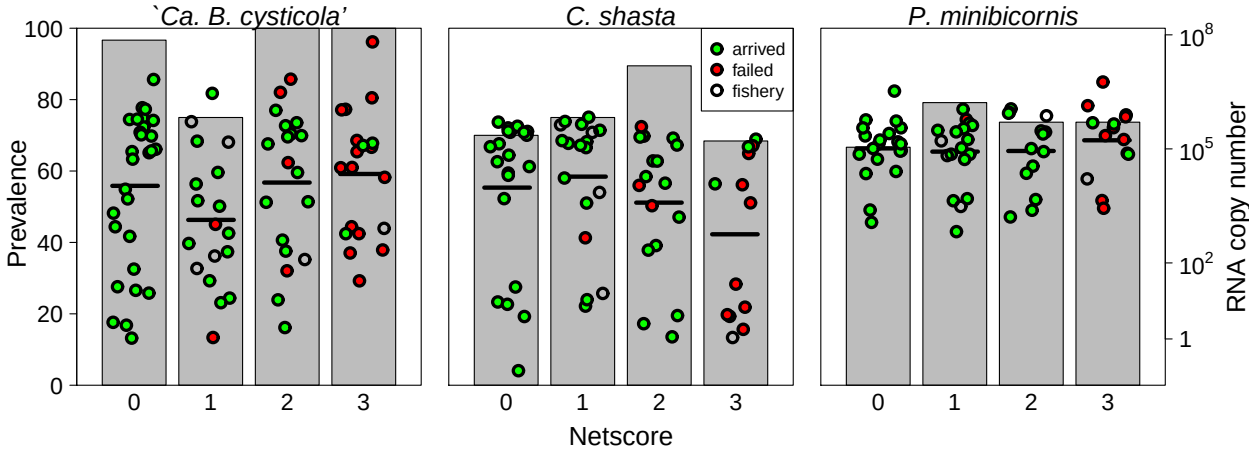

Figure 5.5: Prevalence and load of the three most prevalent infectious agents (names at top) detected in sockeye salmon gill samples collected in the Seton River, British Columbia, 2015. Prevalence and load are presented by gillnet injury severity (netscore). Gray bars indicate prevalence and points indicate individual infectious agent RNA copy number, color coded by migration fate. Horizontal black lines are group means.

trally correlated with PC1, except for IFI44A, which was negatively correlated (Figure 5.6A). Genes associated with the APR were positively correlated with PC1 (Figure 5.6A, 5.7). Genes associated with t-cell activity and the cell-mediated (specific, acquired) immune response were negatively correlated with PC1 (Figure 5.6A, 5.7).

Although not on the magnitude of the APR genes, and therefore not correlated with PC1, there was consistent upregulation of osmoregulatory genes as netscore increased (Figure 5.7). Similarly, VDD genes

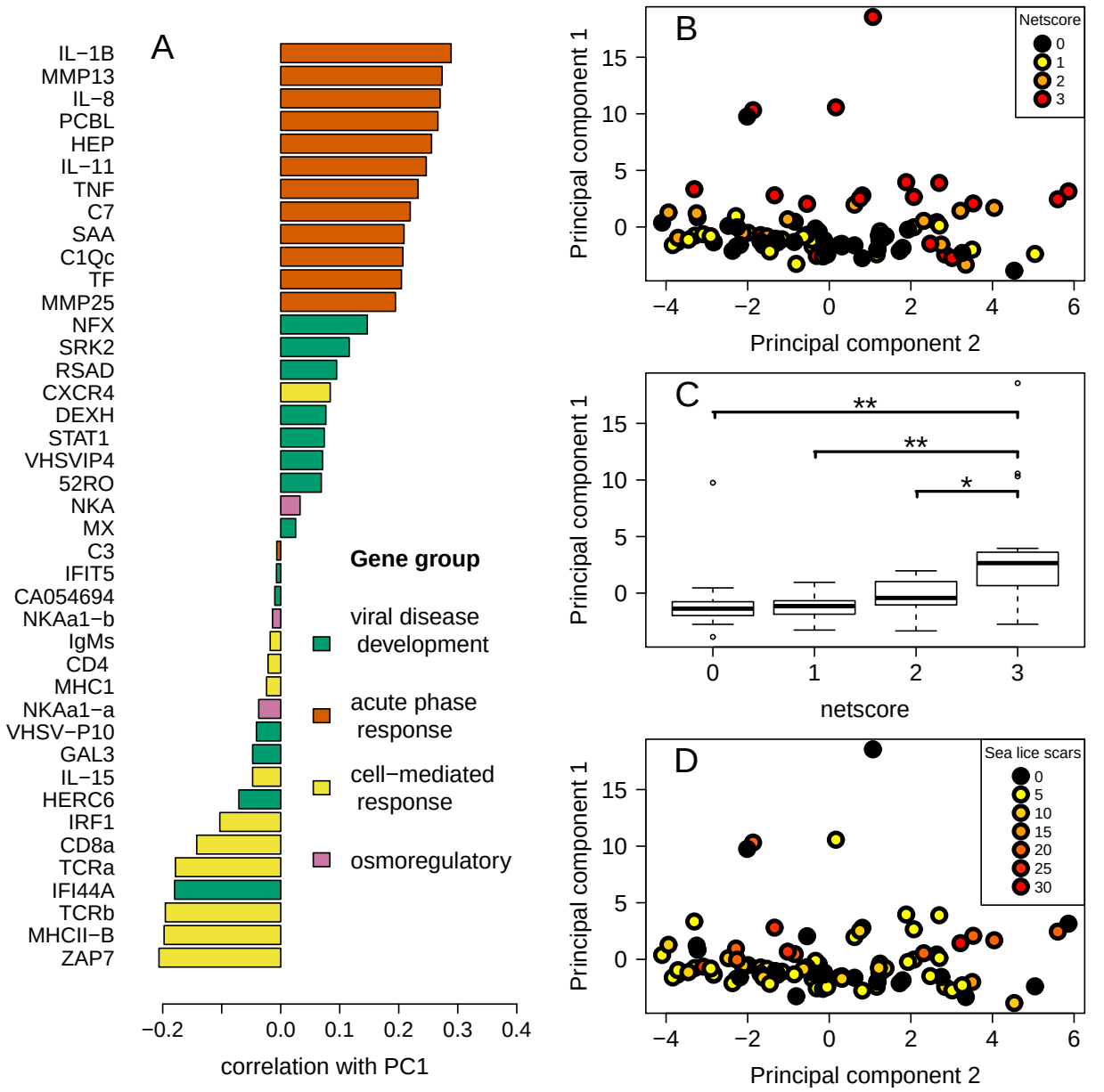

Figure 5.6: Comparison of a principal component (PC) of gene expression (A) in Gates Creek sockeye salmon gill, with gillnet injury severity (B, C), and sea lice scars (D). Bars in panel A represent the correlation of each gene (abbreviations at left), with PC1, and genes were grouped by putative function. In panel C, * indicates $p < 0.01$ and ** indicates $p < 0.001$.

were not correlated with PC1 but showed an overall trend of down-regulation (Figure 5.7).

Because sea lice injuries were associated with migration survival and spawning success, we also compared this covariate to immune gene expression. While there was a slight positive trend between sea lice injury and PC1, linear regression (with netscore 3 individuals removed due to their strong association with PC1) indicated that there was not a significant association ($p = 0.07$).

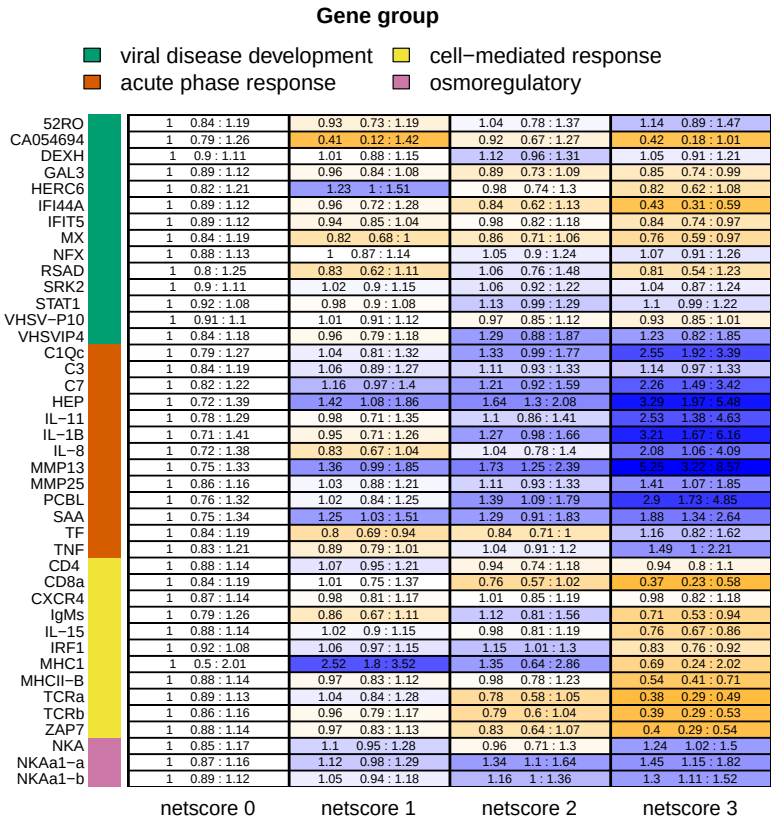

Figure 5.7: Sockeye salmon gene expression fold change as a function of netscore, for fish biopsied in the Seton River, British Columbia, 2015. Fold change is calculated relative to the netscore 0 group mean, therefore a value of 1 indicated no change in gene expression. The mean fold change for each group and gene combination is presented in each cell, with the 95% confidence interval around the mean. As confidence interval overlap becomes more distant from 1, the difference from the netscore 0 group becomes more substantial. Cell color indicates the direction of fold change (orange = down-regulation, white = neutral, blue = up-regulation). Gene names are indicated on the left, and ordered by gene group (legend).

5.4.5 Thermal selection in lakes

Sockeye salmon whose ibuttons were recovered in Gates Creek showed a preference for relatively similar temperatures in Seton and Anderson Lakes (Figure 5.8A, $r^2 = 0.33$), although on average they used signficantly warmer water in Seton Lake (1.3 ± 2.0°C, $p < 0.001$). Regression models revealed that males utilized warmer water than females in both lakes (Tables 5.3,5.4). Fish with netscores 2 and 3 chose significantly cooler water than fish with netscore 0 in Seton Lake (Figure 5.8B, Table 5.4). Females later experiencing

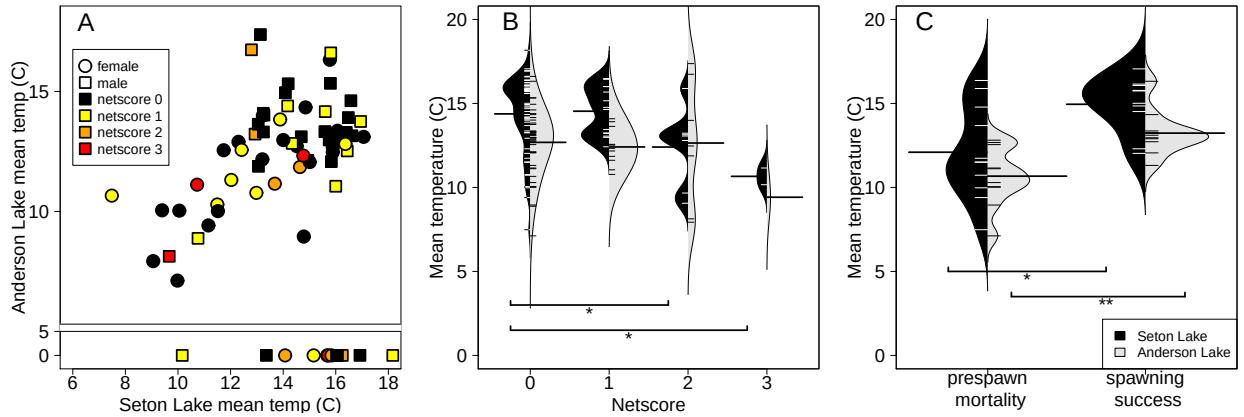

Figure 5.8: Mean temperatures experienced by Gates Creek sockeye salmon migrating through Seton and Anderson Lakes, British Columbia, 2015. Panel A shows the consistent temperature preference across the two lakes, with color indicating gillnet injury severity, and shape indicating sex. Individuals in panel A at 0° for Anderson Lake were recovered in between the lakes and therefore had no temperatures logged in Anderson Lake. The beanplots in panel B and C indicate mean temperature used in each lake (Seton = black, Anderson = gray), split by gillnet injury severity (B) and spawning fate (C). Small hash marks indicate individual means and large black horizontal marks indicate group means. In panels B and C, * indicates $p < 0.05$ and ** indicates $p \leq 0.001$.

prespawn mortality in Gates Creek (and spawning channel) selected colder temperatures in both Seton and Anderson Lakes than females spawning successfully (Figure 5.8C).

5.5 Discussion

The skin of Pacific salmon is an important defensive barrier that protects them along their migration and severe damage to this organ, including abrasions that expose flesh, is likely to result in delayed mortality. We found that sockeye salmon in the final 45 km stretch of their migration with the highest category of skin damage resulting from gillnet NTFI were less likely to arrive at their natal stream, demonstrated increased expression of APR immune genes, and selected cooler water when swimming across stratified lakes. Interestingly, the next lower category of injury did not experience migration survival significantly different from the lowest level of injury and fish without injury. However, there was some evidence that these fish also experienced elevated APR and selected cooler water temperatures. These results indicate that NTFI injury severity and not the NTFI experience alone, is predictive of delayed mortality. Therefore, fishing gears that minimize damage to skin can be expected to minimize the delayed mortality component of FRIM.

Sea lice injury was associated with migration survival, spawning succcess, and thermal experience in Seton Lake. In a three year study of GCss I found that the presence of sea lice injuries was negatively associated with migration survival (Chapter 3). In the current study, similarly to how I parameterized gillnet injuries, I also gave sea lice injuries an ordinal score. This may be responsible for revealing an association between sea lice wounds and spawning success since it appears that the impact occurs at higher sea lice injury levels (similar to gillnet severity) and sea lice injuries in Chapter 3 were only categorized as present or

absent. Similar to gillnet injuries, I would expect that sea lice injuries contribute to decreased barrier function and elevated vulnerability to infections, although our data here did not or could not reveal such relationships. Alternatively, as I expect that almost every sockeye salmon experiences some level of infestation in the ocean (BEAMISH *et al.* 2005), the inability to heal sea lice wounds may be indicative of chronic stress, as sea-lice challenged salmon with experimentally elevated cortisol showed down-regulation of APR genes associated with wound healing (KRASNOV *et al.* 2012). In other words, instead of being the cause of mortality, large sea lice injuries unhealed by the time GCss arrive in the Seton River could be symptomatic of chronic stress that results in mortality. Little evidence has been provided to demonstrate an impact of sea lice wounds on survival for adult migrating Pacific salmon (JOHNSON *et al.* 1996). Here I have intercepted salmon at a point in their migration where they are rarely observed using a low-impact capture method, and coupled this with the ability to determine individual fate by biotelemetry. Visually estimating the surface area of sea lice injuries provided a coarse estimate and did not describe other potentially important information, for example, how deep these injuries extended beyond the epidermis. A more quantitative approach (e.g. photographic methods) for measuring sea lice injuries, paired with biotelemetry as I performed could give more conclusive results regarding the impact of sea lice injuries on adult salmon during spawning migrations. In addition, sea lice injuries could be swabbed or biopsied to determine whether they promote infection.

My hypothesis that GCss with severe injuries would have high infectious agent loads was not supported by the data. On the contrary, the infectious agent with the most variation among netscores, *C. shasta*, showed a negative (non-significant) trend with increasing netscore. Multiple experimental studies have demonstrated that elevated stress and injury leads to elevated infection following exposure to an infectious agent (BADER *et al.* 2006; SMALL and BILODEAU 2005; SVENDSEN and BØGWALD 1997). However, since I did not experimentally infect our fish and instead sampled "the ghost" of prior fisheries interactions, I was likely observing the impact of disease that occurred somewhere downstream. BADER *et al.* (2006) found that channel catfish, *Ictalurus punctatus*, exposed to a bacteria after experimental injury required less bacteria to cause mortality as the wound size increased. It is possible that fish with severe wounds can only maintain low loads of pathogenic infectious agents, and infectious-agent-mediated mortality, catalyzed by severe injury, might have occurred downstream of the fishing fence. Another potential explanation of the observed pattern is that by sampling only non-lethal gill tissue, I was unable to adequately characterize infectious agent burden. *C. shasta* enters a salmonid host through the gill tissue where it enters the gill blood vessel, replicates, ruptures the blood vessel and then move through the blood stream to other target organs (BJORK 2010). In a study with identical molecular analyses to mine, TEFFER *et al.* (2017) found that over a three day period, *C. shasta* loads in gill tissue appeared to decrease and that 31% of 54 sockeye salmon with positive detections in tissue pooled from multiple organs had false negatives in gill tissue alone, while the reverse was true only in 2% of samples. Furthermore gill and pooled tissue *C. shasta* loads were not correlated. In this study, netscore 3 fish with some of the highest *C. shasta* loads in gills were the only fish from that group to arrive at spawning grounds. This could indicate that the myxozoan had not yet left the gills, perhaps as a result of more recent infection or a functional immune response. This could also explain the bimodal pattern of infection seen in C. shasta in this and other studies (Appendices A and B). Clearly any association between visible injury and infectious agent load remains unresolved in this study and additional work (and perhaps a

larger sample size) is needed to better under the relationship.

In fish with the highest category of gillnet injuries, I found upregulation of most genes that are components of the APR. Following injury, cytokines (in this study: Il-1B, IL-8, IL-11, TNF) are released from immune cells in the damaged tissue, and trigger the production and recruitment of proteins that restructure tissue (MMP13, MMP25, SAA), bind available iron to prevent its use by infectious agents (HEP, TF), activate the complement pathway (C1Qc, C7), and have other anti-microbial properties (HEP, SAA, PCBL) (BAYNE and GERWICK 2001; MARTIN *et al.* 2006). Netscore 3 GCss had the lowest expression of genes associated with aspects of the cell-mediated response including: presentation of extracellular non-self material (MHCII-B) and communication between MHCI and T-cells (CD8a, TCRa, TCRb, ZAP7). VDD genes were relatively neutrally regulated with a tendency towards down-regulation, especially for IFI44A, which was strongly downregulated in netscore 3 fish. The lack of a strong pattern for the VDD genes is likely due to a low prevalence of viruses in GCss in 2015. Although Pacific salmon parvovirus has been detected in this system (MILLER *et al.* 2014), it is a DNA-virus and the VDD genes were selected for their response to RNA-viral species (MILLER *et al.* 2017). Osmoregulatory genes were up-regulated in netscore 3 fish, potentially because they were in a state of chronic stress and had elevated cortisol (MCCORMICK *et al.* 2008). Alternatively, this might have been in response to an increased need for osmoregulatory activity due to the loss of barrier function (MATEUS *et al.* 2017). The contrast between APR and CMR (and to a lesser extent, VDD) demonstrated by netscore 3 fish could indicate a trade-off in immune function where, given that resources for GCss are finite, energy was dedicated to synthesis of components of the APR at the expense of components of the CMR (PANNEVIS and HOULIHAN 1992). Regardless, the activation of the APR by GCss with severe injuries represents an immune response to life-threatening injury. Elevated expression of APR genes, especially MMP13, IL-11, IL-1B, TF, and C7, have been associated with infection and mortality in multiple studies of adult Pacific salmon (JEFFRIES *et al.* 2014a; TEFFER *et al.* 2018, 2017, Chapter 4).

Although fish utilizing colder water were more likely to die before complete spawning, there were several females that successfully spawned after swimming through colder strata, suggesting that this could be an effective strategy in some cases. However, utilization of colder strata was primarily associated with prespawn mortality. MINKE-MARTIN *et al.* (2018) found that GCss females that spent more time outside a thermal optimum (primarily in water $<13.4^{\circ}$C) when migrating through Seton and Anderson Lakes were more likely to experience prespawn mortality. In other studies, the utilization of cool strata by sockeye salmon has been attributed to sexual maturation (NEWELL and QUINN 2005; ROSCOE *et al.* 2010) or mediation of infectious agent loads (MATHES *et al.* 2009). However, none of these previous studies evaluated prespawn mortality. MATHES *et al.* (2009) suggested that use of cooler lake strata was necessary for survival of some sockeye salmon, but their results were complex due to the presence of abnormally-timed migrators. Depending upon the characteristics of migrations for independently evolved populations of sockeye salmon, the purpose behind utilizing cool lake strata could vary. A limitation of my study was that I could only recover thermocrons from successful migrators, so the thermal experience of those dying during migration is unknown (and consequently our sample size for thermal experience of severely injured fish is small). The acquisition of this missing data, coupled with the biopsy and biotelemetry techniques used in this study, could further elucidate the motivations and consequences of thermal selection by adult sockeye salmon in

lakes.

My findings indicate that gears which cause less damage to the integument should be expected to cause less delayed mortality. This phenomenon was noted by VANDER HAEGEN *et al.* (2004), who found that larger gillnet mesh sizes caused elevated mortality in Chinook salmon, and visible injury was more severe at the time of capture by larger nets. These findings are applicable to salmon that have experienced a fishery and survived for several days and so I can only speculate on the importance of damage to immediate and short-term mortality. However, I expect that mortality occurring during or shortly after capture is more frequently caused by suffocation, severe damage, extensive bleeding, or crushing (DAVIS 2002; KOJIMA *et al.* 2004). Regardless, this study demonstrates that at a late stage in the migration, severe gillnet injury, but not NTFI alone, is associated with migration failure. Therefore, if fishing gears or techniques can be modified to minimize physical damage to non-retained fish, FRIM will be minimized. Research efforts should be focused on developing or identifying such gears or techniques. For example, at the Bridge River Rapids, a traditional First Nations fishing area on the Fraser River, Xwisten Band gillnet fishers are required by First Nations managers to use multi-filament nylon gillnets based on observations that this causes less damages to non-retained fish compared to monofilament (pers. comm. Gerald Michel, Fisheries Manager, Xwisten Band). A study measuring short-term and delayed FRIM for fish caught by multi-filament compared to monofilament gillnets could assess this management action and potentially encourage others to adopt what may be a lower impact fishing method.

Chapter 6

Conclusion

When considering the long history of fisheries research on Pacific salmon, the study of the impacts of fisheries interactions on escaping or released fish has been a relatively recent occurrence. This is likely due to the fact that when populations are abundant, FRIM is of less concern, and is easily ignored given its covert nature. However, as populations decline and the escapement of fewer and fewer fish becomes more and more crucial, attention shifts towards minimizing any impact that can be controlled quickly by changes in human operations (e.g., fishing regulations). Reductions in the array of gear choices, followed or accompanied by major constriction of fishery opening times allowed in historically abundant fisheries such as the Sacramento, Klamath, Columbia, and Skagit Rivers illustrate this phenomenon. The Fraser River and other abundant fisheries in British Columbia and Alaska have avoided (or experienced to a lesser degree) many of the anthropogenic impacts driving the population reductions in these southern fisheries (dams, habitat loss, overfishing, pollution, etc.). However, salmon populations in British Columbia are currently and will continue to bear the impacts of climate change, which now include elevated water temperature and more volatile weather patterns, and will likely include an increased human population (and its associated impacts) as humans migrate northward (FENG *et al.* 2010). Therefore, there is a pressing need to better quantify, and find methods for minimizing, FRIM.

The goal of this thesis was to provide empirical estimates of FRIM under specific fishery scenarios and further define the factors that regulate FRIM. In Chapter 2, a comparison of capture using both gillnets and beach seines at multiple locations along a salmon migration revealed that gillnets had elevated FRIM relative to beach seines, although this difference did not occur at a site closer to spawning areas. Gillnets caused more physical damage than beach seines and sockeye salmon captured by gillnet were more likely to temporarily delay migration. Chapter 3 further investigated gillnet associated FRIM by observing injuries caused by a fishery occurring downstream of the tagging site and estimating migration survival and spawning success of sockeye salmon over three years. Although these fish were nearing spawning grounds, injuries received earlier in the migration caused significant mortality, and this was estimated on a population scale in the form of effective female spawners lost. This study also revealed that any type of damage to skin is associated with reduced migration survival and may cause upriver populations of salmon to stray into non-natal streams. The importance of condition at the time of capture was demonstrated in Chapter 4, where Chinook salmon were assayed for multiple infectious agents at the time of exposure to a simulated gillnet fishery. In this study, a single infectious agent was the best predictor of mortality, and its impact was demonstrated on the physiological and transcriptomic levels. While gillnet exposure did not result in mortality, fish receiving this treatment did delay migration. The severity of injury was categorized in Chapter 5, with the expectation that infectious agent loads would differ among the groups. While this did

not occur, fish with greater damage to skin generated a significant immune response, selected colder water strata, and had lower migration survival compared to those with minor or no injuries. In total, these chapters demonstrate the considerable impacts that gillnet fisheries interactions have on physiological parameters, immune gene expression, behavior, survival, and spawning success.

Based on my findings from Chapter 2 it appears that the post-release mortality rates used by Fisheries and Oceans Canada for Fraser River Pacific salmon fisheries (60% mortality for gillnet captured fish and 5% mortality for beach-seined fish (DFO 2017)) are conservative for late-run migrating sockeye salmon, especially for those captured by beach seine. The survival endpoint to which the DFO mortality estimates are applied is unclear, as well as whether or not some level of natural mortality is accounted for. If natural mortality is added to the DFO estimates, for example a rate of 30% for upstream migrating salmon (CROSSIN *et al.* 2009), my estimates from Chapter 2 would be fairly similar. However, in the following year of the study described in Chapter 2, post-release mortality for summer-run sockeye salmon was very high, demonstrating the limited applicability of a single value mortality estimate (as opposed to a range) that has no flexibility for incorporating intrinsic and extrinsic variables (especially water temperature). Furthermore, there is no indication that the DFO mortality estimates account for mortality on spawning grounds. In Chapter 3 I found that female sockeye salmon with gillnet marks were more likely to die prior to complete spawning (47% successful spawning for gillnet marked fish, an average 24% lower than the rate for unmarked fish). Given these factors, I recommend that the post-release mortality rates for both gillnet and beach seine capture be raised or be presented as ranges as opposed to single estimates. Due to fewer studies to draw upon for estimating mortality, further research of beach seine post-release survival through migrations and on spawning grounds is warranted.

I will now discuss some of the commonalities amongst the chapters, along with considerations for future research and management pertinent to each topic.

6.1 The importance of fisheries gear

Because my dissertation was primarily focused on delayed mortality, brief gillnet entanglements were applied in chapters 2 and 4, so short term mortality was lower than would likely occur in an actual gillnet fishery (THOMPSON *et al.* 1971). Indeed, when gillnets are fished with short soak times, even up to 20 min, the primary component of FRIM is delayed mortality (DONALDSON *et al.* 2012; TEFFER *et al.* 2017). If fisheries interactions are relatively short and do not cause physical damage, the resultant physiological stress will remain acute (DONALDSON *et al.* 2014). Acute stress is adaptive and provides an organism with the necessary energy to escape a threat (WENDELAAR BONGA 1997). In order for a fisheries interaction to cause chronic stress, it must be prolonged, or induce considerable damage. In studies that sought to compartmentalize the impacts of fisheries captures into physiological and physical impacts, researchers found that capture methods that cause greater physical damage were associated with elevated delayed mortality, while parameters representing physiological stress showed little difference between capture methods (DONALDSON *et al.* 2012; NGUYEN *et al.* 2014). My results support the hypothesis that delayed mortality in Pacific salmon escaping or released from fisheries gear is primarily caused by significant damage to the skin

(NGUYEN *et al.* 2014).

Chapters 3 and 5 demonstrate the importance of physical damage caused by fishing gears. In short, any cause of damage to skin, including natural phenomena like sea lice, will increase the risk of delayed mortality and spawning failure. In chapter 2, the capture method associated with elevated mortality (gillnet), consistently inflicted greater damage to skin. Furthermore, as demonstrated in chapter 5, the magnitude of this damage is crucial as fish with minimal damage show migration survival and spawning success indistinguishable from uninjured fish. This distinction was emphasized by the clear presence of the APR in fish with severe injuries, but not in the groups of fish with lesser injuries. With four studies where gillnet capture (simulated, actual, or observed injuries resulting from) served as a treatment, this dissertation contributes strong evidence to the body of literature suggesting that gillnet fishing has a high impact on escaping or released Pacific salmon (BAKER and SCHINDLER 2009; DONALDSON *et al.* 2012; NGUYEN *et al.* 2014; THOMPSON *et al.* 1971). The results in these studies are very similar to the findings of BAKER and SCHINDLER (2009), suggesting that the relationship between skin loss and mortality is likely a widespread phenomenon for migrating Pacific salmon. Certainly, these results should prompt the prioritization of gears or techniques that minimize damage to the integument.

6.1.1 Recommendations for future research on fisheries gear

It is my belief that whenever possible, field and laboratory studies of FRIM should use actual fisheries captures, as opposed to simulated capture, so that they are maximally applicable. Simulated capture provides experimental control of treatments, and therefore more consistency between treated individuals. However, in a study where simulated captures were used to investigate the impacts of multiple gears on Pacific salmon, NGUYEN *et al.* (2014) stated:

> "It is unknown how severe our experimental treatment was relative to normal fisheries operations; however, the physiological response recorded after 15 min from our treatments resulted in similar physiological values as those measured in coho salmon sampled immediately after capture from an experimental commercial gill net vessel..."

This statement epitomizes a central problem with the use of simulated capture in experiments with implications for fisheries management: the realism of simulated capture relative to actual capture is often unknown. If the hypothesis that physical damage is the primary driver of delayed mortality is true (as suggested by NGUYEN *et al.* 2014), whether or not a simulated capture generates a stress response similar to an actual capture is less relevant than whether or not it causes similar damage. Furthermore, it is easy for stakeholders or managers to undermine the results of a study utilizing simulated capture by questioning its realism, and thus evade assimilation of important findings. When using actual capture events as a treatment in a study of FRIM, more variability between individual experiences will certainly be encountered. However, if the variability between individual experience exceeds the variability between capture methods in the "real world", then one capture method can not be expected to cause more FRIM compared to another, and therefore should not be regulated differently on the basis of FRIM alone. Interestingly, raw survival estimates were very similar between NGUYEN *et al.* (2014) and the closest sampling site in chapter 2 (NGUYEN *et al.*

(2014): 35% gillnet, 50% beach seine; chapter 2: 35% gillnet, 71% beach seine). While this may indicate that the simulated capture by NGUYEN *et al.* (2014) was an accurate representation of the damage caused by a true gillnet fishery, not all simulations can be expected to perform so well. For example, in chapter 4 I attempted to simulate two different gillnet interaction scenarios but found no difference in migration time between these two treatments and no difference in FRIM between any treatments (including biopsy controls). While I attributed this to maturity, it must be maintained that I was unable to test how well these simulations reflect the scenarios they were intended to represent, and inference in this regard is limited (see section 4.5). Of course, simulated capture treatments continue to have an important role in the study of FRIM, and to a certain degree all capture methods used in scientific studies will not perfectly represent the real world. However, I encourage researchers to make concerted efforts to represent fisheries as accurately as possible when designing simulated treatments, which likely involves observing the true fishery in action as well as the physical damage it causes.

In addition to the challenge of accurately portraying the impact of fisheries captures in FRIM studies, estimating baseline mortality of migrating fish is a problem inherent to all telemetry-based studies. The negative effects of capture, handling, and tagging may contribute to mortality, altered behavior, or increased development of infectious agents and therefore preclude accurate estimation of natural mortality. In my dissertation, comparisons were generally made between different capture treatments or levels of injury severity, as opposed to against natural mortality. In chapter 3, I used a low-impact capture method, short handling times, and minimal tagging to produce the best possible estimate of natural mortality. However, the impacts of handling may be reduced by utilizing electro-anesthesia techniques that have recently become available (ABRAMS *et al.* 2018) and I encourage researchers working with Pacific salmon to explore these options. A better estimation of baseline mortality can refine our understanding of the impacts from fisheries gear.

There is considerable variation in the application of any fishing gear. With gillnets, for example, the material used to create the nets (multi- and mono- filament nylon, cotton), the mesh dimensions, how the nets are fished (set, drift, benthic), soak times, and where nets are fished (ocean, estuary, freshwater) are all variables that have consequences for FRIM (PATTERSON *et al.* 2017a). Since FRIM has primarily garnered attention in the last two decades, most gillnet literature that compares different mesh sizes and materials is focused on capture efficiency. Modern research is needed to relate variations in gear to variations in FRIM. For instance, a study in the Columbia River showed that the use of small mesh "tangle" gillnets resulted in higher survival of migrating adult Chinook salmon compared to the use of traditionally sized gillnets (VANDER HAEGEN *et al.* 2004). At the Bridge River Rapids, a traditional First Nations fishing area on the Fraser River, Xwisten Band gillnet fishers are required to use multi-filament nylon gillnets based on observations that this causes less damages to non-retained fish compared to monofilament (pers. comm. Gerald Michel, Fisheries Manager, Xwisten Band). A biotelemetry study where fish caught with these two types of gear side-by-side at this location could provide the empirical evidence to support or refute this management strategy, and potentially inspire more fishers to adopt a technique that minimizes FRIM. As mentioned above, the capture methods used in this sort of experiment must be as realistic as possible. In addition, such studies should be primarily focused on the physical impact of capture, as opposed to the capture induced displacement from physiological homeostasis, which is temporary in the presence of

minimal physical impact.

While managers schedule fisheries openings based upon expectations of take, and sometimes estimates of FRIM, quantification of fisheries interactions (releases, but especially escapes), is sorely lacking. Fish that interact with gear and then escape, potentially experiencing life-threatening damage in the process, are extremely difficult to quantify, especially in turbid systems like the Fraser River. However, while we are accumulating studies that provide FRIM estimates under a diversity of gear types, environmental conditions, and fish-specific context, we must somehow quantify escapes if impact is to be evaluated on the population scale. It is with the goal of quantifying non-take fisheries interactions that I propose the following methodology, which was explored by DFO as a pilot project but not implemented (Jeff Grout, DFO, pers. comm.). Dual-frequency identification sonar (DIDSON) or adaptive resolution imaging sonar (ARIS) could be used to visualize salmon interactions with gillnets *in situ*. This would allow estimates of fisheries interactions and escapes relative to mesh size, filament type, drift and set nets, turbidity, migration size, species, time of day, and many other factors. Estimates of encounter and entanglement rate could be scaled up based on knowledge of gears in use throughout a fishery (creel survey and catch monitoring). These estimates could be related to incidence of gillnet marks on spawning grounds and survival estimates from concurrent biotelemetry studies. Of 796 salmon observed encountering a set gillnet during the pilot project, 95.6% avoided contact with the net, 2.0% were entangled but then escaped, and 2.4% were entangled and retained (Jeff Grout, DFO, pers. comm.). I believe such a study will contribute significantly to our understanding of FRIM and its impact on a population scale, an important component for proper management.

6.1.2 Management recommendations regarding fisheries gear

A simple solution to minimizing gillnet associated FRIM is to minimize the use of gillnets, which has been done in places like the Columbia River, where commercial gillnetting has recently been banned from the mainstem river and isolated to side-channels. However, gillnets are affordable, can be employed in more locations than lower impact gears, and require only a single person for operation (e.g., set nets). Therefore, gillnets are the main tool used to provide access to fish for the Food, Social, and Ceremonial needs of First Nations along the Fraser River as well as other rivers in British Columbia and beyond. While population sizes remain adequate to support in-river fisheries, efforts should be focusing on modifying gillnet fisheries so that FRIM is minimized. To minimize the incidence of FRIM, the optimal mesh size for the target population should be selected to maximize retention once fish are entangled, and materials that inflict minimal damage on fish that do escape must be used to construct nets. Additional research may be needed to determine the ideal mesh size and materials for a diverse array of species and populations, but it would be up to management to implement regulations based around these parameters once they are identified.

6.2 The role of maturity in FRIM

While the type of fisheries gear in use clearly has major implications for FRIM, the condition of fish at capture is a very significant, and often overlooked, factor in studies of FRIM (RABY *et al.* 2015). In two chapters of this dissertation, there is evidence of the importance of maturity. In chapter 2, I found that sock-

eye salmon further along in their migration, and thus more mature, were more resistant to gillnet capture as evidenced by fewer net marks following capture and no apparent difference in survival from beach seine capture. Furthermore, summer run sockeye salmon (which enter the river in a less mature state than late run) captured the following year demonstrated consistently higher levels of injury. In Chapter 3, mature Chinook salmon rarely showed physical damage after experimental gillnet entanglement and did not experience elevated mortatlity compared to non-entangled fish. I suggested several potential explanations for the relative resilience of more mature fish including: thickening of integument and absorption of scales provided greater resistance to net wounds, naturally elevated cortisol in fish nearing spawning grounds desensitizes fish to the stress induced by fisheries interactions, and lower amounts of stored energy equates to less struggle in nets, and therefore less potential for injury and excess plasma lactate.

In general, the pattern of increased resilience to fisheries interactions with maturity should positively increase as salmon are encountered further upstream. For example, RABY *et al.* (2013) found that chum and pink salmon on spawning grounds were resilient to physical and physiological disturbance. Likewise, individuals encountered later throughout the migration period should be more resilient to capture than those encountered at the same location but earlier. By simultaneously capturing two distinct populations of sockeye salmon with overlapping migrations but differing spawning phenologies, DONALDSON *et al.* (2012) demonstrated that those further from spawning (and therefore less mature) experienced elevated mortality following fisheries capture and release compared to those that would spawn sooner. A noteworthy exception to these patterns was observed in a study concurrent with chapter 2, where sockeye salmon captured by gillnet in the Fraser River estuary (rkm 15 and below) survived to spawning areas considerably better than those captured upstream (Katrina Cook, UBC, unpublished data). A holding study conducted during this period using marine and river sourced fish led TEFFER (2018) to conclude that the elevated mortality for fish captured upstream of marine areas was due to infectious-agent-mediated mortality. Fish released upstream of the estuary may have been more vulnerable due to their release into an infectious-agent-rich environment, while those downstream had recovered adequately from injury since vulnerability to exogenous pathogens following injury may be temporary (BADER *et al.* 2006). Although this may seem tangential, I mention this anecdote since it reveals an instance where local conditions may supersede what we may consider a universal pattern (i.e., further along in migration equates to greater resilience).

6.2.1 Recommendations for future research on maturity and FRIM

As the phenomenon of increased resilience to fisheries interactions with increasing maturity has strong implications for management, there is much potential for continued research. First, since I know of no other studies of Pacific salmon that apply similar capture techniques along a migratory corridor as done in chapter 2, repetition of this approach is warranted. Repeating such an experiment, but also measuring egg retention in females on spawning grounds would reveal whether or not there truly was no difference in the impact of gillnet and beach seine capture at the most upstream site, or if such a difference was simply masked by a short migration distance. To focus on the maturity aspect, a single capture method could be employed, but repeated across more locations, and including marine and estuarine sites. This might allow further investigation into the phenomenon described in the previous paragraph and also a more specific identification of

where along the migration maturation starts to play a significant role in ameliorating the effects of fisheries interaction. Because some stakeholders might consider the maturation of salmon to imply a decline in quality of the flesh for human consumption, honing in on the earliest occurrence of elevated resilience is important. The roles of thickening skin, elevated cortisol, and decreasing energy could be investigated as part of such a study by conducting the appropriate biopsies and measurements.

Variability in timing at a single location could also be a productive way to study the importance of maturity in FRIM. Fish could be treated with the same capture experience over the course of a multi-week migration, at a location where fish appearance is known to shift considerably in maturity from early migration to late (Figure 6.1). Biotelemetry and carcass recovery as performed in chapters 3 and 5 would enable the determination of migration survival and spawning success relative to injury following treatment. Biopsises and measurements as described above would allow identification of specific mechanisms underlying the maturity associated pattern. A phenomenon that could confound such a study would be the existence of abnormally-timed early migrating fish, which enter freshwater much earlier than the rest of their cohort and experience high levels of mortality for unknown reasons (HINCH *et al.* 2012).

6.2.2 Management recommendations regarding maturity and FRIM

Given that FRIM is expected to be lowest among mature fish, terminal areas (sub-natal and natal watersheds) might be the most desirable location for Pacific salmon fisheries. An added benefit to prioritizing terminal fisheries would be the reduction of FRIM in downstream areas since non-target species and populations are less frequently encountered in terminal fisheries (HEALEY 2009). A potential downside to this approach would be the loss of fisheries to those who reside downstream.

Another approach to prioritize mature fish could be to target populations in the later portion of their migrations, when available fish are more mature than those in the earlier or peak portions of the run. Under this scenario, salmon escaping fishing interactions should be more likely to survive to spawning. If fishery intensity was too high however, this could decrease the diversity of run timing by selecting against late migrators, which might undermine long-term population stability (HARD *et al.* 2008). From a fisher's perspective, more mature salmon might be less preferred or valued due to decreased fat content.

6.3 The intersection between infectious agents and FRIM remains elusive

Despite the fact that many studies of Pacific salmon FRIM cite infectious disease as a likely ultimate cause of mortality, this relationship still has not been conclusively demonstrated. Laboratory studies have shown that injury to skin followed by exposure to an infectious agent may result in infection and subsequent mortality (BADER *et al.* 2006; SVENDSEN and BØGWALD 1997). Infection observed in wild salmon *in situ* has only been linked anecdotally, and non-empirically, to injuries caused by fisheries interactions (BAKER and SCHINDLER 2009). Perhaps some of the most compelling work linking fisheries interactions to infection was conducted by TEFFER *et al.* (2018), who found that the overall number of infectious agents and the infectious load of one bacteria in particular was elevated in wild salmon exposed to a simulated gillnet treatment. However, this study was conducted in captivity, and the relationship still remains to be demonstrated in a

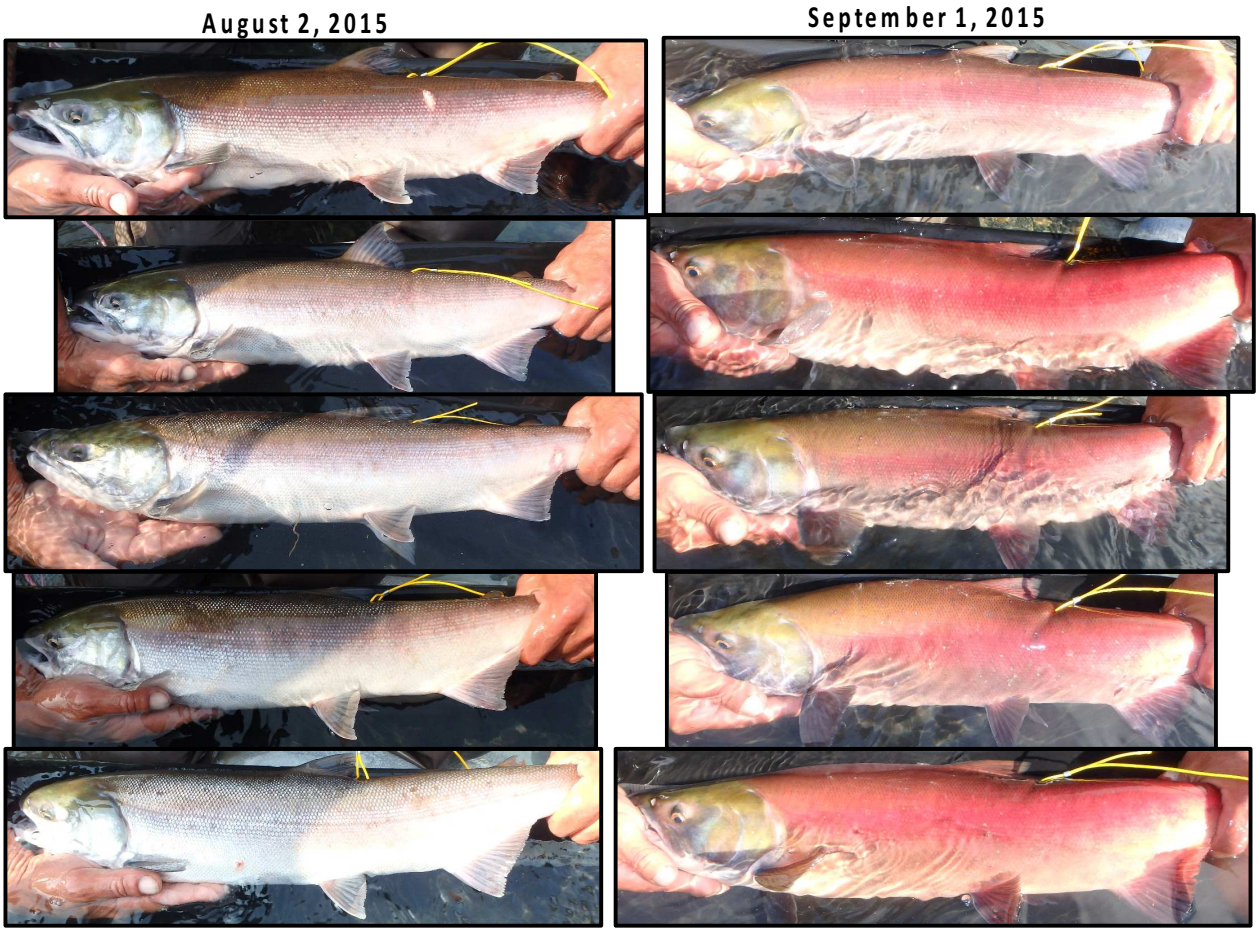

Figure 6.1: Appearance of Gates Creek sockeye salmon sampled at the Seton River fishing fence, British Columbia, 2015. Fish on the left were sampled in early August, immediately after fence installation and fish on the right were sampled in early September, close to the end of the Gates Creek sockeye salmon migration past this location. Note that later sampled fish have more developed secondary sexual characteristics (kype and dorsal hump), are deeper bodied, and have absorbed scales.

natural environment.

Originally, a primary goal of this dissertation was to investigate the relationship between infectious agents and fisheries interactions. However, the complex epidemiology of wild salmon, the limitations of field biotelemetry experiments, and the challenges of quantifying infectious agents base on non-lethal biopsies conspired to prevent conclusive evidence of such a relationship. While non-lethal gill sample biopsies may provide a low rate of type II error for systemic infections such as the blood-borne protozoan, *C. salmositica*, the opposite could occur for infectious agents such as *C. shasta* which show tissue tropism throughout the course of infection (BJORK 2010; TEFFER *et al.* 2017), or infectious agents that are limited to a single tissue, like Pacific salmon parvovirus, which is commonly detected in liver tissue but rarely in gill. In future research, careful planning of sampling is required to encompass windows where infectious-agent-mediated mortality is present, especially as would be revealed in non-lethal gill tissue.

Regardless, this dissertation does provide several valuable contributions to the study of infectious agents

in Pacific salmon and their role in fisheries interactions. First, I provided useful descriptive data regarding the prevalence and abundance of over 20 infectious agents found in Fraser River Chinook and sockeye salmon across their migrations (Appendices A and B). In chapter 4, I demonstrated that infection by a virulent agent, *Cryptobia salmositica*, supersedes the impact of simulated gillnet entanglement on FRIM. Finally, while the relationship between *Ceratonova shasta* load and injury severity was not significant in chapter 5, a pattern worthy of further investigation (with potential to be confirmed by a larger sample size) was revealed.

As Appendix B is one of the only studies detailing the occurrence of multiple infectious agents across the migration of a population of Pacific salmon, our understanding of where migrating salmon contract infections during their migration, when and if they succumb to them, and which agents are most virulent is relatively limited. Therefore, the ideal location (or time) along the migration to sample fish in order to observe infectious-agent-mediated mortality is unknown. Fish could be sampled in the lower river, where salmon display a multitude of infections, but the multiple weeks of migration that pass before delayed mortality occurs could mask associations. Furthermore, without resampling, it is unknown whether infection progressed or if new infectious agents were contracted. A single biopsy provides only a snapshot of the host-pathogen process, and the snapshot is only informative if collected at a moment in this continuum where pathogen-mediated mortality is evident. On the same token, if fish are sampled too late, pathogen-mediated mortality will be missed. This might have prevented observation of pathogen-mediated mortality in severely injured fish in Chapter 5. In the Seton-Anderson Watershed there was evidence of considerable mortality associated with severe injuries occurring prior to arrival in the Seton River (fence mortalities, Chapter 3). Studies have shown that mortality begins to occur around 3–5 days following fisheries interactions (TEFFER *et al.* 2018, 2017, Chapter 4), and so the only high injury fish arriving at the Seton River could have been those that managed to maintain low levels of infectious agents.

Although molecular techniques did not reveal the linkage between fisheries interactions and infectious-agent-mediated mortality in this dissertation, they did demonstrate a strong connection between the acute phase response and subsequent mortality. In Chapter 4, APR genes were highly expressed in the presence of *C. salmositica* and in Chapter 5, in association with severe gillnet injuries; both factors were associated with increased mortality. The association between injuries, infections, increased mortality, and elevated APR gene expression has been observed in other studies (BAYNE and GERWICK 2001; JEFFRIES *et al.* 2014a; SUTHERLAND *et al.* 2014; TEFFER *et al.* 2018, 2017). Combined, these studies and my dissertation indicate that the measurement of APR gene expression could serve as an informative response variable in studies of fisheries induced injuries or infectious agents in salmonids. Furthermore, monitoring APR upregulation on a population level could provide predictive data to inform management decisions.

6.3.1 Recommendations for future studies of infectious agents and FRIM

Future studies investigating the linkage between fisheries interactions and infectious agents would benefit strongly from a better understanding of the limitations of non-lethal gill biopsies. For example, TEFFER *et al.* (2017) showed that pooled tissue from multiple organs more consistently revealed the presence of infectious agents including *C. shasa*, *Flavobacterium psychrophilum*, and *Loma salmonae* and that where positive in both substrates, loads of *C. shasta* from gill were not correlated with those from mixed tissue.

Controlled studies that span the infection cycle of an agent, featuring comparisons against pooled tissue and between multiple gill samples (to determine consistency in loads and sensitivity between biopsies), would contribute greatly to confidence in interpretations of single biopsies collected in the field.

Because gill samples may be prone to type II errors, the collection of mixed tissue (lethal samples) pertinent to a hypothesis is warranted where feasible and appropriate. In retrospect, it would have been ideal to collect mixed tissue from at least 10 individuals from each category of gillnet injury severity in the Seton River (Chapter 5). Such samples could be procured by working with those harvesting salmon upstream of a fishery (e.g., the Bridge River Rapids). Detailed documentation and photographs of phsyical injuries could be compared to infectious agent prevalence and load to provide a more certain test of the hypotheses regarding infectious agents from Chapter 4.

Another approach for investigating the change in infection status following fisheries interaction is to non-lethally sample individuals on multiple occassions. This is not logistically feasible in most remote or large rivers, but may be possible where proper infrastructure (fishing fence, spawning channels) is available. Gates Creek is an ideal system for recaptures, and recently, gill biopsies were collected following a simulated capture below Seton Dam and once again when fish arrived at Gates Creek (Laura Elmer, Carleton University, pers. comm.). In addition to determining how simulated capture influences infectious agent development, the acquisition of thermal experience data will provide an opportunity to test whether selection of cooler strata while transiting lakes impacts infectious agent development. Coupling the multiple sampling approach with an expanded window of the migration would improve the likelihood of observing the intersection of infectious agents and fisheries interactions. A study in the Nass River (British Columbia) featuring experimental purse seining in the ocean followed by multiple biopsy events as radio tagged fish migrated to spawning grounds should provide such a dataset (Andrea Reid, Carleton University, pers. comm.). A drawback to the multiple sampling approach is that each successive handling and biopsy event likely further impacts migration survival and spawning success, so those variables become less representative of their true rates (in non-biopsied fish). However, the data from these multi-biopsy approaches are novel, and the aforementioned projects are the next logical step from the studies of infectious agents in my dissertation.

While I only collected gill biopsies, there may be other non-lethal methods that could be more informative. It may be possible to swab visible wounds with a sterile material, such as filter paper, that could then be homogenized. This way, bacteria, fungi or other infectious agents colonizing wounds could be detected, even though they might not be present in simultaneously sampled gill tissue. The success of such an approach could be contingent upon assaying for the appropriate freshwater infectious agents. For instance, fungus from the genus *Saprolegnia* commonly occurs on the skin of adult salmon, is opportunistic where mucus and skin are removed (PICKERING and WILLOUGHBY 1982), and has been associated with gillnet FRIM (BAKER and SCHINDLER 2009). I observed lesions that were reminiscent of *Saprolegnia* on gillnet marked fish but the assay was not available to include with those I ran on the BioMark. In summary, direct sampling of gillnet injured tissue coupled with an expanded range of assays for infectious agents likely to colonize injured tissue might reveal patterns of infectious-agent-mediated FRIM.

6.3.2 Recommendations for management regarding infectious agents and FRIM

Due to technological innovations in molecular biology, including high-throughput qPCR and next-generation sequencing, we are on the verge of a much greater understanding of the interactions between Pacific salmon and infectious agents. The ability to determine whether dozens of infectious agents are infecting salmon from non-lethal samples (as demonstrated in this dissertation) and the ability to apply the same techniques to environmental samples will rapidly broaden our knowledge of infectious agent taxa, distributions, life histories, virulence, persistence in the environment, transmission, and response to anthropogenic activities. Fisheries management should take an active role in furthering the use of this technology in studies of Pacific salmon.

Natural levels of infectious agents will always occur in populations of Pacific salmon and there is probably little that can or should be done by managers in terms of control. However, management should strive to provide the best possible baseline information and monitoring regarding the distribution of infectious agents in Pacific salmon habitats (similar to Appendices A and B). This knowledge will enable determination of how infectious agent dynamics change over time, most importantly in regards to anthropogenic activities. If this type of monitoring determines that certain areas are hotspots for infectious agents, fishing efforts could be shifted geographically or temporally to minimize FRIM resulting from exposure of released or escaped salmon to infectious agents. The need for infectious agent monitoring is especially timely given rapid climate change. As climate change extends the growing season (earlier spring), fish parasites, which usually produce one or two generations per year, may produce additional generations annually (MARCOGLIESE 2001). It is unknown how this will impact fisheries and regulation of FRIM, but proactive monitoring is the best way to prepare.

6.4 Conclusion

This dissertation contributes significantly to the fields of Pacific salmon biology and fishing-related incidental mortality. I combined the disciplines of fisheries biology, biotelemetry, physiology, epidemiology, and immunology to produce unique studies that are applicable to management and also inform future research. I demonstrated that gillnets are consistently associated with elevated FRIM, more so than another frequently used gear, the beach seine. I provided the first direct study of the spawning success of any fish escaping fisheries encounters, which indicated that female salmon experiencing gillnet interactions had an elevated risk of mortality on spawning grounds, as well as during migration. I provided an example of an instance where an infectious agent proved more influential than a fisheries interaction, as well as evidence of the physiological impact of this agent. I demonstrated that injury severity, and not fisheries interactions alone, is the best predictor of mortality, and this phenomenon was confirmed on the molecular level by elevated immune response in severely injured fish. Finally, I provided two studies of the distribution and abundance of infectious agents in two species of Pacific salmon. I am optimistic that these studies will contribute to the large body of knowledge regarding Pacific salmon, and ultimately to the conservation of this iconic group of species.

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Appendix

Appendix A

A survey of microparasites present in adult migrating Chinook salmon (*Oncorhynchus tshawytscha*) in southwestern British Columbia determined by high-throughput quantitative polymerase chain reaction

A.1 Synopsis

Microparasites play an important role in the demography, ecology, and evolution of Pacific salmonids. As salmon stocks continue to decline and the impacts of global climate change on fish populations become apparent, a greater understanding of microparasites in wild salmon populations is warranted. I used high-throughput, quantitative PCR (HT-qRT-PCR) to rapidly screen 82 adult Chinook salmon from 5 geographically or genetically distinct groups (mostly returning to tributaries of the Fraser River) for 45 microparasite taxa. I detected 20 microparasite species, four of which have not previously been documented in Chinook salmon, and four of which have not been previously detected in any salmonids in the Fraser River. Comparisons of microparasite load to blood plasma variables revealed some positive associations between *Flavobacterium psychrophilum*, *Cryptobia salmositica*, and *Ceratonova shasta* and physiological indices suggestive of morbidity. I include a comparison of our findings for each microparasite taxa with previous knowledge of its distribution in British Columbia.

A.2 Introduction

Microparasites, here defined as viruses, bacteria, myxozoa, protozoa, and fungi that inhabit other organisms and have the capacity to cause disease, play a role in shaping virtually all ecosystems found on earth. Parasitism represents the most common life history strategy on earth (PRICE 1980), and parasites are capable of regulating host population sizes, trophic interactions, competition, and biodiversity (MINCHELLA and SCOTT 1991). The influence microparasites exert over their hosts warrants their inclusion in 'whole organism' and population ecology studies, but this is often precluded by the difficulties of studying these microorganisms, their extensive diversity, and the complexity of their life cycles.

Compared to microparasites infecting many other animals, those infecting salmonids have been relatively well-studied due to the economic importance of their hosts (for a recent synthesis, see MILLER *et al.* (2014)). Much of our understanding of salmonid microparasites comes from aquaculture. Cultivating fish often facilitates (1) stressful conditions that promote infection and disease, (2) high densities of fish that enable easy transmission of microparasites, and (3) easy observation and collection of mortalities for assessment (BAKKE and HARRIS 1998). The prevalence and impact of these organisms in wild salmon populations is far more difficult to study as salmon inhabit large environments and mortalities often go unnoticed due to predation and disappearance (BAKKE and HARRIS 1998). Perhaps the easiest place to observe microparasites and disease in wild salmon is along the upstream spawning migration. While mature fish may return to the river with low level infections occurring in seawater or during juvenile lifestages, in-river conditions experienced by adult salmon provide abundant exposure to freshwater pathogens and the factors which may increase their prevalence such as starvation, elevated temperature, and stress (DOLAN *et al.* 2016; MATHES *et al.* 2009; MAULE *et al.* 1989).

In addition to their vast cultural and economic value, Pacific salmon are considered to be keystone species since they feed many organisms throughout their life cycle (WILLSON and HALUPKA 1995). The introduction of microparasites through natural and anthropogenic routes has, in some instances, caused mass mortality of keystone species followed by drastic alterations to the associated ecosystem (LESSIOS 1988). Large-scale epizootics are occasionally observed in salmonid aquaculture (ASCHE *et al.* 2009) and also have been noted in wild salmonid populations (KRKOŠEK *et al.* 2006; TRAXLER *et al.* 1998). Considering the importance of salmonids to the ecosystems they inhabit, understanding microparasite dynamics is an important aspect of conservation, necessary for avoiding management decisions that unintentionally encourage microparasite outbreaks. This is especially important in the context of changing climate, where shifts in hydrology, water temperature, and species composition may upset balances between microparasites and their hosts (MARCOGLIESE 2001).

Recent advances in molecular technology have greatly improved our ability to study microparasites infecting wild salmon (MILLER *et al.* 2014). High-throughput real-time quantitative polymerase chain reaction (hereafter, HT-qRT-PCR) allows for the detection and quantification (to extremely low concentrations and for many assays and samples simultaneously) of genetic material specific to known microparasites. While this technology is not capable of identifying clinical indications of disease, it is capable of detecting sub-clinical "carrier" levels that may not be identified using histopathology techniques. Most previous microparasite surveys on migratory salmon have been conducted using either histopathology, which provides good dif-

ferentiation of parasites but limited species identification of bacteria or viruses, or molecular assays, which due to cost are generally limited to only a few species of interest. These investigations are often undertaken following an epizootic (KOCAN *et al.* 2004; PAULEY 1967; TRAXLER *et al.* 1998), so may fail to capture the normal variation of microparasites within and among populations. Therefore, although much research has been conducted on microparasites that infect salmonids, baseline information on the microparasite community is lacking for many stocks of Pacific salmon. The speed, sensitivity, and affordability of HT-qRT-PCR makes it an ideal tool for providing this baseline data.

The Fraser River watershed is the largest Canadian producer of Chinook salmon, *Oncorhynchus tschawytscha*, consisting of four major stock complexes and three migration timing groups (DFO 1999). Many southern British Columbia Chinook salmon stocks have experienced declines in population abundance over the last three decades, and Fraser River stocks have displayed noticeable declines since the early 2000s (RIDDELL *et al.* 2013). What role, if any, infectious disease may play in the decline of British Columbia Chinook salmon is currently unknown. Disease has been identified as a potential driver of declines in sockeye salmon (*Oncorhynchus nerka*) in the Fraser River (COHEN 2012). Microparasites including *Parvicapsula minibicornis* and *Ichthyophtherius multifiliis* have been implicated in several major sockeye salmon prespawn mortality events (BRADFORD *et al.* 2010b; TRAXLER *et al.* 1998). In systems to the north and south of the Fraser river, microparasites including *Dermocystidium salmonis*, *Ceratonova shasta*, and *Ichthyophonus hoferi* have been associated with significant mortality for wild Chinook salmon populations (FUJIWARA *et al.* 2011; KOCAN *et al.* 2004; PAULEY 1967) . Before we can determine whether infectious disease plays a role in Fraser River Chinook salmon declines, we must provide a better understanding of the presence and distribution of microparasites in Fraser River stocks.

In this study, I used HT-qRT-PCR on the Biomark^{TM} microfluidics platform, recently analytically validated for research monitoring of salmon microparasites (MILLER *et al.* 2016), to document the prevalence and relative quantity of 45 microparasite taxa known or suspected to cause disease in salmon. My analysis included four genetically or geographically distinct populations of Chinook salmon concluding their spawning migrations and one group sampled in the marine environment. While I was primarily interested in documenting prevalence and loads of microparasites, I also determine whether (1) microparasite prevalence and load changed over time for a stock sampled twice, (2) microparasite loads differed between sexes, (3) microparasite load was correlated with physiological indices (osmoregulatory, stress, and reproductive) measured in blood plasma, and (4) there was evidence of co-infections between all pairs of microparasites.

A.3 Methods

A.3.1 Fish, blood, and tissue collection

Adult Chinook salmon were collected at two hatcheries and two locations in the wild from August to November 2013 (Table A.1, Figure A.1). The summer Chilliwack River Chinook salmon stock (CRS) was sampled at the Chilliwack Hatchery (approximately 125 km upstream from the mouth of the Fraser River - the Chilliwack is a tributary to the Fraser River). From 1985-1989, this stock was created from the transplantation of Upper Fraser populations including Quesnel River, Bowron River, Slim Creek, Finn Creek and Chilko

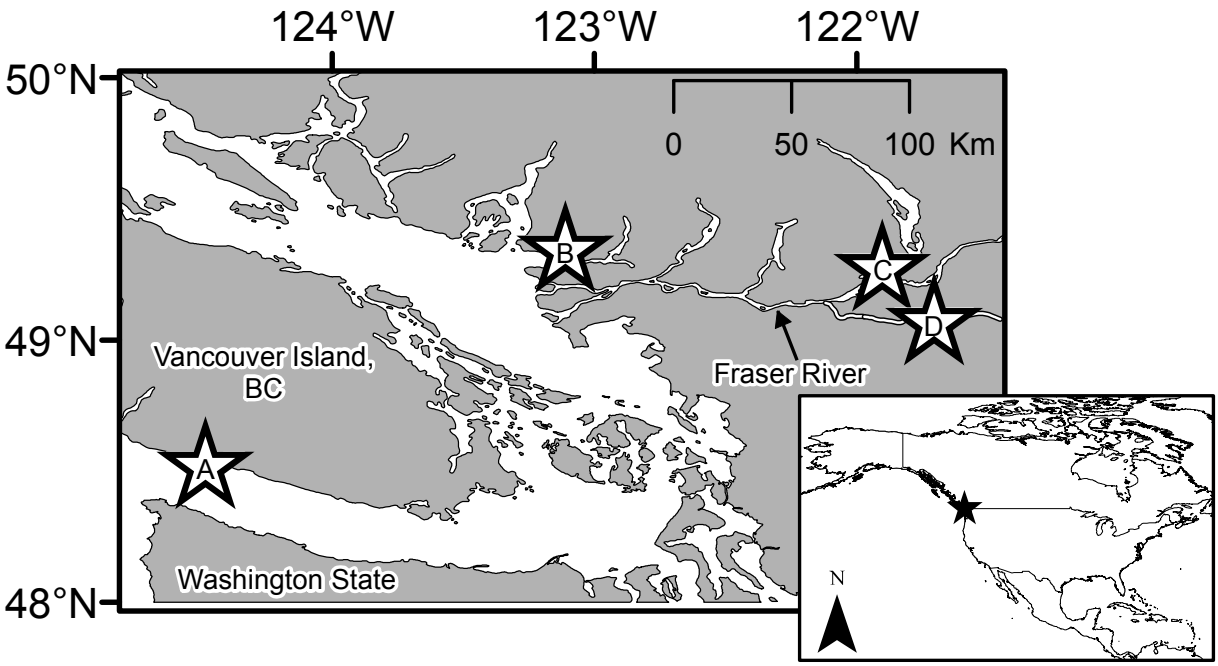

Figure A.1: Map of collection sites of adult Chinook salmon, British Columbia, Canada, 2013. A = Port Renfrew (Aug 30 - Sep 2), B = Capilano River Hatchery (Nov 14), C = Harrison River (Oct 17, 29), and D = Chilliwack River Hatchery (Aug 19, Oct 10, Oct 21).

River (Robert Stanton, pers. comm., Chilliwack Hatchery). This stock begins to arrive in early July and fish become sexually mature in early September. Summer Chilliwack Chinook are expected to encounter higher water temperatures than all other stocks during migration and they descend from stocks evolved to migrate through summer water temperatures (elevated relative to fall). The fall Chilliwack Chinook stock (sampled twice at the hatchery; CRF1, CRF2) was established from transplantation of Harrison River and Pitt River fall Chinook from 1981 to 1988. This stock arrives in late September and early October and fish are sexually mature as they arrive. Harrison River fall Chinook (HR) were sampled at the Chehalis First Nations beach seine site (49.287 N, 121.903 W), which is a short distance upstream of the primary spawning area and approximately 105 km upstream from the Fraser River mouth (also a Fraser River tributary). Capilano River Chinook (CR) were sampled at the Capilano River Hatchery (located 6 km upstream of saltwater in the Burrard Inlet). Although this stock has been sourced from multiple populations over time, the broodstock is currently supplemented from the Chilliwack River Hatchery and these adults resemble the Harrison River stock in appearance and timing (pers. comm. Jeremy Smith, Capilano Hatchery). The Port Renfrew samples (PR) were captured by purse seine between Sombrio Point (48.485 N, 124.293 W) to the east and Bonilla Point to the west (48.591 N, 123.704 W), and are expected to be primarily Harrison River stock based on run timing (PARKEN *et al.* 2008). University of British Columbia (UBC) researchers collected CRS and CRF groups under Fisheries and Oceans Canada (DFO) permits XR 201 2013 and XR 355 2013. DFO staff collected the CR, HR, and PR groups under a section 52 scientific collection permit. This work was performed according to the UBC animal care and use permit, A12-0250.

Table A.1: Sampling information for adult Chinook salmon collected in British Columbia, 2013. CRS = Chilliwack River summer, PR = Port Renfrew (marine), CRF1 = Chilliwack River fall first sampling, CRF2 = Chilliwack River fall second sampling, HR = Harrison River, CR = Capilano River. Means ± standard error for each sex are reported for length, weight, and physiological variables. Some standard errors were not available (NA) when variables were only measured for one individual. P.O.H. = distance from post orbital to hypurnal (fish length). Blank spaces indicate that samples were not collected.

Variable	Sex	CRS	PR	CRF1	CRF2	HR	CR
sample dates		Aug 19	Aug 30,31, Sep 1,2	Oct 10	Oct 21	Oct 17, 29	Nov 14
n	m	8	7	9	8	4	7
	f	8	3	9	11	2	6
P.O.H. (cm)	m	53.94 ± 2.01	56.86 ± 2.11	59.67 ± 1.02	56.62 ± 3.36	37.62 ± 1.57	51.23 ± 1.89
	f	63.00 ± 2.12	56.83 ± 8.85	61.44 ± 0.96	65.11 ± 1.57	66.25 ± 1.85	69.53 ± 1.36
Weight (kg)	m	3.69 ± 0.43		5.21 ± 0.37	4.53 ± 0.70	1.37 ± 0.16	3.47 ± 0.34
	f	5.99 ± 0.59		5.84 ± 0.29	6.68 ± 0.53	6.49 ± NA	8.28 ± 0.62
Hematocrit	m			34.44 ± 2.69	41.71 ± 3.41	45.00 ± 2.04	43.57 ± 3.40
(% Red Blood Cell)	f			43.89 ± 2.61	41.44 ± 1.56	52.50 ± 2.50	45.83 ± 2.39
Plasma glucose	m	3.46 ± 0.47	7.18 ± 0.87	4.50 ± 0.25	5.68 ± 0.30	5.25 ± 0.68	10.74 ± 2.15
(mmol/L)	f	3.71 ± 0.51	4.51 ± 0.22	5.69 ± 0.49	5.68 ± 1.16	4.00 ± 0.80	8.85 ± 1.11
Plasma lactate	m	7.51 ± 1.42	20.68 ± 2.53	5.73 ± 0.99	4.99 ± 0.49	10.78 ± 0.99	5.30 ± 0.85
(mmol/L)	f	6.27 ± 1.49	13.17 ± 1.34	5.60 ± 0.80	7.02 ± 1.11	14.25 ± 0.65	4.05 ± 0.68
Plasma chloride	m	125.61 ± 2.69	144.36 ± 4.67	120.11 ± 2.56	119.88 ± 1.77	121.83 ± 2.61	114.29 ± 3.30
(mmol/L)	f	126.17 ± 3.84	148.17 ± 3.03	121.87 ± 1.22	117.27 ± 4.27	122.95 ± 2.65	116.65 ± 3.71
Plasma osmolality	m	310.94 ± 5.83	401.36 ± 7.89	298.17 ± 4.92	299.07 ± 3.07	314.25 ± 4.66	304.00 ± 3.23
(mmol/L)	f	307.44 ± 5.81	386.67 ± 4.04	311.72 ± 2.70	294.50 ± 6.72	307.00 ± 7.50	297.83 ± 4.27
Plasma sodium	m	153.38 ± 3.17	189.57 ± 2.72	143.36 ± 2.30	144.10 ± 1.98	152.25 ± 3.17	142.71 ± 3.36
(mmol/L)	f	148.75 ± 3.51	178.00 ± 2.31	145.08 ± 1.34	138.75 ± 3.90	148.5 ± 1.50	142.00 ± 3.31
Plasma potassium	m	2.25 ± 0.63	1.14 ± 0.46	2.57 ± 0.43	1.76 ± 0.30	2.88 ± 0.49	1.80 ± 0.31
(mmol/L)	f	2.36 ± 0.63	0.60 ± 0.12	3.12 ± 0.45	2.20 ± 0.47	0.75 ± 0.05	1.37 ± 0.39
Plasma cortisol	m	123.55 ± 19.13	199.82 ± 21.53	419.67 ± 78.07		72.56 ± 10.19	
(ng/mL)	f	213.91 ± 49.72	187.99 ± NA	586.67 ± 62.29		191.70 ± 48.65	
Plasma testosterone	m	26.27 ± 9.45	95.95 ± 8.95	30.93 ± 10.79			
(ng/mL)	f	123.11 ± 30.05	79.18 ± NA	156.65 ± 41.78			
Plasma estradiol	m	0.22 ± 0.02	0.17 ± 0.05	0.21 ± 0.02			
(ng/mL)	f	7.90 ± 3.43	16.46 ± NA	4.97 ± 2.61			

Fish were euthanized by cerebral percussion. Immediately afterwards, blood was drawn from the caudal vein using a heparinized vacutainer. Samples were immediately centrifuged for 7 min at 7,000 g and plasma was stored at -80° C. Hematocrit and leukocrit were calculated as the percentage of packed red blood cells and leukocytes in whole blood, measured after spinning at 10,000 g in capillary tubes for 2 min. Plasma osmolality, sodium, potassium, chloride, glucose, and lactate were measured in duplicate or triplicate using the procedures outlined in FARRELL *et al.* (2000). Plasma cortisol, testosterone, and 17b-estradiol were

measured using commercial ELISA kits (Neogen Corporation, Lansing, MI, USA), and run in duplicate at appropriate dilutions. Testosterone and 17b-estradiol samples were extracted in ethyl ether according to manufacturer's protocols.

Sterile technique was used to acquire small sections of tissue (approximately 0.3 g) from the gills, spleen, liver, heart, white muscle, and head kidney. The muscle tissue was collected at the lateral line, immediately posterior to the dorsal fin. The entire brain was sampled from every second fish. All tissues were suspended in RNAlater® (Life Technologies, Carlsbad, CA, USA), according to the manufacturer's recommendations and after 24 hrs at $4°$C were stored at $-20°$C for 1-2 months and then $-80°$C until extraction.

A.3.2 Molecular Methods

Tissue samples were screened for the presence of 45 microparasite taxa (Table C), using HT-qRT-PCR on the Fluidigm Biomark Dynamic Array™ microfluidics platform (Fluidigm, San Francisco, CA, USA). This platform has been used to test for human pathogens found in food and water in Japan as well as tick-bourne pathogens sampled over a broad geographic region in Europe (ISHII *et al.* 2013; MICHELET *et al.* 2014). The platform was analytically validated for research application in salmon (MILLER *et al.* 2016) and utilizes the same TaqMan™ (Applied Biosystems, Carlsbad, CA, USA) assays as other single assay platforms. Like other qRT-PCR platforms, using this technology alone on non-sequential samples, it is difficult to differentiate a carrier state from an active infection, and the data cannot discern whether a fish is, in fact, diseased.

Microparasite taxa were chosen based on knowledge of their presence in Canada or evidence of their association with disease worldwide (MILLER *et al.* 2016). Taqman assays (Table C) were designed to target RNA which allows the inclusion of some RNA viruses and focuses on microparasites in an active state (MILLER *et al.* 2016). A potential drawback to this approach is that the actual number of a given microparasite may be distorted by high gene expression. However, using these techniques our group has found associations between microparasite RNA copy number and immune response (JEFFRIES *et al.* 2014a), mortality (experimental and field, juveniles and adults (JEFFRIES *et al.* 2014a; MILLER *et al.* 2014)), and tissue damage (K. Miller, unpublished data). The Biomark platform, running all assays presented in this study, has been evaluated for specificity, sensitivity, and repeatability (MILLER *et al.* 2016). For more details regarding the selection of assays, assay development, and evaluation of the platform, see MILLER *et al.* (2016).

To limit risk of false positive contamination, the laboratory pipeline was set up to flow between work stations whereby amplified products and high concentration controls were separated from extractions and other sample processing steps (outlined in MILLER *et al.* (2016)). Moreover, the artificial construct controls contained an extra probe to track any potential contamination (see SNOW *et al.* (2009)).

Total RNA was extracted using methods previously described (JEFFRIES *et al.* 2014a; MILLER *et al.* 2016, 2011, 2014). Tissues were homogenized separately in TRI-reagent™ (Ambion Inc., Austin, TX, USA). Next, 1-bromo-3-chloropropane was added to the homogenate, and aqueous phase aliquots were pipetted into 96-well plates. Equal volumes of the aqueous phase from each tissue were combined prior to extraction. Extractions were carried out using MagMAX™-96 for Microarrays Total RNA Isolation Kits (Ambion Inc.) with a Biomek NXP™ automated liquid-handling instrument using the "spin method" according

to the manufacturer's instructions. RNA quantity and purity was assessed by measuring the A260/A280 using a Beckman Coulter DTX 880 Multimode Detector (Brea, CA, USA). Samples were normalized to $62.5\,\mathrm{ng}/\mu\mathrm{L}$.

Normalized RNA ($1\,\mu\mathrm{g}$) was reverse transcribed to cDNA using the SuperScript VILO MasterMix Kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The assay volume used for qPCR on the BioMark is small (7 nL) and therefore a pre-amplification step is required. Thus, $1.25\,\mu\mathrm{L}$ of cDNA from each sample was pre-amplified with primer pairs corresponding to all 48 assays (45 microbes and 3 reference genes) in a $5\,\mu\mathrm{L}$ reaction volume using 1X TaqMan Preamp Master Mix (Applied Biosystems, Foster City, California) according to the BioMark protocol. Unincorporated primers were removed using ExoSAP-IT^{TM} (Affymetrix, Santa Clara, California), and samples were diluted 1:5 in DNA Suspension Buffer (Teknova, Hollister, California).

All assays were run in duplicate. A serial dilution of artificial positive constructs (APC clones) corresponding to all assays was run as six samples on the dynamic array. This serial dilution allowed for the calculation of assay efficiency. The APC clones contain an additional probe labeled with NED^{TM} (Life Technologies) that allows for the detection of vector contamination (see MILLER *et al.* (2016)).

A $5\,\mu\mathrm{L}$ sample mix was prepared containing 1X TaqMan Universal Master-Mix (Life Technologies), 1X GE Sample Loading Reagent (Fluidigm PN 85000746), and amplified cDNA, which was added to each assay inlet of the array following manufacturer's recommendations. Five µL of assay mix was prepared containing $10\,\mu\mathrm{M}$ primers (microparasite in FAM-MGB and APC in NED-MGB) and $3\,\mu\mathrm{M}$ probes for the TaqMan assays. After loading the assays and samples into the chip using an IFC controller HX (Fluidigm), PCR was performed with the following conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Cycle threshold was determined using the Biomark Real-Time PCR analysis software. Reaction curves for each positive sample-assay combination were visually evaluated for abnormal curve shapes, close correspondence between replicates, and presence of APC contamination as indicated by NED positives. Using scripts created in R statistical software (R CORE TEAM 2015), I calculated efficiency for each assay, omitted results where only one duplicate was positive for a sample-assay combination, removed NED positive samples, and averaged duplicates. In this study, the limit of detection (LOD) is defined as the estimated cycle threshold (Ct) number under which true positive results are expected 95% of the time for a given assay (MILLER *et al.* 2016). Because this is a conservative estimate, in this study I present data exceeding the LOD. Since the purpose of the LOD is to reduce the number of false positives, I note when LODs were exceeded. Note that I only included detections beyond the LOD for microparasites that were also detected within the LOD and that microparasites only detected beyond the LOD were considered to be false positives. Values beyond the LOD were included in all analyses described below.

Microparasite prevalence was calculated for each sampling event simply as the proportion of each site's total sample that was positive. To make the data more approachable to those not familiar with qPCR (where a lower cycle threshold (Ct) indicates a higher quantity of genetic material), I chose to express measures of microparasite RNA quantity as "relative load" instead of Ct, where: Relative load = 40 - Ct. Note that this reverses the relationship to the LOD.

A total of 86 adult Chinook salmon were sampled across the five sample locations. I removed all fish that had poorly expressed reference genes, an indication of low sample quality (Average ref. gene Ct $>$ 16, PR = 1, CR = 3) for a total of 82 samples for analysis (Table A.1).

A.3.3 Statistical Analyses

All analyses were performed using R statistical software (R CORE TEAM 2015). Due to the large number of comparisons between microparasites and factors of interest from our multiple analyses, I chose to only present those that appear of importance based on significance tests (α = 0.05). While I recognize that this may be construed as data dredging (IOANNIDIS 2005), I have restricted type 1 errors (false positives) by adjusting all p values using the False Discovery Rate (FDR) approach (BENJAMINI and HOCHBERG 1995). This adjustment limits the rate of falsely rejecting the null hypothesis according to a ranking of p values from multiple hypothesis tests (BENJAMINI and HOCHBERG 1995).

To test differences in microparasite relative load associated with sampling period for the Chilliwack Hatchery fall stock, I used Welch's two sample t-tests to compare time 1 to time 2 for each microparasite. Since the prevalence of some microparasites resulted in unbalanced groups for the t-tests, only some microparasites could be tested in this analysis (Table A.2).

Table A.2: A list of all the microparasite taxa detected in Chinook salmon (British Columbia, 2013) with an indication of which statistical tests they were included in: ΔCRF = changes in relative load between two sampling events for the Chinook River Fall stock, Sex = differences in relative load with sex, and Blood = correlations between relative load and blood plasma physiological indices.

Microparasite	ΔCRF	Sex	Blood
A. salmonicida	Y	Y	Y
'*Ca.* B. cysticola'	Y	Y	Y
F. psychrophilum	Y	Y	Y
Rickettsia-like organism	Y	Y	Y
D. salmonis	Y	Y	Y
I. hoferi	N	Y	N
S. destruens	N	Y	N
F. margolisi	N	Y	N
L. salmonae	Y	Y	Y
P. theridion	N	Y	N
C. shasta	Y	Y	Y
K. thyrsites	Y	Y	Y
M. arcticus	N	Y	N
P. minibicornis	Y	Y	Y
P. pseudobranchicola	N	Y	N
T. bryosalmonae	Y	Y	Y
C. salmositica	N	Y	Y
I. multifiliis	Y	Y	Y
Piscine reovirus	Y	N	N
Viral erythrocytic necrosis virus	Y	Y	Y

To compare microparasite loads between sexes I created general linear models for each microparasite

and included sample group as a nuisance parameter (essentially an ANCOVA with load as the dependent variable, sex as the explanatory variable, and sample group as the covariate). Sample group was considered a nuisance parameter since I expect inherent differences between sites to contribute to variance in microparasite loads but I was interested in the differences between sexes. This analysis could not be conducted for every microparasite (Table A.2). To test for differences in likelihood of infection with a given microparasite based on sex, I also parameterized microparasites as simply present or absent and repeated this analysis as a logistic regression.

To test for associations between blood plasma physiological indices and microparasite loads, I again employed the general linear model approach, this time including both sample group and sex as nuisance parameters and a given blood variable as the response. This analysis was conducted for 13 microparasites (Table A.2). Due to a lack of normality, I log transformed plasma cortisol, testosterone, and 17b-estradiol.

To test for differences in the number of microparasite taxa detected per individual (hereafter microparasite richness) between sampling events, I used Dunn's test which allows multiple comparisons for rank sum data. Using the the dunn.test package in R, I adjusted the p value using the FDR approach. For the same analysis but between sexes instead of sampling events, I used ordinal multiple linear regression including sampling event as a nuisance parameter.

In search of correlations between microparasites suggestive of co-infection or a biological relationship between microparasites, I used simple linear regression for each pair of microparasites with positive detections.

A.4 Results

Twenty distinct microparasite taxa were detected in our samples (Figure A.2). Patterns of prevalence and relative load varied by microparasite and across sampling locations (Figure A.2). For example, '*Candidatus* Branchiomonas cysticola' was found in nearly every fish and at consistent relative load across sites, while in contrast *Facilispora margolisi* was found at high prevalence only at PR, the marine site. Relative load of *Flavobacterium psychrophilum* was 1.74 relative Ct higher for females (FDR-adjusted p = 0.01). The greatest difference between sexes was found for *K. thyrsites*, which was 10.8 times more likely to infect females (adjusted $p = < 0.001$) and 4.9 relative Ct higher in females (adjusted p = 0.004). For the group that was sampled twice (CRF), *F. psychrophilum* load increased significantly by 3.0 relative Ct (adjusted p = 0.01). *Aeromonas salmonicida* and *Cryptobia salmositica* showed noteworthy increases in prevalence for this group (Figure A.2).

The relative load of *F. psychrophilum* was negatively correlated with chloride (Table A.3). *C. shasta* relative load was negatively associated with plasma glucose (Table A.3). *C. salmositica* relative load was negatively associated with the log of plasma testosterone and *D. salmonis* had a similar relationship with the same parameter, although non-significant. It is noteworthy that for 10 measurements of blood plasma physiological indices, *F. psychrophilum* and *C. salmositica* appeared among the top three most associated for 5 and 9 variables respectively (Table A.3). This is contrasted with some of the other prevalent microparasites, including *I. multifiliis*, '*Ca.* B. cysticola', *Kudoa thyrsites*, *P. minibicornis*, and *Tetracapsuloides*

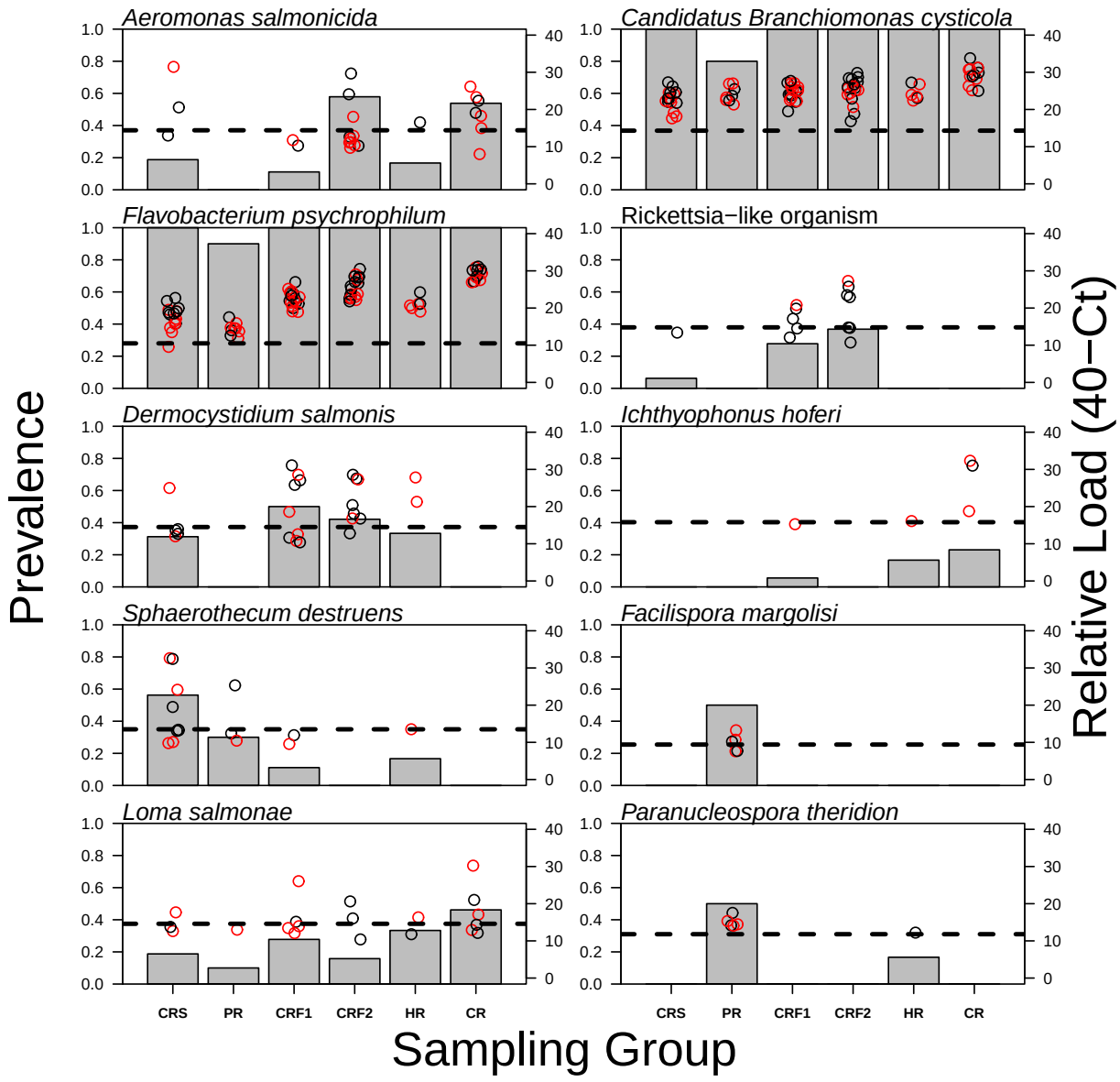

Figure A.2: Bar plots of microparasite prevalence (gray bars, primary y-axis) overlaid with jitter plots of relative load (open points, secondary y-axis) for microparasites in adult Chinook salmon at seven sampling events, British Columbia, 2013. CRS = Chilliwack River summer (n=16), PR = Port Renfrew (marine, n=10), CRF1 = Chilliwack River fall first sampling (n=18), CRF2 = Chilliwack River fall second sampling (n=19), HR = Harrison River (n=6), CR = Capilano River (n=13). The horizontal dashed line depicts assay specific limit of detection. Relative load is shown for each individual by sex (black=female, red=male).

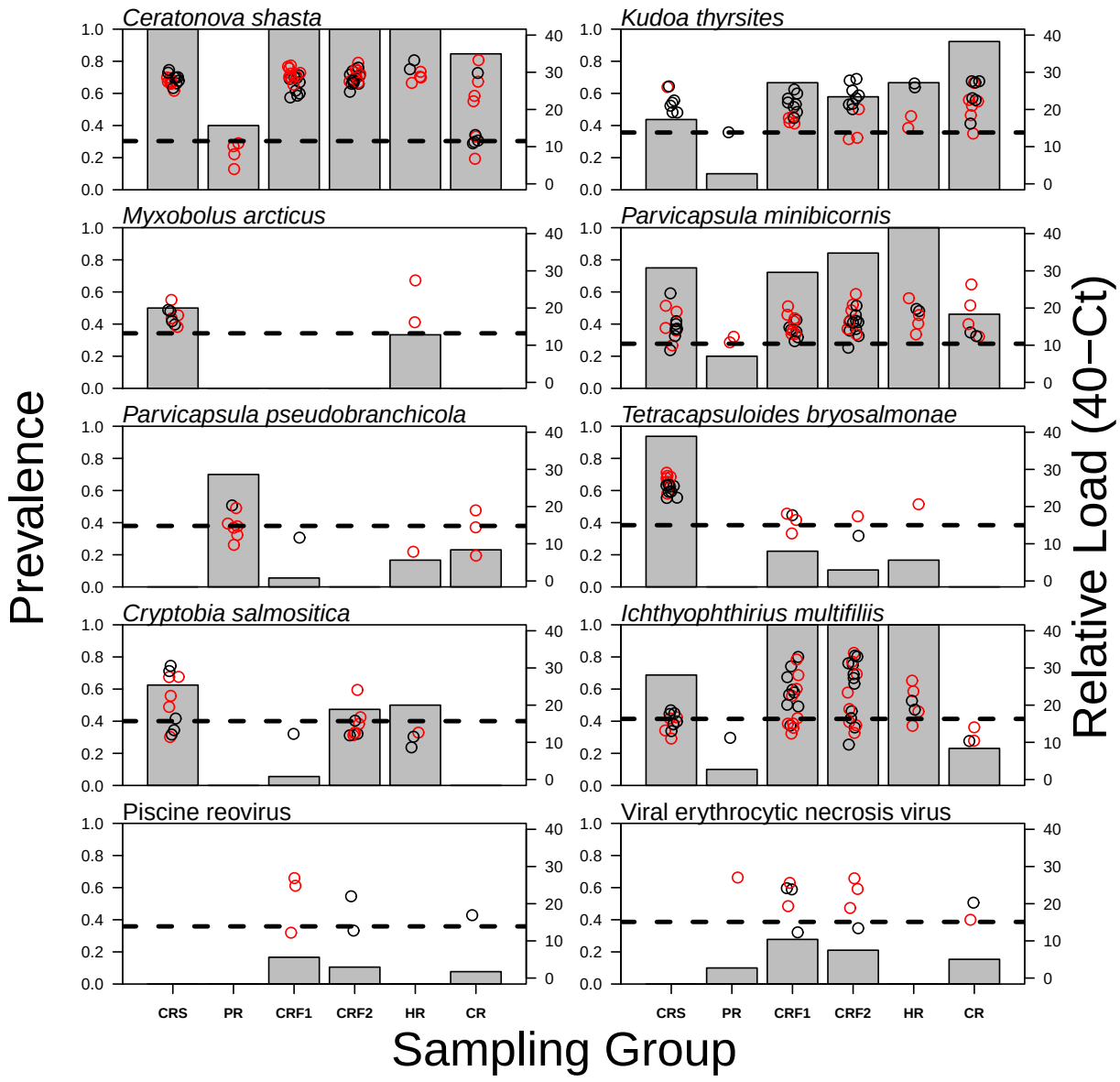

Figure A.2: Bar plots of microparasite prevalence (gray bars, primary y-axis) overlaid with jitter plots of relative load (open points, secondary y-axis) for microparasites in adult Chinook salmon at seven sampling events, British Columbia, 2013. CRS = Chilliwack River summer (n=16), PR = Port Renfrew (marine, n=10), CRF1 = Chilliwack River fall first sampling (n=18), CRF2 = Chilliwack River fall second sampling (n=19), HR = Harrison River (n=6), CR = Capilano River (n=13). The horizontal dashed line depicts assay specific limit of detection. Relative load is shown for each individual by sex (black=female, red=male).

bryosalmonae, that were rarely associated with blood parameters.

Table A.3: Results from multiple linear regression of blood plasma physiological indices compared to microparasite load in adult Chinook salmon, British Columbia, 2013. Sample group and sex were included in each model but estimates are not included here as they are considered nuisance parameters. Although 143 models were run, only the top three (based on significance of microparasite as a predictor of plasma variable) for each variable are presented. *P* values were adjusted according to the FDR approach (BENJAMINI and HOCHBERG 1995). *P* values significant at the $\alpha = 0.05$ level are in boldface.

Plasma variable	Microparasite	β	t-statistic	df	*p*	adjusted *p*
Hematocrit	*C. shasta*	-0.29	-1.20	45	0.24	0.67
(% of Total Blood)	*C. salmositica*	0.59	1.01	7	0.34	0.74
	A. salmonicida	-0.27	-0.81	15	0.43	0.75
Chloride	*F. psychrophilum*	-1.44	-3.44	70	0.001	**0.04**
(mmol/L)	*C. salmositica*	-0.82	-2.76	16	0.01	0.20
	'*Ca.* B. cysticola'	-0.94	-2.37	69	0.02	0.27
Potassium	*T. bryosalmonae*	-0.29	-1.61	16	0.13	0.51
(mmol/L)	*F. psychrophiluma*	0.10	1.42	70	0.16	0.60
	C. salmositica	0.08	1.34	16	0.20	0.65
Sodium	*C. salmositica*	-0.72	-2.47	16	0.03	0.28
(mmol/L)	*F. psychrophilum*	-0.95	-2.29	70	0.03	0.28
	T. bryosalmonae	2.36	2.32	16	0.03	0.28
Osmolality	*A. salmonicida*	-1.56	-4.17	15	0.001	**0.04**
(mOsm/kg)	*C. salmositica*	-1.25	-3.04	16	0.01	0.14
	F. psychrophilum	-1.68	-2.19	70	0.03	0.28
Glucose	*C. shasta*	-0.29	-4.07	63	< 0.001	**0.01**
(mmol/L)	*C. salmositica*	-0.11	-2.06	16	0.06	0.33
	D. salmonis	-0.14	-1.83	18	0.08	0.42
Lactate	*F. psychrophilum*	0.52	2.77	70	0.01	0.14
(mmol/L)	*C. salmositica*	0.28	2.32	16	0.03	0.28
	L. salmonae	-0.19	-1.37	11	0.20	0.65
Cortisol	*C. salmositica*	0.09	2.37	8	0.05	0.33
(log ng/mL)	*A. salmonicida*	-0.03	-8.39	1	0.08	0.41
	'*Ca.* B. cysticola'	0.08	1.76	35	0.09	0.42
17b-estradiol	*P. minibicornis*	-0.20	-2.70	21	0.01	0.20
(log ng/mL)	*D. salmonis*	-0.08	-2.36	10	0.04	0.32
	C. shasta	-0.22	-2.05	30	0.05	0.33
Testosterone	*C. salmositica*	-0.19	-7.93	7	< 0.001	**0.01**
(log ng/mL)	*D. salmonis*	-0.09	-4.01	10	0.002	0.07
	C. shasta	-0.16	-1.67	30	0.11	0.45

Microparasite richness was not significantly different between sexes when sampling event was taken into account but there were significant differences between sampling events (Figure A.3). The PR and CR sites had significantly lower microparasite richness than the sampling sites in the Fraser watershed. The greatest pairwise difference was between the CRS and the PR groups (adjusted $p<0.001$).

From 65 pairwise correlations of microparasite loads, two associations clearly stood out. The bacteria

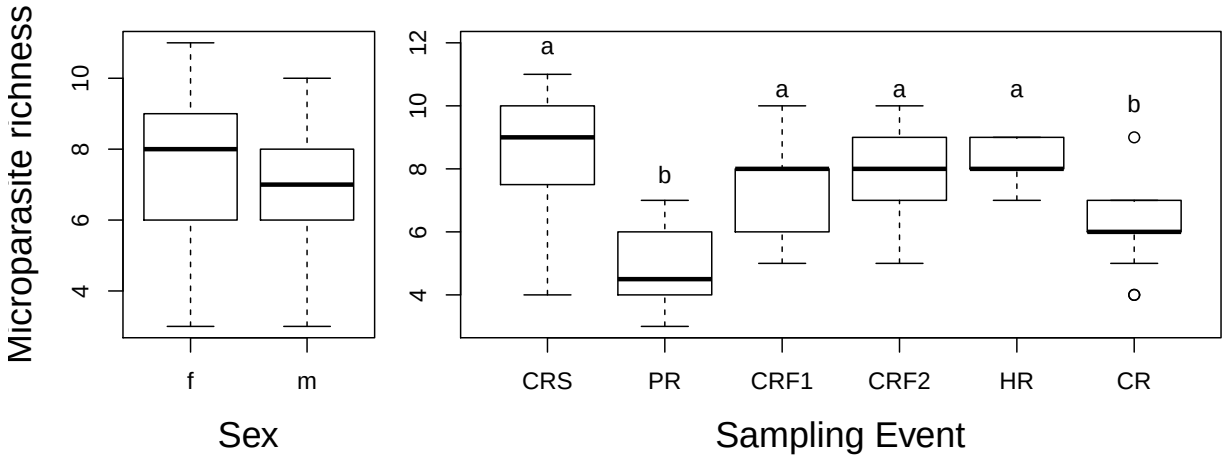

Figure A.3: Boxplots showing differences in microparasite richness between sexes (left) and sampling events (right). Heavy black line indicates the median, the box represents the first and third quartiles, the whiskers represent 1.5 times the inter-quartile range, and the circles represent outliers. CRS = Chilliwack River summer, PR = Port Renfrew (marine), CRF1 = Chilliwack River fall first sampling, CRF2 = Chilliwack River fall second sampling, HR = Harrison River, CR = Capilano River. Significant differences for pairwise comparisons (Dunn's test) are indicated when letters are not shared between groups.

'*Ca.* B. cysticola' and *F. psychrophilum* were positively correlated (β = 0.91, R^2 = 0.34, df = 78, adjusted $p = < 0.001$). The relative load of the myxozoan, *T. bryosalmonae*, was negatively correlated with that of *F. psychrophilum* ($\beta = -0.98$, R^2 = 0.70, df = 20, adjusted $p = < 0.001$). RLO only occurred in the presence of *I. multifiliis*, but relative loads were not significantly correlated likely due to low occurrence of RLO (n = 13) coupled with the restriction imposed by adjusting *p* values.

A.5 Discussion

High-throughput quantitative polymerase chain reaction is an effective, low-cost tool for rapidly determining the prevalence and load of dozens of microparasites across a large number of samples. By applying this tool to a sample of 82 Chinook salmon, I conducted one of the most comprehensive surveys of salmonid microparasites (in terms of target pathogens) for adult salmon in British Columbia. I found evidence of 20 different microparasites across 6 sampling events, four of which ('*Ca.* B. cysticola', *F. margolisi*, *P. theridion*, and *P. pseudobranchicola*) have not been previously described in Chinook salmon and four of which (to our knowledge) have not been detected in any salmonid in the Fraser River (*A. salmonicida*,

'*Ca.* B. cysticola', *I. hoferi*, *S. destruens*). I also found that microparasite richness, a measure shown to be associated with predation of sockeye salmon smolts (MILLER *et al.* 2014), was lower for adult Chinook sampled in the ocean and in a much smaller river outside the Fraser River watershed.

In this study, while I did not directly ascertain linkages between microparasites and disease, I used correlations between loads of individual microparasite taxa with clinical blood variables (indicative of stress, osmoregulation, maturation, and senescence) to identify infectious agents with potential for impact. *C. salmositica* was the most frequently associated with clinical variables that together characterize advanced senescence or morbidity. *F. psychrophilum*, which was ubiquitous in all sample locations and present at high relative loads in freshwater, was negatively associated with chloride levels, which have previously been associated with imminent mortality (HRUSKA *et al.* 2010; JEFFRIES *et al.* 2011). Load of *C. shasta*, also common in freshwater, was highly associated with glucose levels. Importantly, many of these microparasites associated with clinical data that could indicate potential for negative impact are known from previous studies to cause premature mortality of adult salmon (e.g. *C. shasta* (HALLETT and BARTHOLOMEW 2012), *C. salmositica* (WOO 2012)), suggesting that our approach is able not only to broadly identify the microparasites present within the system, but also to aid in identifying those that may be pathogenic.

This study (with the exception of the marine samples) focused on mature salmon close to spawning. It is essential to note that these fish were migratory survivors, and therefore our results do not reveal which microparasites may cause mortality during migration or at other life stages. Highly pathogenic microparasites might not be detectable on spawning grounds since they have already caused high mortality in migrating fish. Interestingly, *F. margolisi, P. theridion,* and *P. pseudobranchicola* were 44, 56, and 78% prevalent (respectively) at the marine site but 0, 17, and 6-23% prevalent at freshwater sites. Further investigations are needed to determine whether these reductions in prevalence resulted from a successful immune response or microparasite-associated mortality.

Since most of my results are specific to each microparasite, I now discuss the key findings for each taxon that was detected in abundance and provide a brief summary of previously published information regarding its distribution in British Columbia.

A.5.1 Bacteria

Aeromonas salmonicida

This bacteria, the infectious agent causing furunculosis, was deemed by KENT (2011) to be of high risk to Fraser River sockeye salmon, *O. nerka*, due to its virulence. While *A. salmonicida* has been detected in a Chinook salmon in a coastal BC netpen facility (KENT *et al.* 1998) and in a private freshwater hatchery (STEPHEN *et al.* 2011), I found no previous records of its presence in Fraser River salmon. It was present at all hatchery sampling events and, for the CRF group, appeared to show an increase in prevalence (40%) and relative load (non-significant) over time. Increases in prevalence and load are not surprising as *A. salmonicida* is commonly detected at hatcheries, where close contact between fish allows transmission of this bacteria, which does not persist for long in the absence of a host (WOO *et al.* 2011). Similar to my results for the CRF group, LOCH *et al.* (2012) found that levels of *A. salmonicida* increased throughout the spawning run of introduced Chinook salmon collected at a weir on a tributary to Lake Michigan. I did not detect this bacteria

in the group sampled in the marine environment. Although *A. salmonicida* is found in sea water netpens, it is likely carried over from freshwater and can be horizontally transferred at high fish densities (KENT *et al.* 1992).

As *A. salmonicida* relative load increased, plasma osmolality decreased, indicating a potential impact of the bacteria on osmoregulatory homeostasis. ROBERTSON *et al.* (1987) found that plasma osmolality became more reduced as a bacterial infection (species not disclosed) progressed in red drum (*Sciaenops ocellatus*) (Linnaeus, 1766) , although this was not determined experimentally so causality was not established.

Candidatus Branchiomonas cysticola

Recently discovered in Norway, this bacteria is a member of the gill microbiota that has been linked to proliferative gill inflammation (TOENSHOFF *et al.* 2012). It was present in 98% of Chinook salmon in this study, and our research group has found it across multiple genera and life stages of salmonids (K. Miller, unpublished data). MITCHELL *et al.* (2013) found '*Ca.* B. cysticola' to be highly prevalent over a 7 year period from a large geographical area in Norway and Ireland. Where epitheliocysts (bacteria filled cysts in gill lamellae) were abundant, qPCR found higher levels of '*Ca.* B. cysticola' RNA (MITCHELL *et al.* 2013).

Relative load of '*Ca.* B. cysticola' was well correlated with that of *F. psychrophilum*. As these are both bacteria, it is possible that they share similar requirements for infection including host immune status, host density, and environmental factors. However, the two other bacterial species detected, *A. salmonicida* and RLO, were not correlated with either of the above. Studying Atlantic salmon, *Salmo salar* (L.), in Norwegian fish farms, GUNNARSSON *et al.* (2017) suggested that '*Ca.* B. cysticola' may benefit from infections of *P. theridion* after observing a positive correlation between these two microparasites.

Since this bacteria was present in 98% of fish sampled, detected at fairly constant levels, equally common between sexes, and not associated with any phsyiological indices, I do not expect that it is pathogenic. Other authors have noted the ubiquitous nature of '*Ca.* B. cysticola' and its lack of association with mortality (GUNNARSSON *et al.* 2017). As this microparasite has recently been described, this is the first publication documenting its presence in British Columbia salmon.

Flavobacterium psychrophilum

This bacteria, with virtually a worldwide distribution (STARLIPER 2011), is the aetiological agent of "bacterial cold-water disease" (ROBERTS 2012), typified by necrotic skin patches which can develop at relatively low temperatures (4-12°C). Epizootics occur frequently among young fish at hatcheries in the north-western United States (INGLIS *et al.* 1993).

F. psychrophilum, considered to be an opportunistic microparasite, was present in 99% of the sampled fish and showed a significant increase in load between multiple sampling events at the Chilliwack Hatchery. This increase in load is likely not singular to hatcheries, as high levels of *F. psychrophilum* have been observed in other Pacific salmon species on spawning grounds (MILLER *et al.* 2014). DOLAN *et al.* (2016) found that the bacterial killing capability of the innate anti-bacterial components in Chinook salmon plasma diminished throughout the spawning migration, and especially in moribund fish on spawning grounds. The load of this bacteria appeared to track senescence well, as evidenced by negative correlations with plasma

osmolality, chloride, sodium, and a positive correlation with plasma lactate. These physiological trajectories are all indicative of moribidity (HRUSKA *et al.* 2010), suggesting that individuals in this study with high loads of *F. psychrophilum* were closer to death than others. Another potential explanation of the correlations between ion variables and *F. psychrophilum* load is that this microparasite causes necrosis of flesh and most organs (BARNES and BROWN 2011), which might impact the ability of the host to osmoregulate.

F. psychrophilum relative load was significantly higher in females compared to males. Typically, male Chinook salmon arrive on spawning grounds prior to females and reside longer (MORBEY 2000) so it is unlikely that these differences in load are merely based on time on spawning grounds. Alternatively, sex hormone mediated alterations to immune system function may allow greater infection of *F. psychrophilum* in females. WENGER *et al.* (2011) exposed juvenile rainbow trout to 17b-estradiol and then challenged them with *Yersinia ruckeri*. The authors found that 17b-estradiol suppressed the ability of trout to up-regulate complement (an innate component of the immune system that defends against bacterial infection) gene expression in response to bacterial infection. Hence, it is possible that elevated levels of 17b-estradiol in female migrating Pacific salmon (SLATER *et al.* 1994) may lead to reductions in immune function and subsequently higher bacterial infections compared to males. However, I did not see any association between *F. psychrophilum* relative load and plasma 17b-estradiol concentration in this study.

Rickettsia-like organism

The Taqman assay that I employed to target this bacteria (hereafter RLO), has been associated with an inflammatory skin disorder known as "strawberry disease" (LLOYD *et al.* 2011). Since causation has not been confirmed and it is uncertain whether researchers describing strawberry disease were targeting the same organism as our study, establishing previous records of detection for this microparasite is challenging. However, I saw no mention of the disease or RLO occurring in BC, aside from a study using this same platform on coho salmon, *Oncorhynchus kisutch* (MILLER *et al.* 2014). RLO was detected at the Chilliwack Hatchery but only occurred in the presence of *I. multifiliis*. SUN *et al.* (2009) found *Rickettsia* spp. to be endosymbionts of *I. multifiliis* infecting channel catfish.

A.5.2 Mesomycetozoea

Dermocystidium salmonis

This microparasite, which has defied categorization to some degree (now considered a mesomycetozoean), has been associated with prespawn mortality events at hatcheries and spawning channels in the Columbia River claiming approximately 25% of the spawning Chinook population in some years (ALLEN *et al.* 1968; PAULEY 1967). In British Columbia, a species from the *Dermocystidium* genus was implicated as the primary cause of a large scale fish kill in the Nipkish River on Vancouver Island (HOSKINS *et al.* 1976). ARAI and MUDRY (1983) documented a *Dermocystidium spp.* occurring in the gill of multiple salmonids inhabiting tributaries to the Fraser River.

ALLEN *et al.* (1968) mention that *D. salmonis* was most virulent at temperatures below 15.5^{o}C. *D. salmonis* was most prevalent in the CRF group, in concordance with this temperature preference. This

microparasite was not detected at the PR or CR sites, and I have seen no accounts of *D. salmonis* being transmitted in the marine environment. Several authors have mentioned co-infections involving *D. salmonis* and *Flavobacterium columnare* (HÖGLUND *et al.* 1997; PAULEY 1967; WOOD 1974). I found a positive correlation between *D. salmonis* and another *Flavobacterium* species, *F. psychrophilum*, although this relationship was not significant ($p = 0.058$, adjusted $p = 0.251$).

OLSON *et al.* (1991) found that laboratory held juvenile Chinook salmon infected with *D. salmonis* were capable of shedding the microparasite and appeared to recover from infection, something that I believe I have witnessed in wild adult sockeye salmon held *in situ* (A. Bass, unpublished data). As OLSON *et al.* (1991) found that *D. salmonis* had a free swimming life stage infective to salmonids, this shedding may be the mechanism of transmission for the microparasite.

Ichthyophonus hoferi

Another mesomycetozoean, *I. hoferi* is readily transmitted from prey to predator as demonstrated by Chinook salmon in British Columbia contracting this microparasite after eating infected herring, *Clupea pallasii* (JONES and DAWE 2002). The microparasite was unreported in the Yukon River, Alaska prior to 1985 but in the mid-2000s was infecting more than 40% of returning Chinook salmon (KOCAN *et al.* 2004). Considered a marine microparasite in origin, *I. hoferi* has been detected in the Salish Sea (HERSHBERGER *et al.* 2013) but I found no prior records of infection in the Fraser River. I detected *I. hoferi* at multiple freshwater locations, with the highest loads and prevalence occurring at the Capilano hatchery.

Sphaerothecum destruens

Previously known as the Rosette Agent, this intracellular mesomycetozoean was discovered after a high mortality event of Chinook salmon held in marine netpens in Washington State (HARRELL *et al.* 1986). ARKUSH *et al.* (1998) suspected that the freshwater supply used to rear juvenile Sacramento River Chinook salmon was the source of *S. destruens* that manifested in high mortality upon transfer of fish to seawater netpens. ARKUSH *et al.* (1998) also asserted that the microparasite has disseminated and nodular forms in the host, the former resulting in higher mortality than the latter. KENT (2011) referred to this as a "deadly marine pathogen", although ARKUSH *et al.* (1998) found *S. destruens* in returning Sacramento River spawners and mentioned that some infected fish were able to spawn successfully.

I found no published cases of *S. destruens* occurring in adult salmon in British Columbia, although MILLER *et al.* (2014) detected low prevalence in juvenile sockeye salmon in the Salish Sea. *S. destruens* was most common in Chilliwack River summer Chinook, with over half of the CRS group with positive detections. The second highest abundance occurred at the marine site.

A.5.3 Microsporidia

Facilospora margolisi

This recently discovered microsporidian was first isolated from sea lice, *Lepeophtheirus salmonis*, that were collected from Atlantic salmon held in BC marine netpens (JONES *et al.* 2012). There is little information

regarding its life cycle, salmonid hosts, or distribution. *F. margolisi* was only detected at the marine sampling location and at low relative load. To my knowledge, this is the first documentation of this microparasite in Chinook salmon and any salmonid species in the Fraser River.

Loma salmonae

This pathogen causes "microsporidial gill disease of salmon" which tends to become visible in British Columbia aquaculture in the late summer and early autumn, and is transmissible in seawater (SPEARE and LOVY 2012). *L. salmonae* xenoma formation has an optimal temperature range overlapping with that of Pacific salmon (11-19°C) (SPEARE and LOVY 2012).

L. salmonae has been documented in Chinook salmon in coastal waters of British Columbia (KENT *et al.* 1998) and in other species of Pacific salmon in the Fraser River (MILLER *et al.* 2014), but I found no records of the microparasite in Fraser River Chinook salmon. This microparasite is found in Fraser River sockeye salmon, and its presence in marine sampled fish from the Chilko River stock was associated with reduced migratory survival in a telemetry study (MILLER *et al.* 2014). In Alaska, *L. salmonae* was linked to the mortality of 10% of juvenile Chinook at a freshwater enhancement facility (HAUCK 1984). I found *L. salmonae* at all sampling locations and time periods, with prevalence of infection low to moderate.

Paranucleospora theridion

Also known as *Desmozoon lepeophtherii* (FREEMAN and SOMMERVILLE 2011), this marine microsporidian was first discovered in 2008 and is suspected to be associated with proliferative gill inflammation (NYLUND *et al.* 2011). Like *F. margolisi*, this microparasite was isolated from sea lice, which are suspected to act as reservoirs capable of transmitting *P. theridion* to salmonids (NYLUND *et al.* 2010).

P. theridion has been observed in sea lice collected from British Columbia marine netpens (JONES *et al.* 2012) and detected in juveniles sockeye salmon sampled in the Salish Sea (MILLER *et al.* 2014). Here I report the first account of the microparasite in Chinook salmon. Of the Chinook sampled at the marine location, 50% tested positive for *P. theridion* and there was one positive detected in freshwater. After using qPCR to test for 5 microparasites associated with gill disease in Norwegian Atlantic salmon, GUNNARSSON *et al.* (2017) concluded that higher loads of *P. theridion* were associated with mortality more so than for other microparasites. Those findings, combined with my findings of 50% prevalence in the ocean and almost 0% prevalence in freshwater, are grounds for further investigation of this microparasite in British Columbia wild salmon.

A.5.4 Myoxozoa

Ceratonova shasta

Ceratonova shasta, a myxozoan parasite endemic to many rivers draining into the Northeast Pacific Ocean, has severe impacts on Chinook salmon in some locations (FUJIWARA *et al.* 2011). This myxozoan has an obligate freshwater invertebrate host which releases the actinospores that infect adult salmon (BARTHOLOMEW *et al.* 2006). Using "sentinel fish" (caged juvenile Chinook salmon), CHING and MUNDAY (1984a) surveyed

several sites in the Upper and Lower Fraser River for the presence of *C. shasta*. The authors concluded that *C. shasta* was present in the Upper Fraser River (722 km upstream) but at very low levels. CHING (1984) used water sampled in September from the Fraser River Delta (7 km upstream from ocean) to successfully infect hatchery juvenile Chinook salmon.

In this study, *C. shasta* was found in 97% of freshwater samples, with consistently high relative loads. The exception was the Capilano River Hatchery, where fish showed a unique, bimodal distribution of load, with some loads similar to other sites and others close to and below the LOD, potentially indicating a recent infection or carrier state. All Chinook salmon sampled at the marine site had loads below the LOD, similar to the low load at the Capilano Hatchery. CHING and MUNDAY (1984b) found that juvenile Chinook salmon could maintain *C. shasta* infections in seawater, results that have been confirmed by our surveys of out-migrating juvenile salmon (Strahan Tucker, unpublished data). Whether the positive detections at Port Renfrew were a result of infection carried to the ocean by juvenile salmon and maintained into adulthood or adult salmon encountering infective actinospores in the Fraser River plume is unclear.

At elevated river temperatures, *C. shasta* can rapidly complete its life cycle, resulting in more generations and higher production of infective actinospores in one season, although longevity of actionspores outside of hosts does decrease as water temperature increases (BJORK 2010). Development of *C. shasta* is considered to be retarded at water temperatures below 10°C (ROBERTS 2012). This pathogen inflicts higher host mortality as water temperature increases (RAY *et al.* 2012). In a study using caged juvenile Chinook salmon placed *in situ*, infection rate in the Lower Fraser River (up to 100 km upstream from ocean) was high, with the highest prevalence and mortality occurring in July and August when water temperatures were greater than 16 °C (CHING and MUNDAY 1984a). The CRS group was most likely to experience similar conditions, although load and prevalence does not appear different between this group and the other Fraser River groups.

I found that *C. shasta* relative load was negatively associated with plasma glucose concentration. As fish senesce on spawning grounds, glucose levels decrease (HRUSKA *et al.* 2010). It is possible that fish that were closer to death had both reduced glucose and elevated *C. shasta* infections. The main organ impacted by *C. shasta* is the intestine, which does not play a role in the movement of glucose into the bloodstream for adult salmon during their freshwater spawning migration. Therefore, it seems unlikely that *C. shasta* myxospores were directly blocking glucose as found by KODAMA *et al.* (2014) for another myxosporean species that attacked the intestine of puffer fish, *Takifugu rubripes* (Temminck Schlegel, 1850) . A challenge study with experimentally manipulated infections of *C. shasta* would be the best way to further investigate this association.

Kudoa thyrsites

K. thyrsites is considered non-pathogenic in salmonids but is highly important commercially as it causes myoliquefaction of salmon flesh post-mortem (ST-HILAIRE *et al.* 1997). It is widespread in Pacific salmonids and expected to be of marine origin (KENT 2011; MORAN *et al.* 1999), but has received very little attention in wild Pacific salmon.

Prevalence of *K. thyrsites* ranged from 44-92% across the freshwater sites but was only 11% at the marine location despite the expectation of marine origin. *K. thyrsites* was at consistently higher relative

loads in females compared to males. As in the case of *F. psychrophilum*, I suspect that this is associated with differences in immune function between males and females (WENGER *et al.* 2011). An alternative explanation could involve the life history and transmission of *K. thyrsites*, but these are not currently well understood (JONES *et al.* 2016).

Myxobolous arcticus

This myxozoan infects salmonids in their juvenile stage and is present in the brain and spine for the remainder of their lives. *M. arcticus* is considered to be relatively non-pathogenic although MOLES and HEIFETZ (1998) demonstrated reduced swimming ability in infected fish. *M. arcticus* has been used to determine stock of origin for Chinook salmon captured at sea (URAWA *et al.* 1998). It is unclear which rivers URAWA *et al.* (1998) sampled in British Columbia, but they only reported positives occurring in Chinook salmon sampled on Vancouver Island. In this study, I only sampled brains for molecular analysis from every other fish, and every one of these from the CRS group were positive for *M. arcticus*. Therefore, while it appears that only half of the CRS group were positive for *M. arcticus* in Figure 2, it is likely that most or all fish in this group were positive.

Parvicapsula minibicornis

This myxozoan parasite shares a similar life cycle with *C. shasta*, including the same freshwater invertebrate host (BARTHOLOMEW *et al.* 2006). ST-HILAIRE *et al.* (2002) proposed that *P. minibicornis* infection is a significant cause of premature mortality for Fraser River sockeye. JONES *et al.* (2003) found that *P. minibicornis* was detected by PCR in roughly 50% of Chinook salmon sampled from the Chilliwack and Chehalis (a tributary to the Harrison) hatcheries. I detected this microparasite at higher prevalence in Chinook salmon sampled at the Chilliwack Hatchery and on the Harrison River relative to other locations. The prevalence of *P. minibicornis* slightly increased between sampling periods for the Chilliwack River fall Chinook. This is to be expected, as BRADFORD *et al.* (2010a) noted that virtually all Fraser River sockeye become infected with *P. minibicornis* and infections become very intense on spawning grounds. BRADFORD *et al.* (2010a) found that *P. minibicornis* infection was negatively correlated with osmolality in adult sockeye salmon, a pattern I did not see with Chinook.

ST-HILAIRE *et al.* (2002) found that this pathogen was first detectable in sockeye salmon via histological or molecular methods (using kidney tissue) around the Hell's Gate area of the Fraser River, and therefore speculated that it is contracted in the lower Fraser River. Here I found positive detections at Port Renfrew and the Capilano River Hatchery, suggesting that the microparasite could be contracted in the Fraser River plume within the Salish Sea and beyond, or alternatively, that it can be retained from an earlier life stage. Indeed, *P. minibicornis* has been detected in juvenile Chinook salmon outmigrating from the Klamath River, California (TRUE *et al.* 2009).

Parvicapsula pseudobranchicola

Discovered in Norwegian netpen-reared Atlantic salmon with clinical signs of gill infection in 2002 (KARLSBAKK *et al.* 2002), *P. pseudobranchicola* has since been detected in predated juvenile sockeye salmon in the Salish Sea (MILLER *et al.* 2014). This myxozoan parasite has been associated with impacts on swimming activity and possibly vision (JØRGENSEN *et al.* 2011). In my study, this microparasite was 78% prevalent at the marine sampling site but less than 25% prevalent elsewhere. The next highest prevalence was at the Capilano hatchery, which is a short distance from the marine environment. To my knowledge, this is the first documented occurrence of this microparasite in Chinook salmon.

Tetracapsuloides bryosalmonae

This myxozoan, which has an obligatory life stage in freshwater bryozoans, is the infectious agent that causes proliferative kidney disease. Since the rate of development of this microparasite within its bryozoan host and its pathogenic effects both increase with water temperatures, some researchers suspect that the range and severity of *T. bryosalmonae* infections will increase with climate change (BRUNEAUX *et al.* 2016; TOPS *et al.* 2006). Although detected at freshwater hatcheries on Vancouver Island (varying from 40 to 100% over the course of five years), I found no published record of the microparasite in the marine environment in British Columbia, although our group has identified the parasite in out-migrating smolts in the ocean (Strahan Tucker, unpublished data). *T. bryosalmonae* was recently detected in coho salmon, sampled at the Chilliwack Hatchery (MILLER *et al.* 2014). *T. bryosalmonae* has been previously documented in a single juvenile Chinook salmon in freshwater on Vancouver Island (KENT *et al.* 1995).

I detected *T. bryosalmonae* in the Chilliwack and Harrison rivers, with 94 % prevalence in Chilliwack summer Chinook, in contrast to less than 25% for fall Chinook. This may be due to the timing of actinospores shedding from bryozoans. Alternatively, the water temperatures experienced by the CRS and CRF groups could explain the differences in prevalence (Fraser River temperature at Hope, BC: early July, 2013 approx. 17°C, early October, 2013 approx. 12°C). MILLER *et al.* (2014) found that prevalence and load of *T. bryosalmonae* in coho salmon experimentally exposed to a warm water treatment was much greater than that for fish exposed to cool water.

I found that the relative load of *T. bryosalmonae* was negatively correlated with the relative load of *F. psychrophilum*. Recently, DOLAN *et al.* (2016) demonstrated that the bacterial killing properties of blood plasma were greater in fish that were infected with another myxozoan, *P. minibicornis*. The authors suggested that the inflammation of the kidney caused by this species, a physiological impact similar to that of *T. bryosalmonae*, could have boosted the innate immune response. This provides a plausible mechanism behind the negative association we found between *T. bryosalmonae* and *F. psychrophilum*. However, this would be contrary to the findings of CHILMONCZYK *et al.* (2002); that infection with *T. bryosalmonae* impacted innate immunity and left infected *Oncorhynchus mykiss* (Walbaum, 1792) more vulnerable to secondary infection. It is possible that immuno-suppression by *T. bryosalmonae* is species specific.

A.5.5 Protozoa

Cryptobia salmositica

C. salmositica is a blood borne protozoan parasite that has been reported in all species of Pacific salmon (WOO 2012). It is carried in a non-pathogenic state by sculpins (*Cottus* spp.) and is transferred to salmonids by the freshwater leech, *Piscicola salmositica* (Meyer, 1946) (WOO 2003). Horizontal transmission between infected fish at high densities is possible (WOO 2012). BOWER and MARGOLIS (1984) surveyed 29 rivers in British Columbia and the Yukon and found the distribution of this microparasite to be limited to the Fraser River watershed and Vancouver Island. *C. salmositica* was as far upstream as the Bowron River (in Chinook salmon) and Thompson River systems and as close to the ocean as Weaver Creek, and Chinook salmon from the upriver source populations for the CRS group (Bowron River, Slim Creek) displayed infections (BOWER and MARGOLIS 1984). Epizootics have occurred at freshwater hatcheries and in sea pens (likely fish already infected in freshwater (WOO 2012)). Infections of *C. salmositica* are often lethal, although there are no accounts of epizootics in wild salmon (KENT 2011).

I detected *C. salmositica* in both the Chilliwack and Harrison Rivers but not at Port Renfrew or the Capilano hatchery. The lack of *C. salmositica* in coho salmon from the Capilano River was specifically noted as a surprising finding by BOWER and MARGOLIS (1984). Prevalence of infection for fall Chinook at the Chilliwack hatchery increased from 6 to 47% in 11 days. At a hatchery on Vancouver Island in September, adult Chinook recently entering freshwater were all negative for *C. salmositica* (BOWER and MARGOLIS 1984). One month later, 70% of these fish were infected; a similar pattern to our Chilliwack fall Chinook. Hatcheries, especially those with areas of gravel substrate hospitable to *P. salmositica*, may provide conditions suitable for the transmission of *C. salmositica*. I did see this leech on Chinook salmon at the Chilliwack Hatchery.

I found that testosterone was negatively correlated with relative load of *C. salmositica*. HRUSKA *et al.* (2010) found that moribund sockeye salmon in a spawning channel (both sexes) had significantly lower levels of testosterone than fish at arrival. It is more likely that the fish with high loads in our samples had been at the hatchery for long enough to both experience this decline in testosterone and accumulate higher loads of *C. salmositica* than there existing a causal relationship between load and testosterone. Although non-significant after FDR adjustment, several other plasma variables appeared associated with *C. salmositica* load. The direction of the relationship for each was in agreement with the senesence trajectory (HRUSKA *et al.* 2010).

Ichthyophthirius multifiliis

Considered by KENT (2011) to be a high risk to Fraser River sockeye salmon, *I. multifiliis* is a ciliate protozoan that is the causative agent of "white-spot" disease in freshwater fish. This microparasite develops more quickly at high water temperatures (GRATZEK 1993) and epizootics tend to occur in locations with high host density (e.g. hatcheries, spawning grounds). In 1995, *I. multifiliis* was identified as the pathogen causing sockeye salmon pre-spawn mortality in spawning channels in the Skeena River (PSM as high as 80%), and in the Fraser River at the Weaver Creek and Nadina River spawning channels (TRAXLER *et al.* 1998). While

this microparasite has been well-documented in Fraser sockeye, I was unable to find any documentation of *I. multifiliis* in Fraser River Chinook salmon.

We found *I. multifiliis* infection to be highly prevalent in Chilliwack and Harrison Chinook salmon. Fall Chinook had a broad range of relative loads for the microparasite, with some particularly high loads. RLO was only found in the presence of *I. multifiliis* and relative loads between the two were positively correlated, although non-significantly. Using molecular methods and microscopy, SUN *et al.* (2009) found a *Rickettsia* bacteria and several other bacteria living in *I. multifiliis* theronts as endosymbionts. It is unknown whether these endosymbionts play a role in pathogenesis of the *I. multifiliis* infection or the host immune response (SUN *et al.* 2009).

A.5.6 Viruses

Piscine Reovirus

While there is not consensus among researchers, many assert that the Piscine Reovirus (hereafter PRV) is associated with Heart and Skeletal Muscle Inflammation (HSMI) in Norway (FINSTAD *et al.* 2012), Chile (GODOY *et al.* 2016), and Canada (K. Miller, unpublished data), but whether the virus alone can cause the disease is still under debate (GARSETH *et al.* 2012; GARVER *et al.* 2016). First associated with HSMI in 2010 (PALACIOS *et al.* 2010), PRV is widely distributed in Norway, Scotland, and in the Northeast Pacific Ocean (MARTY *et al.* 2015). Using PCR, MARTY *et al.* (2015) found PRV present in 9% of 66 adult Chinook salmon captured on the British Columbia coast in 2013. Similarly, this virus was found in 8 - 18 % of the Chinook salmon I sampled in 2013. These collections may be the first in British Columbia showing positives in Chinook salmon adults returning to freshwater. MILLER *et al.* (2014) detected a weak association between PRV and migration survival in sockeye salmon returning to the Chilko Lake spawning grounds, within the Fraser River watershed. GARSETH *et al.* (2013) sampled returning Atlantic salmon in 36 Norwegian rivers and found average prevalence of 13, 24, and 55 % across wild, hatchery, and escaped farmed salmon, respectively.

Viral erythrocytic necrosis virus

EVELYN and TRAXLER (1978) reported this marine-origin virus in British Columbia Pacific salmon, but noted that the disease did not appear to naturally occur in Chinook salmon and exposing Chinook to the virus failed to lead to infection. BELL and TRAXLER (1985) found VEN present in pink salmon, *Oncorhynchus gorbuscha* (Walbaum, 1792), migrating up the Fraser River, appearing to increase in prevalence from Langley to Lillooet, BC. I detected this virus at prevalence between 11 - 28 % at multiple locations, it was most abundant in the Chilliwack fall group.

A.5.7 Future Research

While this study provided useful information regarding the distribution of microparasites in southern British Columbia, it is descriptive in nature and all analyses are correlative. Future studies applying HT-qRT-PCR to experimental manipulations in the field and laboratory will allow stronger inference. Pairing HT-qRT-PCR

data from non-lethal gill biopsies with telemetric data could reveal connections between microparasites and mortality throughout the freshwater migration. Finally, comparison of microparasite data with host gene expression and clinical signs of disease determined via histopathology will further our understanding of the mechanisms underlying the impact of microparasites on the survival of wild Pacific salmon.

Appendix B

Infectious agent dynamics in adult sockeye salmon during spawning migration

B.1 Synopsis

Pacific salmon experience rapid changes in morphology and physiology during their spawning migrations but little is known about the changes that occur in the infectious agent community (viruses, bacteria, protozoans, etc.) infecting migrating salmon. Baseline information describing the infectious agent community can indicate changes over time and elucidate infectious agent life histories. I used high throughput, quantitative PCR to describe the prevalence and load of 44 infectious agent taxa in one stock of sockeye salmon across multiple locations throughout the spawning migration. In pooled samples created from six organs, nineteen different taxa were present and the majority (12 of 19) followed a pattern of infection characterized by an increase in prevalence and load as fish approached their natal spawning area. My results add several new infectious agent taxa to those known to infect Fraser River sockeye salmon. I discuss noteworthy results for several infectious agent taxa possibly influencing the survival of sockeye salmon in the Fraser River.

B.2 Introduction

Pacific salmon are semelparous fishes that often conduct long-distance, energy intensive spawning migrations that are accompanied by rapid physiological and morphological transformations. Studies of Pacific salmon stocks along their spawning migrations, featuring multiple sampling locations, have been conducted to investigate changes in energy (GILHOUSEN 1980; JONSSON *et al.* 1997), osmoregulation (SHRIMPTON *et al.* 2005), stress hormones (BAKER and VYNNE 2014), morphology (ROBERTSON and WEXLER 1960), and metabolic processes (MILLER *et al.* 2009). In addition, "infectious agents", including viruses, bacteria, protozoans, and myxozoans, are known to vary along adult salmon migrations in terms of the percentage of a population infected (prevalence) and the magnitude of infection within individuals (load) (MILLER *et al.* 2014). However, few studies with multiple sampling locations along a migratory route have been conducted to describe the dynamics of infectious agents in Pacific salmon (but see KOCAN *et al.* 2004; MILLER *et al.* 2014; ST-HILAIRE *et al.* 2002).

Our knowledge of the dynamics of infectious agents along salmon migrations is limited due to several restrictions intrinsic to wild salmon populations. In contrast to their counterparts in aquaculture, wild salmon are typically not sampled for the study of infectious agents unless large scale morbidity is observed (BAKKE and HARRIS 1998). Therefore, we have little sense of what "normal" or "baseline" levels of prevalence

and infection should be. Also stemming from the focus on epizootics, studies of infectious agents along migrations tend to focus on one taxa at a time (KOCAN *et al.* 2004; ST-HILAIRE *et al.* 2002). As a result, studies rarely reveal the diverse infectious agent community infecting wild salmon and how its components interact with one another. In contrast to aquaculture, if an infectious agent infecting a wild salmon population is virulent, it may remain undetected since dying fish are difficult to recover for sampling (BAKKE and HARRIS 1998). Finally, the gold standard in fish epidemiology, histopathology, is not sensitive to subclinical infection, the early stage of infection when virulent infectious agents are tractable in wild populations but not causing disease. As a result of these limitations, there is little baseline information regarding infectious agent infections of adult Pacific salmon in freshwater.

Baseline infectious agent information, in the form of many samples across multiple years assessed for a wide breadth of infectious agent taxa, is important because it : 1) provides a temporal snapshot which is useful in regions where vectors for the introduction of exotic salmon infectious agents (e.g. aquaculture) exist, 2) facilitates insight into the life history patterns of salmonid infectious agents, and 3) provides a comparison against future samples collected during periods of high mortality or stressful environmental conditions, including those resulting from climate change (MARCOGLIESE 2001; MILLER *et al.* 2014).

The Fraser River, British Columbia, is the most productive river for wild Pacific salmon in Canada and supports ecologically, culturally, and economically important runs of sockeye salmon, *Oncorhynchus nerka*. Studies of infectious agents in Fraser River sockeye salmon have typically focused on single infectious agents suspected of causing prespawn mortality in spawning areas (BRADFORD *et al.* 2010b; COLGROVE and WOOD 1966; TRAXLER *et al.* 1998). However, recent work has taken a broader approach, incorporating multiple infectious agents. Surveying the liver tissue of sockeye salmon collected from 2005 – 2010 in the ocean, river, and spawning areas, MILLER *et al.* (2014) detected 9 infectious agent taxa from 11 assayed. As declining Fraser River sockeye salmon populations led to interest in the role of infectious agents in mortality (COHEN 2012), KENT (2011) compiled a relatively comprehensive review of infectious agents considered likely to pose a threat to Fraser River sockeye salmon and designated a hypothesized threat level to each. This review revealed that several infectious agents known to impact salmonids elsewhere were not known to occur in Fraser River sockeye salmon, in some cases due to a lack of monitoring, and therefore deemed by KENT (2011) to be of low risk.

The objective of this study was to provide a record of infectious agent prevalence and load in one population of sockeye salmon throughout their spawning migration. To conduct such a survey across a broad range of infectious agent taxa, I employed high-throughput quantitative polymerase chain reaction (HT-qPCR) with an assay platform featuring 44 different infectious agent taxa (MILLER *et al.* 2016). Sampling sites extended from the coastal marine environment, through the Fraser River, and to a spawning area. Herein, I provided a descriptive analysis of the patterns observed across the migration. Additionally, I compared infectious agent richness (i.e. the number of different infectious agents present) among sampling locations. My results contribute to the baseline knowledge of infectious agents in Fraser River sockeye salmon and their dynamics during a migration.

B.3 Methods

B.3.1 Fish collection and tissue sampling

University of British Columbia (UBC) researchers and Fisheries and Oceans Canada (DFO) staff collected sockeye salmon under Fisheries and Oceans Canada (DFO) permit XR 318 2014 and a DFO section 52 scientific collection permit. This work was performed according to the UBC animal care and use permit, A12-0250.

This study was conducted on a dominant cycle year (2014) for late-run Shuswap sockeye salmon, so that the vast majority of migrating fish during the sampling period would be from this population complex. In some Fraser River tributaries, a persistent, 4 yr cycle occurs where the run size is persistently larger than other years and the Adams River (480 km from the ocean, one of several populations whose young rear in Shuswap Lake) hosts the largest Fraser River sockeye salmon run. Fish were captured from the Strait of Georgia near the Fraser River mouth (hereafter SOG, 49.232 N, 123.271 W, river kilometer [rkm] 0), the Fraser River estuary (EST, 49.123 N, 123.076 W, rkm 16), the Fraser River at McMillan Island (MCM, 49.180 N, 122.567 W, rkm 48), the Fraser River at Peter's Road (PET, 49.299 N, 121.667 W, rkm 131), the Thompson River downstream from Kamloops Lake (KAM, 50.743 N, 120.895 W, rkm 363), and the Adams River spawning area (ADA, 50.896 N, 119.566 W, rkm 480) (see Figure B.1 and Table B.1). A purse seine

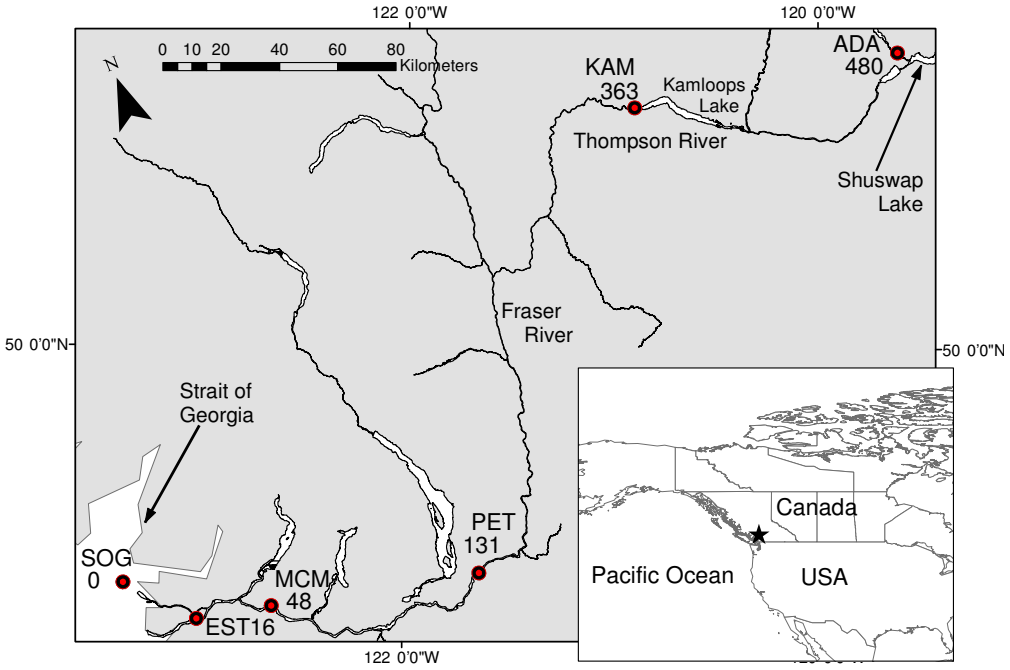

Figure B.1: Map of sampling locations (red circles) for late-Shuswap sockeye salmon collected from the Fraser and Thompson Rivers, British Columbia, 2014. Sampling locations are indicated along with river kilometer (distance from ocean): SOG = Strait of Georgia, EST = estuary, MCM = McMillan Island, PET = Peters Rd, KAM = Kamloops Lake, and ADA = Adams River.

was used to capture fish at SOG, gillnets were used to capture fish at EST and MCM, and beach seines were used to capture fish at PET, KAM, and ADA. Water temperature varied from 10 to 15°C across the sampling sites, with coldest temperatures at the spawning area (ADA).

Table B.1: Sampling information for adult sockeye salmon collected in British Columbia, 2014. SOG = Strait of Georgia, EST = Fraser River estuary, MCM = Fraser River at McMillan Island, PET = Fraser River at Peters Road, KAM = Thompson River downstream of Kamloops Lake, ADA = Adams River. Means ± standard deviation for each sex are reported for length, weight, and physiological variables. Length = distance from post orbital to hypural bone.

Variable	Sex	SOG	EST	MCM	PET	KAM	ADA
sample dates		Sept 4	Sept 27	Sept 23	Sept 30	Oct 7,8	Oct 17, 29
n	m	6	3	7	7	7	7
	f	6	6	8	3	7	7
Length (cm)	m	51.8 ± 2.0	48.7 ± 1.3	49.5 ± 3.2	48.9 ± 1.6	47.9 ± 1.3	49.1 ± 1.3
	f	50.7 ± 4.4	49.5 ± 1.4	48.1 ± 1.6	50.9 ± 1.7	47.3 ± 1.8	48.9 ± 1.2
Weight (kg)	m	2.9 ± 0.5	2.6 ± 0.03	2.6 ± 0.2	2.5 ± 0.2	2.6 ± 0.3	3.4 ±0.3
	f	2.6 ± 0.4	2.6 ± 0.2	2.1 ± 0.3	2.5 ± 0.1	2.2 ± 0.3	2.6 ± 0.4

Late-run Shuswap sockeye salmon in 2014 likely spent 10 – 14 days milling near the mouth of the Fraser River (CROSSIN *et al.* 2009) but how long those sampled at the SOG location had been in the area prior to collection can not be determined. Based on radio tag detections from multiple studies (Chapter 2, CROSSIN *et al.* 2009), we estimate that sockeye salmon travel from the SOG location to the EST location in 1 day, from EST to MCM in 1–2 days, from MCM to PET in 3–5 days, from PET to KAM in 11–15 days, and from KAM to ADA in 4–7 days for a total in-river migration time of 20–30 days. It was not possible to determine how long sampled fish had been at the ADA location prior to collection.

Fish were euthanized by cerebral percussion. Sterile technique was used to acquire small sections of tissue (approximately 0.3 g) from the gills, spleen, liver, heart, white muscle, and head kidney. The muscle tissue was collected at the lateral line, immediately posterior to the dorsal fin. All tissues were suspended in RNAlater® (Life Technologies, Carlsbad, CA, USA), according to the manufacturer's recommendations and after 24 hrs at 4°C were stored at −20°C for 1-2 months and then −80°C until extraction.

Only individuals from the late-run Shuswap population were selected for analysis on the BioMark platform. Because the Weaver, Harrison, and Portage populations co-migrate with the late-run Shuswap population, an adipose fin punch was collected from each fish during dissection for genetic stock identification by microsatellite analysis (performed at the Department of Fisheries and Oceans Pacific Biological Station [Nanaimo, BC]) as outlined in BEACHAM *et al.* (2004)). Genetic analyses were conducted for all individuals collected at the first four sampling locations; at sampling sites in the Thompson River and beyond it would be very unlikely to encounter fish not belonging to the late-run Shuswap population.

B.3.2 Molecular Methods

Tissue samples were screened for the presence of 44 infectious agent taxa (Table C), using HT-qPCR on the Fluidigm Biomark Dynamic Array™ microfluidics platform (Fluidigm, San Francisco, CA, USA) at the Pacific Biological Station. This platform has recently been analytically validated for quantitative infectious agent profiling in salmon tissue (MILLER *et al.* 2016) and applied to multiple studies of Pacific salmon (DI CICCO *et al.* 2017; MILLER *et al.* 2017; TEFFER *et al.* 2017). Infectious agent taxa were chosen based on knowledge of their presence in Canada or evidence of their association with disease worldwide (MILLER *et al.* 2016). Assays utilizing taqman probes (Table C) were designed to target RNA which allows the inclusion of some RNA viruses and focuses on infectious agents in an active state (MILLER *et al.* 2016).

Total RNA was extracted using methods previously described (JEFFRIES *et al.* 2014a; MILLER *et al.* 2016, 2014). Tissues were homogenized separately in TRI-reagent™ (Ambion Inc., Austin, TX, USA). Next, 1-bromo-3-chloropropane was added to the homogenate, and equal volumes of the aqueous phase from each tissue type were combined for extraction. Extractions were carried out using MagMAX™-96 for Microarrays Total RNA Isolation Kits (Ambion Inc.) with a Biomek NXP™ automated liquid-handling instrument. RNA quantity and purity was assessed by measuring the A260/A280 ratio using a Beckman Coulter DTX 880 Multimode Spectrophotometer (Brea, CA, USA).

Normalized RNA (1 µg) was reverse transcribed to cDNA using the SuperScript VILO MasterMix Kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The assay volume used for qPCR on the BioMark is small (7 nL) and therefore a pre-amplification step is recommended by the manufacturer. Thus, 1.25 µL of cDNA from each sample was pre-amplified with primer pairs corresponding to all 48 assays (45 microbes and 3 reference genes) in a 5 µL reaction volume using 1X TaqMan Preamp Master Mix (Applied Biosystems, Foster City, California) according to the BioMark protocol. Unincorporated primers were removed using ExoSAP-IT™ (Affymetrix, Santa Clara, California), and samples were diluted 1:5 in DNA Suspension Buffer (Teknova, Hollister, California).

All assays were run in duplicate. Artificial positive constructs (APC clones) corresponding to all assays were run in six serial dilutions on the dynamic array to construct a standard curve and calculate efficiency for each assay and estimate RNA copy number for each positive sample. The APC clones contained an additional probe labeled with NED™ reporter dye (Life Technologies) that allowed for the detection of vector contamination (see MILLER *et al.* 2016).

A 5 µL sample mix was prepared containing 1X TaqMan Universal Master-Mix (Life Technologies), 1X GE Sample Loading Reagent (Fluidigm PN 85000746), and amplified cDNA, which was added to each assay inlet of the array following the manufacturer's recommendations. Five µL of assay mix was prepared containing 10 µM primers (infectious agent in FAM-MGB and APC in NED-MGB) and 3 µM probes for the TaqMan assays. After loading the assays and samples into the chip using an IFC controller HX (Fluidigm), PCR was performed with the following conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Cycle threshold was determined using the BioMark Real-Time PCR analysis software. Reaction curves for each positive sample-assay combination were visually evaluated for abnormal curve shapes, close correspondence between replicates, and presence of APC contamination as indicated by NED positives. Using

scripts created in R statistical software (R CORE TEAM 2015), I calculated efficiency for each assay (standard curve method (LARIONOV *et al.* 2005)), omitted results where only one duplicate was positive for a sample-assay combination, removed NED positive samples, and averaged duplicates. In this study, the limit of detection (LOD) is defined as the estimated cycle threshold (Ct) number under which true positive results are expected 95% of the time for a given assay (MILLER *et al.* 2016). Because LOD was established for maximum compliance with OIE standards but limits the sensitivity of the BioMark to detect low-level infection, in this study I present data exceeding the LOD. Note that I only included detections beyond the LOD for infectious agents that were also detected within the LOD and infectious agents only detected beyond the LOD were considered to be false positives.

B.3.3 Statistical Analyses

Infectious agent prevalence was calculated for each sampling event as the percentage of each site's sampled fish that were positive for a given infectious agent. To make the data more approachable to those not familiar with qPCR (where a lower cycle threshold (Ct) indicates a higher quantity of genetic material), I chose to express measures of infectious agent RNA quantity as "relative load" instead of Ct, where: Relative load = 40 - Ct. Forty is significant because this is the number of PCR cycles performed by the BioMark. By reporting these data in relative load, LOD was also converted for visual representation (for example, a LOD that was 27 would be 13 after converting to relative load).

Infectious agent richness was calculated as the total number of infectious agents with a positive detection in a given individual. I tested for a significant difference in infectious agent richness among sites using Tukey's test with $\alpha = 0.05$. Given that previous studies have found sex-based differences in the survival of Pacific salmon (JEFFRIES *et al.* 2012a; MARTINS *et al.* 2011; TEFFER *et al.* 2017) I conducted t-tests to compare infectious agent relative load between sexes for each infectious agent at each site where sample size allowed ($n \geq 3$ per sex). To test for correlations between infectious agents suggestive of co-infection or a biological relationship between infectious agents, I used simple linear regression for each pair of infectious agents at each site with 6 or more positive detections. To control for Type I errors due to repeated hypothesis testing I adjusted *p* values using the False Discovery Rate (FDR) approach (BENJAMINI and HOCHBERG 1995). All analyses were performed using R statistical software (R CORE TEAM 2015).

For interpretation, each infectious agent was subjectively assigned to one of the following categories based on its pattern of prevalence and load from SOG to ADA: "increasing", "decreasing", or "neutral" (i.e. where prevalence and load were relatively level across sites).

B.4 Results

A total of 74 adult sockeye salmon were assessed over six sampling locations for 44 infectious agent taxa (Table B.1). Of those 44 taxa, a total of 19 were detected in fish at one or more of the six sampling locations (Figure B.2, Table B.2). Twelve of the 19 showed an increasing pattern of prevalence and load, three were decreasing, and four were neutral (Figure B.2).

Infectious agent richness ranged from 3 at the marine location (SOG) to 12 at the spawning area (ADA)

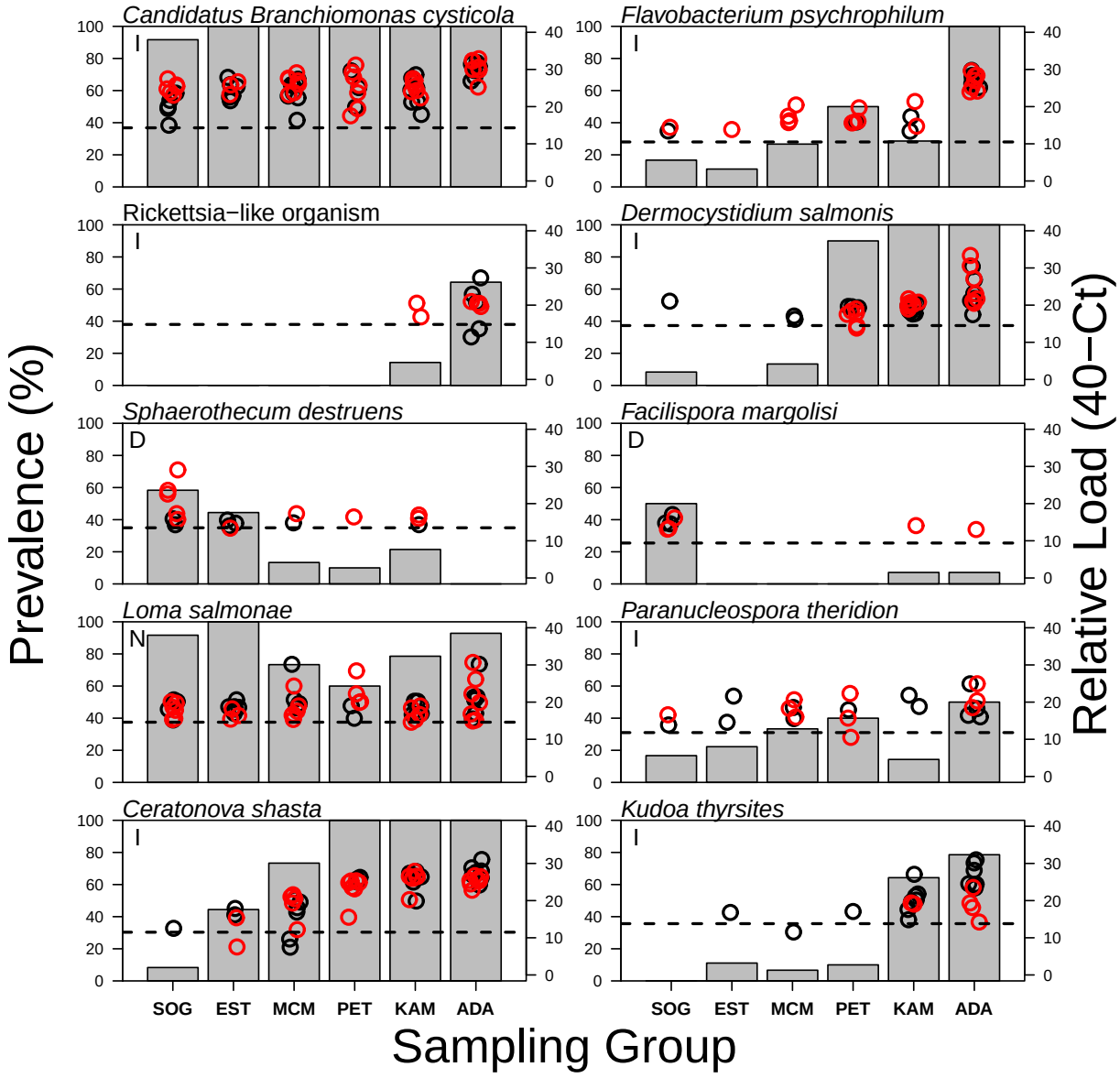

Figure B.2: Bar plots of infectious agent prevalence (gray bars, primary y-axis) overlaid with jitter plots of relative load (open points, secondary y-axis) for infectious agents in sockeye salmon at SOG = Strait of Georgia, EST = Fraser River estuary, MCM = Fraser River at McMillan Island, PET = Fraser River at Peters Road, KAM = Thompson River downstream of Kamloops Lake, ADA = Adams River. The horizontal dashed line depicts assay specific limit of detection. Relative load is shown for each individual by sex (black=female, red=male). A letter in the upper left corner of each plot indicates how the infectious agent was categorized (I = increasing, D = decreasing, N = neutral).

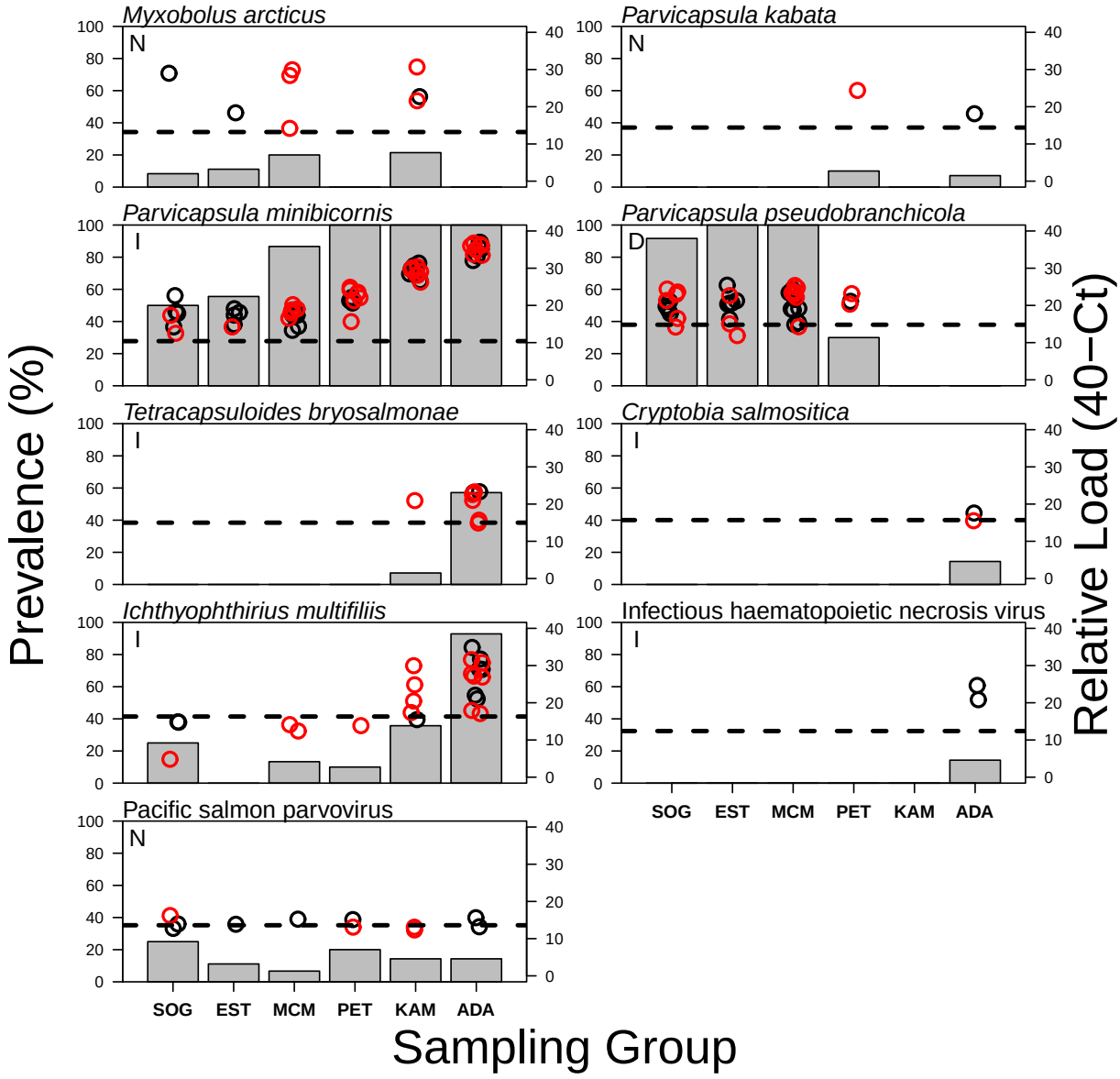

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Table B.2: Infectious agent taxa assayed in late-run Shuswap sockeye salmon (2014, Fraser River, BC) using the BioMark HT-qPCR platform. For primer and probe sequences, see supplementary material. Infectious agents mentioned by KENT (2011) are labeled with their suggested risk level to Fraser River sockeye salmon (H = high, M = moderate, L = low, U = unknown). The prevalence of infectious agents detected in a multi-year, multi-population study of Fraser River sockeye salmon (MILLER *et al.* 2014) is provided (based on liver and gill tissue only). Prevalence for each site in this study is provided, with limit of detection applied. Hyphens indicate that a given infectious agent was not mentioned or assayed, single zeroes indicate that an infectious agent was assayed but not detected.

Infectious Agent Category	Scientific Name	Assessed risk in KENT (2011)	Prevalence (%) in MILLER *et al.* (2014)			Prevalence across sites (%)					
			ocean	river	spawning	SOG	EST	MCM	PET	KAM	ADA
Bacteria	*Aeromonas hydrophila*	–		–		0	0	0	0	0	0
	Aeromonas salmonicida	H		–		0	0	0	0	0	0
	'*Candidatus* Branchiomonas cysticola'	–		–		92	100	100	100	100	100
	Flavobacterium psychrophilum	M	2	0	65	17	11	27	50	29	100
	Gill Chlamydia	–	0	0	0	0	0	0	0	0	0
	Candidatus Piscichlamydia salmonis'	–		–		0	0	0	0	0	0
	Piscirickettsia salmonis	L		–		0	0	0	0	0	0
	Renibacterium salmoninarum	H		–		0	0	0	0	0	0
	Rickettsia-like organism	–		–		0	0	0	0	14	50
	Vibrio anguillarum	H		–		0	0	0	0	0	0
	Vibrio salmonicida	–		–		0	0	0	0	0	0
Mesomycetozoa	*Dermocystidium salmonis*	L		–		8	0	13	70	100	100
	Ichthyophonus hoferi	M		–		0	0	0	0	0	0
	Sphaerothecum destruens	L		–		58	33	13	10	21	0
Microsporidian	*Facilispora margolisi*	–		–		50	0	0	0	7	7
	Loma salmonae	L		–		92	100	73	60	71	93
	Nucleospora salmonis	L	14	18	17	0	0	0	0	0	0
	Paranucleospora theridion (syn. *Desmozoon lepeophtherii*)	–	0	0	3	17	22	33	30	14	50
Myxozoa	*Ceratonova shasta*	L		–		8	33	60	100	100	100
	Kudoa thyristes	L	0	0	3	0	11	0	10	64	79
	Myxobolus arcticus	L		–		8	11	20	0	21	0
	Myxobolus cerebalis	–		–		0	0	0	0	0	0
	Myxobolus insidiosus	–		–		0	0	0	0	0	0
	Parvicapsula kabatai	–		–		0	0	0	10	0	7
	Parvicapsula minibicornis	H		–		50	56	87	100	100	100
	Parvicapsula pseudobranchicola	–	0	0	0	83	89	93	30	0	0
	Tetracapsuloides bryosalmonae	M		–		0	0	0	0	7	50
Platyhelminthes	*Gyrodactylus salaris*	–		–		0	0	0	0	0	0
	Nanophyetus salmincola	–		–		0	0	0	0	0	0
Protozoa	*Cryptobia salmositica*	M		–		0	0	0	0	0	7
	Ichthyophthirius multifiliis	H		–		0	0	0	0	29	93
	Neoparamoeba perurans	–		–		0	0	0	0	0	0
	Spironucleus salmonicida	–		–		0	0	0	0	0	0
Viruses	Atlantic Salmon Paramyxovirus	–		–		0	0	0	0	0	0
	Infectious hematopoietic necrosis virus	H	2	0	5	0	0	0	0	0	14
	Infectious pancreatic necrosis virus	L		–		0	0	0	0	0	0
	Pacific salmon parvovirus	U	37	30	21	17	11	7	10	0	7
	Piscine myocarditis virus	–		–		0	0	0	0	0	0
	Piscine reovirus	–	0	6	0	0	0	0	0	0	0
	Salmon alphavirus	–		–		0	0	0	0	0	0
	Salmonid herpesvirus	–		–		0	0	0	0	0	0
	Viral erythrocytic necrosis virus	L		–		0	0	0	0	0	0
	Viral hemorrhagic septicemia virus	L		–		0	0	0	0	0	0
	Viral encephalopathy and retinopathy virus	–	2	0	0	0	0	0	0	0	0

(Figure B.3). Richness was not significantly different between the first four locations but richness at KAM was significantly greater than SOG and EST, and richness at the spawning area (ADA) was significantly greater than all other locations ($p = < 0.001$, Figure B.3).

Following FDR correction, there were no infectious agent by sex and site comparisons that were significantly different. The strongest difference between sexes was for *Kudoa thyrsites* which was 7.7 ± 5.7 relative Ct higher in females than males at the ADA site (unadjusted $p = 0.02$). The only statistically significant pairwise correlation between infectious agent taxa was between *Ichthyophthirius multifiliis* and Rickettsia-like organism at the ADA site ($\beta = 0.89$, adjusted $p = 0.01$).

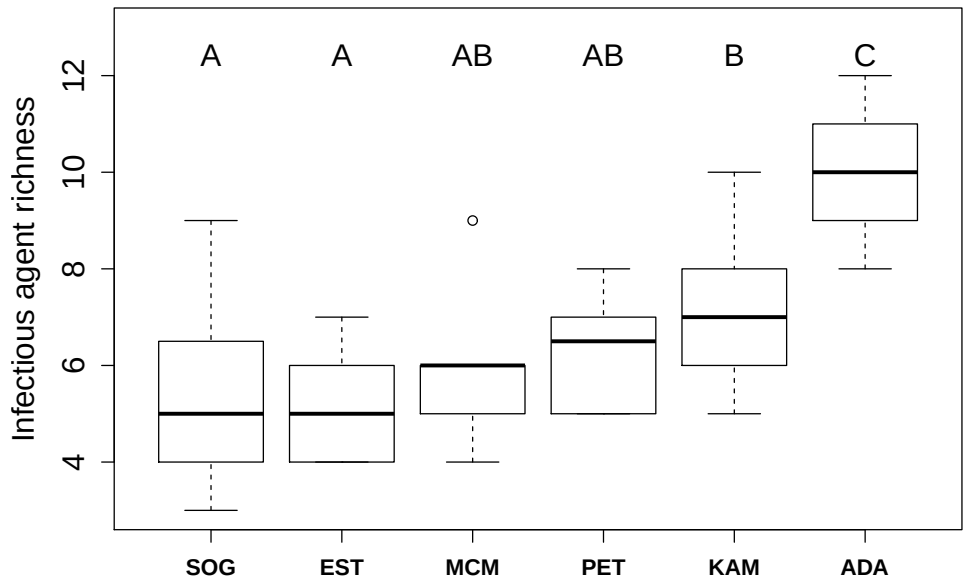

Figure B.3: Boxplots showing differences in infectious agent richness between sampling lcoations. Heavy black line indicates the median, the box represents the first and third quartiles, the whiskers represent 1.5 times the inter-quartile range, and the circles represent outliers. SOG = Strait of Georgia, EST = Fraser River estuary, MCM = Fraser River at McMillan Island, PET = Fraser River at Peters Road, KAM = Thompson River downstream of Kamloops Lake, ADA = Adams River. Significant differences for pairwise comparisons (Tukey's Test) are indicated when letters are not shared between groups.

B.5 Discussion

This study provides important baseline prevalence and load data for a broad range of infectious agent taxa at multiple locations along the spawning migration of the Fraser River's largest sockeye salmon population complex. With positive detections for six infectious agent taxa seldom or never documented in Fraser River salmon, my results considerably expand the list of infectious agents known to occur in sockeye salmon

migrating through this important river. These additions to the understanding of infectious agent taxa in a single sockeye salmon population emphasizes the need for more (and more frequent) baseline studies that cover a wide breadth of infectious agents in wild salmon.

This study included assays for 21 of the 24 infectious agent taxa described in KENT (2011), and I detected 13 of those 21. I detected six infectious agent taxa (*Flavobacteria psychrophilum*, *Sphaerothecum destruens*, *Dermocystidium salmonis*, *Cryptobia salmositica*, *Ceratonova shasta*, and *Tetracapsuloides bryosalmonae*) that were considered in the report to be very rare or non-existent in Fraser River sockeye salmon (KENT 2011). Of these, all but *C. salmositica* were detected in $> 50\%$ of the population at their most prevalent locations. While KENT (2011) rated *C. shasta* as "low risk" due to no previous knowledge of its occurrence in these fish, this infectious agent has since been associated with mortality in a study featuring a population of Fraser River sockeye salmon (TEFFER *et al.* 2017). In addition, I detected six infectious agent taxa not mentioned in KENT (2011), ('*Candidatus* Branchiomonas cysticola', Rickettsia-like organism, *Facilispora margolisi*, *Paranucleospora theridion*, *Parvicapsula kabata*, and *Parvicapsula pseudobranchicola*), several of which were first described since 2011. This study updates the list of known taxa infecting Fraser River sockeye salmon compiled by KENT (2011), contributing to a new baseline for future monitoring and research.

The prevalence and load of 12 infectious agent taxa were consistent with the "increasing" pattern of infection, culminating with highest prevalence and loads at the spawning area (ADA). In accordance, infectious agent richness was significantly greater at spawning grounds compared to every other sampling location, corroborating the findings of MILLER *et al.* (2014). Since spawning grounds are the final location successfully migrating Pacific salmon will inhabit, and where senesence dramatically accelerates, this result is not surprising. The potential for infectious agent transmission is elevated on spawning grounds due to the high densities of conspecifics and the relatively low volume of water (ALTIZER *et al.* 2011; RENO 1998). There is evidence that sexual maturity impacts immune function (SLATER and SCHRECK 1993) and both innate and adaptive immunity are known to decline at spawning, potentially as a means of reallocating resources towards spawning activities and away from defense against pathogens (DOLAN *et al.* 2016; MILLER *et al.* 2009). Therefore, a natural pattern of increased infectious agent prevalence and load is expected across all Pacific salmon spawning grounds.

Three infectious agent taxa, *S. destruens, F. margolisi, P. pseudobranchicola*, displayed the "decreasing" pattern, potentially suggestive of a marine origin followed by a decline in prevalence and load in freshwater. In this study, I was unable to determine whether this decline in prevalence was a result of clearance of the infectious agent by a successful host immune response, an inability of the infectious agent to persist in the freshwater environment, or infectious agent-associated mortality. However, if one expects the prevalence reported here to accurately reflect the larger population, the latter explanation seems unlikely as 50 - 90% (depending on the infectious agent taxa) of the fish sampled here were positive for these "marine-oriented" infectious agent taxa at the SOG site.

Four infectious agent taxa, *Myxobolus arcticus, Loma salmonae, Parvicapsula kabata* and Pacific salmon parvovirus, demonstrated the "neutral" pattern of relatively little change in prevalence and load. This pattern could be indicative of infectious agents that invade salmon at an early life stage and remain relatively stable across subsequent life stages (e.g. *M. arcticus*) or those that are contracted in marine environments and inflict

mortality at a certain infection intensity (potentially *L. salmonae*, (MILLER *et al.* 2014)). As I observed interesting patterns for specific infectious agent taxa, the remainder of this discussion focuses on findings in each of the broad groups of infectious agents.

B.5.1 Bacteria

The three bacteria species detected, '*Candidatus* Branchiomonas cysticola', *Flavobacterium psychrophilum*, and Rickettsia-like organism, all presented greatest prevalence and load on spawning grounds. *Ca.* B. cysticola was present in nearly every salmon we collected (only one negative), consistent with previous studies on other salmon populations (GUNNARSSON *et al.* 2017; TEFFER *et al.* 2017, Appendix A). While this bacteria has been associated with gill epitheliocysts in Norway (MITCHELL *et al.* 2013), no studies to date have found it to be pathogenic in salmon (GUNNARSSON *et al.* 2017; TEFFER *et al.* 2017).

Flavobacterium psychrophilum was present at all sampling sites but prevalence was 50% or lower until the spawning grounds, where all fish were infected and loads were two orders of magnitude greater than at any other site (similar to results of MILLER *et al.* (2014)). Salmon on spawning grounds are highly susceptible to bacterial infection, as demonstrated by a decline in the bacterial killing components in plasma (DOLAN *et al.* 2016). Recent evidence suggests that, under laboratory conditions, high loads of *F. psychrophilum* were associated with mortality in sockeye salmon sampled early in their migration (TEFFER *et al.* 2017). This could explain why prevalence and load were relatively level until spawning grounds - high load individuals may have been lost from the population. It is unlikely that colder temperatures on spawning ground favored *F. psychrophilum* growth and spread since, according to HOLT *et al.* (1989), optimal water temperatures for this bacteria occurred prior to arrival on spawning grounds (12–15°C, Chapter 2). However, high densities of salmon at the spawning area would be conducive to bacterial transmission (RENO 1998).

Rickettsia-like organism, the agent associated with strawberry disease in *Onchorhynchus mykiss* (LLOYD *et al.* 2011), was primarily prevalent on spawning grounds. This bacteria is an endosymbiont of *Ichthyophthirius multifiliis*, and is found within that infectious agent's cytoplasm (SUN *et al.* 2009). Recent evidence suggests that some form of rickettsia bacteria is always present in *I. multifiliis* (ZAILA *et al.* 2017). Correspondingly, we observed a strong positive correlation between the two infectious agents at the ADA site (where they were both most prevalent), as observed by TEFFER *et al.* (2017).

The three bacteria rated by KENT (2011) as presenting the highest risk to Fraser River sockeye salmon, *Aeromonas salmonicida*, *Vibrio anguillarum*, and *Renibacterium salmoninarum*, were not dected in this study.

B.5.2 Mesomycetozoa

The two detected species of mesomycetozoa presented opposing patterns of infection, with *Dermocystidium salmonis* demonstrating an increasing pattern and *Sphaerothecum destruens* showing a decreasing pattern. The increasing pattern is consistent with our current understanding of *D. salmonis*, which is known as a freshwater pathogen (OLSON *et al.* 1991; OLSON and HOLT 1995). Free-swimming zoospores of this species are sloughed from gill cysts of infected salmon and can infect other fish in freshwater (OLSON *et al.* 1991).

Although *S. destruens* was first associated with salmon mortality in the marine environment (HARRELL *et al.* 1986), it is currently associated with epizootics in Europe across multiple species of freshwater fish (ANDREOU and GOZLAN 2016). In contrast, the infectious agent was most prevalent in the marine environment in this study. The decline in prevalence seen as sockeye salmon migrate upstream is reminiscent of pathogen induced mortality (LESTER 1984), especially with high loads only observed at the first sampling location. As this infectious agent is known to cause mortality, and was 58% prevalent in the marine location but absent on spawning grounds, we believe that further research into its transmission dynamics in the marine environment and pathogenicity for sockeye salmon is warranted.

B.5.3 Microsporidia

All three microsporidian species present in this study have been considered to have a marine origin. Both *Facilospora margolisi* and *Paranucleospora theridion* are transmitted between salmon by sea lice, *Lepeoptheirus salmonis* (JONES *et al.* 2012). *Loma salmonae* is transmitted horizontally or through consumption of infected fish (SHAW *et al.* 1998).

Since little information exists regarding the life history and pathogenicity of *F. margolisi*, it is difficult to interpret our observation that this infectious agent was present in the marine environment and rarely elsewhere. I found a similar scenario in Chinook salmon, *O. tshawytscha* (Appendix A). It may be possible that this infectious agent does not persist in freshwater.

Loma salmonae displayed a neutral pattern of prevalence and load when compared to other taxa. MILLER *et al.* (2014) found that *L. salmonae* was associated with the mortality of Chilko River, but not late-run Shuswap sockeye salmon. In this study, an increase in variability and decrease in prevalence for *L. salmonae* following freshwater entry (MCM and PET sites) could represent pathogen-associated mortality, although this is only speculative given the scope of these data. Mortality observed by MILLER *et al.* (2014) for *L. salmonae* positive Chilko River sockeye salmon was high roughly four to seven days after freshwater entry, which would correspond to when salmon would be passing through the MCM and PET sites. However, sockeye salmon captured shortly beyond the PET location and held in tanks where migration temperatures were simulated showed a neutral pattern of *L. salmonae* load in mortalities throughout the study period (TEFFER *et al.* 2017). Our findings, coupled with those of MILLER *et al.* (2014) warrant further investigation of *L. salmonae*-mediated mortality in migrating salmon.

Paranucleospora theridion was recently identified as the most important infectious agent taxa (out of four studied) associated with gill disease among marine netpen Atlantic salmon, *Salmo salar*, in Norway (GUNNARSSON *et al.* 2017). In GUNNARSSON *et al.* (2017), the infectious agent was ubiquitous in the marine environment, but in Fraser River sockeye salmon, we found marine prevalence at only 17% with a gradual increase to 50% on spawning grounds. Prevalence was lowest at the KAM site (14%), which is located at the end of a long section of hydraulic challenges that starts after the PET site (HINCH *et al.* 2002). Individuals infected with *P. theridion* might be unable to negotiate this section of the migration as the associated gill disease could preclude elevated respiratory demands (POWELL *et al.* 2005).

Based on previous studies by our group (JEFFRIES *et al.* 2014a; MILLER *et al.* 2014), I was surprised that we did not detect *Nucleospora salmonis*. This microsporidian has been detected at 15–20% prevalence

in dominant cycle late-run Shuswap sockeye in marine and freshwater environments, and in the parental population for the late-Shuswap sockeye salmon in this study, sampled at the same spawning area (MILLER *et al.* 2014). MILLER *et al.* (2014) stated that the infections were at low "carrier" levels, and it is possible that by pooling multiple tissues (MILLER *et al.* (2014) tested gill and liver individually), I diluted *N. salmonis* RNA to the degree that it was undetectable.

B.5.4 Myxozoa

Seven myxozoan species were detected in this study, although both *Myxobolus arcticus* and *Parvicapsula kabata* were detected at relatively low prevalence ($< 10\%$). *Tetracapsuloides bryosalmonae*, a species which has an obligate life stage parasitizing freshwater bryozoans was only detected in the subnatal river and on spawning grounds. This may reflect the location of infected bryozoans throughout the Fraser River watershed. While surveying surface waters in Eastern Canada, RICCIARDI and REISWIG (1994) found that *Fredericella indica*, the species of bryozoan associated with the presence of *T. bryosalmonae* on Vancouver Island (OKAMURA and WOOD 2002), was primarily located in the littoral zone and outflow of oligotrophic lakes. Late-run shuswap sockeye salmon encounter an oligotrophic lake immediately upstream of the KAM location, and swim through two more before arriving at the ADA location. While BRADEN *et al.* (2010) confirmed *T. bryosalmonae* infections in spawning pink salmon (*O. gorbuscha*) collected from Vancouver Island, they found 0% prevalence of the myxozoan in 40 adult Fraser River sockeye salmon. However, the authors did not report the collection location of the sockeye salmon samples, and our results indicate that *T. bryosalmonae* prevalence varies dramatically along a sockeye salmon migration.

In contrast, *Manayunkia speciosa*, the intermediate host of both *Parvicapsula minibicornis* and *Ceratonova shasta* (BARTHOLOMEW *et al.* 2006), was found throughout the mainstream Klamath River (California, USA) in low velocity areas with silty substrate (STOCKING and BARTHOLOMEW 2007), similar to habitats found in the lower Fraser River. This distribution is reflected in the "freshwater-associated" prevalence of *P. minibicornis* and *C. shasta*. An interesting contrast between these two species is that while *P. minibicornis* relative load increased steadily to the spawning grounds, *C. shasta* relative load plateaued from the PET sampling location to the spawning grounds. This may be indicative of a threshold above which high loads of *C. shasta* caused mortality. This hypothesis is supported by the findings of TEFFER *et al.* (2017) where early mortality was associated with high loads of *C. shasta* while *P. minibicornis* load increased steadily for the duration of the experiment. *C. shasta* is an OIE (World Organisation for Animal Health) reportable agent, with a demonstrated potential to cause high levels of mortality in freshwater, especially in juvenile salmonids (HALLETT and BARTHOLOMEW 2012).

The prevalence of *P. minibicornis* at the marine sampling site (50%, SOG) is consistent with data from MILLER *et al.* (2014) but indicates earlier infection than was found by ST-HILAIRE *et al.* (2002) and JONES *et al.* (2003), who first detected *P. minibicornis* in kidney tissue at 200 and 440 km upstream from the Fraser River mouth, respectively. The inclusion of multiple tissues and/or the sensitivity of the BioMark platform, probably led to the earlier detections in this study. Our observations, as well as those in Appendix A, indicate that adult salmon approaching the Fraser River either encounter infective actinospores in the coastal marine environment, or temporarily enter freshwater habitats where they are exposed to actinospores.

Disease and mortality induced by *P. minibicornis* infection is a function of degree days in the river post-exposure (WAGNER *et al.* 2005) and this infectious agent has been implicated in sockeye salmon prespawn mortality (BRADFORD *et al.* 2010b). With warming conditions and unusual migration timings (e.g. early river entry of some late-run sockeye salmon), impacts from this parasite may increase.

Parvicapsula pseudobranchicola demonstrated a marine-associated pattern in prevalence with a rapid decrease occurring in the Lower Fraser River. In Norway, it has been suggested that although this infectious agent was first identified in Atlantic salmon, the primary host is actually seatrout, *Salmo trutta* (HANSEN *et al.* 2015). Recently, NYLUND *et al.* (2018) demonstrated that this infectious agent was cleared from the pseudobranchs of Atlantic salmon smolts, likely by disrupting and exiting the tissue, 6-8 months after the fish were placed in seawater. This study also revealed that fish had acquired immunity to *P. pseudobranchicola* by the following year. If late-run Shuswap sockeye salmon have been previously exposed to *P. pseudobranchicola* as smolts, as shown in MILLER *et al.* (2014), and are re-exposed close to river entry, for example, (the unknown invertebrate vector of *P. pseudobranchicola* could reside in estuaries, as hypothesized by HANSEN *et al.* (2015)), the pattern I observed could be due to rapid clearing of the infectious agent via acquired immunity.

Kudoa thyrsites, a myxozoan considered non-pathhogenic but commercially important, infects penned Atlantic salmon in British Columbia approximately a month after placement in seawater (MARSHALL *et al.* 2016). However, in our study, *K. thyrsites* displayed a pattern of increasing prevalence and load throughout the sockeye salmon migration. This was also the case in a recent study of infectious agents found in adult Chinook salmon (Appendix A) and is likely a result of the over-dispersed distribution of this infectious agent in muscle tissue. When *K. thyrsites* is at low loads in a host, multiple skin samples are required to ensure detection of this agent, due to its discontinuous distribution (FUNK *et al.* 2007). However, as the infection progresses (as individuals swim upstream), the likelihood of encountering this infectious agent in a given sample increases. Although not significant after FDR correction, *K. thyrsites* had the strongest difference in load between sexes for all infectious agent taxa, consistent with other recent studies (Appendix A, TEFFER *et al.* 2017).

B.5.5 Protozoa

Cryptobia salmositica, a blood-borne protozoan, was only detected in two samples at spawning grounds. It is likely that this location provided the only suitable habitat for the primary vector of *C. salmositica*, the Pacific salmon leech, *Piscicola salmositica* (BECKER and KATZ 1965). *Ichthyopthirius multifiliis* showed a freshwater-associated pattern of prevalence with a sudden strong increase in prevalence and load on spawning grounds. TRAXLER *et al.* (1998) observed a similar pattern of infection on spawning grounds of the Babine River (where significant prespawn mortality occurred). The authors attributed high prevalence of *I. multifiliis* in that system to transfer of the infectious agent from a resident fish species to high densities of sockeye salmon corralled behind spawning channel weirs (TRAXLER *et al.* 1998). Together, these results suggest that although *I. multifiliis* may be present earlier in the migration, spawning areas provide appropriate conditions for higher prevalence and load.

B.5.6 Viruses

Only two viruses were detected and their prevalence was relatively low, as has been the case in other recent studies of adult Pacific salmon in the Fraser River (Appendix A; MILLER *et al.* 2014; TEFFER *et al.* 2017), (but see MORTON *et al.* 2017). Infectious haematopoietic necrosis virus (IHNV) was only detected at low prevalence on spawning grounds. Sampling adults across freshwater and marine habitat, MULCAHY *et al.* (1984) also found IHNV first detected on spawning grounds but TRAXLER *et al.* (1997) later found positive detections in adults in the ocean and proposed a marine reservoir for the virus. Since those studies, invertebrate vectors and a wide range of fish species that can carry IHNV have been identified (DIXON *et al.* 2016). The transmission and vectors of the virus in natal habitats remain unknown, but it is possible that juvenile Chinook salmon, rainbow trout (*O. mykiss*), and returning adults themselves are vectors in the spawning area (ADA) (BREYTA *et al.* 2017).

Pacific salmon parvovirus was first described by MILLER *et al.* (2014) and its pathogenic potential is currently unknown. As in the Chilko Lake population in MILLER *et al.* (2014), I observed prevalence around 20% throughout the spawning migration with similar loads to those reported. Higher prevalence levels of this virus are observed at juvenile and smolt stages throughout BC (Miller, unpublished data), hence the lower infection rates in returning adults may reflect acquired immunity. The lower prevalence seen in this study compared to MILLER *et al.* (2014) (Table E.1) is likely due to dilution from pooling tissues (currently, PSPV only known to occur in liver tissue). Since so little is known regarding this virus, further research is warranted.

Based on previous regional work (MILLER *et al.* 2014), I was surprised at the lack of detections of piscine orthoreovirus (PRV) within my samples. While not associated with en route mortality like in the Chilko River population, PRV was present in the late-run Shuswap population in 2010 (MILLER *et al.* 2014), the brood year for the 2014 samples, and those samples were non-lethal gill, a less sensitive sampling approach in comparison to mixed tissue TEFFER *et al.* (2017). It was also detected in 1.7% of 60 sockeye salmon sampled from the Fraser River in September 2013 (MARTY *et al.* 2015). While the aforementioned studies found PRV in single tissue samples, and pooling tissues could decrease sensitivity, we have previously detected PRV in pooled tissue of adult salmon (Appendix A).

Recently, MORTON *et al.* (2017) reported prevalence of PRV at 40% in ocean sampled salmonids and 20% in those sampled in the Fraser River in 2012-13. However, none of the salmonids sampled by MORTON *et al.* (2017) in the ocean areas that overlapped with this study were adult sockeye salmon. Furthermore, the occurrence of PRV in the Fraser River was limited to high prevalence at two sampling locations and not a single detection at six others (MORTON *et al.* 2017). This suggests a patchy distribution for the infectious agent in freshwater, which my sampling approach might have failed to detect.

B.5.7 Study limitations

While this study provides a unique account of infectious agent dynamics over a relatively detailed spatial scale, it must be noted that this was based on a small number of samples (9–15) per location. However, these numbers were sufficient for detecting a number of infectious agents not previously detected in Fraser River sockeye, and observing clear patterns in infectious agent prevalence and load. On a similar note, the results

presented here are from a single year of sampling. The lack of detections for both *N. salmonis* and PRV, infectious agents previously detected in this population, demonstrate the shortcomings of a study with such a temporal limitation.

While this study provides one of the broadest accounts of infectious agent taxa in a population of Pacific salmon, samples were only drawn from a single population of salmon. Since infectious agent prevalence and load can differ between populations with overlapping migrations (MILLER *et al.* 2014), it is important to exercise caution when applying the results from this study to other Fraser River populations and those further afield.

Although testing multiple tissues individually might be the ideal approach for infectious agent surveys, it is time consuming and expensive. Due to the multi-target nature of this molecular analysis, I chose to pool multiple tissues to maximize the likelihood of detecting infectious agents within our budgetary means. In the case of an infectious agent only found in one or two tissues and occurring at low loads, pooling multiple tissues could dilute the infectious agent to a point where false negatives occur.

Finally, pairing the molecular data in my study with histological samples would have been ideal. While I am confident that positive detections in this study represent the presence of infectious agent RNA, I am unable to relate these data to actual disease without histological or physiological data.

B.5.8 Conclusion

By sampling the late-run Shuswap sockeye salmon population across multiple locations during the freshwater migration, this study provides unique information illustrating the infection dynamics of 19 taxa found in Pacific salmon. I identified multiple infectious agents that, in light of our results here and those from previous studies, may warrant further investigation since they may mediate mortality for migrating Fraser River sockeye salmon: *C. shasta, S. destruens, F. psychrophilum*, and *L. salmonae*. I suggest that more baseline monitoring of the occurrence of infectious agents is needed for wild Pacific salmon, so that their role in salmon mortality and their life histories can be better understood.

Appendix C

Table of infectious agent assays

Table C.1: Taqman assays run for 45 microparasites and 3 host reference genes in 2013 Chinook salmon mixed-tissue samples using the Fluidigm Biomark HT-qRT-PCR platform (DFO Pacific Biological Station, Nanaimo, BC). Microparasites are categorized here based on the most current taxonomic assignment. Below the limit of detection Ct value, positive samples are detected 95% of the time.

Scientific Name	Type	Limit of Detection (95% detected $<$ Ct)	Forward Primer Sequence (5'-3') Reverse Primer Sequence (5'-3') Probe Sequence (FAM-5'-3'-MGB)
			F: ACCGCTGCTCATTACTCTGATG
Aeromonas hydrophila	bacteria	28.7	R: CCAACCCAGACGGGAAGAA
			P: TGATGGTGAGCTGGTTG
			F: TAAAGCACTGTCTGTTACC
Aeromonas salmonicida	bacteria	25.6	R: GCTACTTCACCCTGATTGG
			P: ACATCAGCAGGCTTCAGAGTCACTG
			F: AATACATCGGAACGTGTCTAGTG
'*Candidatus* Branchiomonas cysticola'	bacteria	25.7	R: GCCATCAGCCGCTCATGTG
			P: CTCGGTCCCAGGCTTTCCTCTCCCA
			F: GATCCTTATTCTCACAGTACCGTCAA
Flavobacterium psychrophilum	bacteria	29.5	R: TGTAAACTGCTTTTGCACAGGAA
			P: AAACACTCGGTCGTGACC
			F: GGGTAGCCCGATATCTTCAAAGT
Gill Chlamydia	bacteria	27.9	R: CCCATGAGCCGCTCTCTCT
			P: TCCTTCGGGACCTTAC
			F: TCACCCCCAGGCTGCTT
'*Candidatus* Piscichlamydia salmonis'	bacteria	23.3	R: GAATTCCATTTCCCCCTCTTG
			P: CAAAACTGCTAGACTAGAGT
			F: TCTGGGAAGTGTGGCGATAGA
Piscirickettsia salmonis	bacteria	23.3	R: TCCCGACCTACTCTTGTTTCATC
			P: TGATAGCCCCGTACACGAAACGGCATA
			F: CAACAGGGTGGTTATTCTGCTTTC
Renibacterium salmoninarum	bacteria	25.9	R: CTATAAGAGCCACCAGCTGCAA
			P: CTCCAGCGCCGCAGGAGGAC
			F: GGCTCAACCCAAGAACTGCTT
Rickettsia-like organism	bacteria	25.2	R: GTGCAACAGCGTCAGTGACT
			P: CCCAGATAACCGCCTTCGCCTCCG
			F: CCGTCATGCTATCTAGAGATGTATTTGA
Vibrio anguillarum	bacteria	26.4	R: CCATACGCAGCCAAAAATCA
			P: TCATTTCGACGAGCGTCTTGTTCAGC
			F: GTGTGATGACCGTTCCATATTT
Vibrio salmonicida	bacteria	25.8	R: GCTATTGTCATCACTCTGTTTCTT
			P: TCGCTTCATGTTGTGTAATTAGGAGCGA
			F: TGCCGCGTGTGTGAAGAA
Yersinia ruckeri	bacteria	25.8	R: ACGGAGTTAGCCGGTGCTT
			P: AATAGCACTGAACATTGAC
			F: CAGCCAATCCTTTCGCTTCT
Dermocystidium salmonis	mesomycetozoean	25.5	R: GACGGACGCACACCACAGT
			P: AAGCGGCGTGTGCC
			F: GTCTGTACTGGTACGGCAGTTTC
Ichthyophonus hoferi	mesomycetozoean	24.2	R: TCCCGAACTCAGTAGACACTCAA
			P: TAAGAGCACCCACTGCCTTCGAGAAGA
			F: GGGTATCCTTCCTCTCGAAATTG
Sphaerothecum destruens	mesomycetozoean	26.5	R: CCCAAACTCGACGCACACT
			P: CGTGTGCGCTTAAT
			F: AGGAAGGAGCACGCAAGAAC
Facilispora margolisi	microsporidian	30.6	R: CGCGTGCAGCCCAGTAC
			P: TCAGTGATGCCCTCAGA
			F: GGAGTCGCAGCGAAGATAGC
Loma salmonae	microsporidian	25.4	R: CTTTTCCTCCCTTTACTCATATGCTT
			P: TGCCTGAAATCACGAGAGTGAGACTACCC
			F: GCCGCAGATCATTACTAAAAACCT

Nucleospora salmonis	microsporidian	26.1	R: CGATCGCCGCATCTAAACA P: CCCCGCGCATCCAGAAATACGC F: CGGACAGGGAGCATGGTATAG
Paranucleospora theridion (syn. *Desmozoon lepeophtherii*)	microsporidian	28.2	R: GGTCCAGGTTGGGTCTTGAG P: TTGGCGAAGAATGAAA F: CCAGCTTGAGATTAGCTCGGTAA
Ceratonova shasta	myxozoan	28.5	R: CCCCGGAACCCGAAAG P: CGAGCCAAGTTGGTCTCTCCGTGAAAAC F: TGGCGGCCAAATCTAGGTT
Kudoa thyristes	myxozoan	26.2	R: GACCGCACACAAGAAGTTAATCC P: TATCGCGAGAGCCGC F: TGGTAGATACTGAATATCCGGGTTT
Myxobolus arcticus	myxozoan	26.8	R: AACTGCGCGGTCAAAGTTG P: CGTTGATTGTGAGGTTGG F: GCCATTGAATTTGACTTTGGATTA
Myxobolus cerebalis	myxozoan	26.2	R: ACCATTCATGTAAGCCCGAACT P: TCGAAGCCTTGACCATCTTTTGGCC F: CCAATTTGGGAGCGTCAAA
Myxobolus insidiosus	myxozoan	26.4	R: CGATCGGCAAAGTTATCTAGATTCA P: CTCTCAAGGCATTTAT F: CGACCATCTGCACGGTACTG
Parvicapsula kabatai	myxozoan	25.6	R: ACACCACAACTCTGCCTTCCA P: CTTCGGGTAGGTCCGG F: AATAGTTGTTTGTCGTGCACTCTGT
Parvicapsula minibicornis	myxozoan	29.6	R: CCGATAGGCTATCCAGTACCTAGTAAG P: TGTCCACCTAGTAAGGC F: CAGCTCCAGTAGTGTATTTCA
Parvicapsula pseudobranchicola	myxozoan	25.2	R: TTGAGCACTCTGCTTTATTCAA P: CGTATTGCTGTCTTTGACATGCAGT F: GCGAGATTTGTTGCATTTAAAAAG
Tetracapsuloides bryosalmonae	myxozoan	25.0	R: GCACATGCAGTGTCCAATCG P: CAAAATTGTGGAACCGTCCGACTACGA F: CGATCGTCACTCGGAATCG
Gyrodactylus salaris	platyhelminthes	26.4	R: GGTGGCGCACCTATTCTACA P: TCTTATTAACCAGTTCTGC F: GATCTGCATTTGGTTCTGTAACA
Nanophyetus salmincola	platyhelminthes	24.3	R: CCAACGCCACAATGATAGCTATAC P: TGAGGCGTGTTTTATG F: TCAGTGCCTTTCAGGACATC
Cryptobia salmositica	protozoan	24.3	R: GAGGCATCCACTCCAATAGAC P: AGGAGGACATGGCAGCCTTTGTAT F: AAATGGGCATACGTTTGCAAA
Ichthyophthirius multifiliis	protozoan	23.7	R: AACCTGCCTGAAACACTCTAATTTTT P: ACTCGGCCTTCACTGGTTCGACTTGG F: GTTCTTTCGGGAGCTGGGAG
Neoparamoeba perurans	protozoan	25.4	R: GAACTATCGCCGGCACAAAAG P: CAATGCCATTCTTTTCGGA F: GCAGCCGCGGTAATTCC
Spironucleus salmonicida	protozoan	26.1	R: CGAACTTTTTAACTGCAGCAACA P: ACACGGAGAGTATTCT F: CCCATATTAGCAAATGAGCTCTATCTT
Atlantic Salmon Paramyxovirus	virus	26.2	R: CGTTAAGGAACTCATCATTGAGCTT P: AGCCCTTTTGTTCTGC F: AGAGCCAAGGCACTGTGCG
Infectious hematopoietic necrosis virus	virus	27.6	R: TTCTTTGCGGCTTGGTTGA P: TGAGACTGAGCGGGACA F: GCAACTTACTTGAGATCCATTATGCT
Infectious pancreatic necrosis virus	virus	27.6	R: AGACCTCTAAGTTGTATGACGAGGTCTCT P: CGAGAATGGGCCAGCAAGCA F: CCCTCAGGCTCCGATTTTTAT
Pacific salmon parvovirus	virus	26.4	R: CGAAGACAACATGGAGGTGACA P: CAATTGGAGGCAACTGTA F: TTCCAAACAATTCGAGAAGCG
Piscine myocarditis virus	virus	26.3	R: ACCTGCCATTTTCCCCTCTT P: CCGGGTAAAGTATTTGCGTC F: TGCTAACACTCCAGGAGTCATTG
Piscine reovirus	virus	26.1	R: TGAATCCGCTGCAGATGAGTA P: CGCCGGTAGCTCT F: CCGGCCCTGAACCAGTT
Salmon alphavirus	virus	26.3	R: GTAGCCAAGTGGGAGAAAGCT P: TCGAAGTGGTGGCCAG F: GCCTGGACCACAATCTCAATG
Salmonid herpesvirus	virus	26.6	R: CGAGACAGTGTGGCAAGACAAC P: CCAACAGGATGGTCATTA F: CGTAGGGCCCCAATAGTTTCT
Viral erythrocytic necrosis virus	virus	24.9	R: GGAGGAAATGCAGACAAGATTTG

			P: TCTTGCCGTTATTTCCAGCACCCG
			F: ATGAGGCAGGTGTCGGAGG
Viral hemorrhagic septicemia virus	virus	26.9	R: TGTAGTAGGACTCTCCCAGCATCC
			P: TACGCCATCATGATGAGT
			F: TTCCAGCGATACGCTGTTGA
Viral encephalopathy and retinopathy virus	virus	26.2	R: CACCGCCCGTGTTTGC
			P: AAATTCAGCCAATGTGCCCC
			F: GTCAAGACTGGAGGCTCAGAG
78d16.1	reference gene	NA	R: GATCAAGCCCCAGAAGTGTTTG
			P: AAGGTGATTCCCTCGCCGTCCGA
			F: GCTCATTTGAGGAGAAGGAGGATG
COIL-P84-2	reference gene	NA	R: CTGGCGATGCTGTTCCTGAG
			P: TTATCAAGCAGCAAGCC
			F: CCCAGTATGAGGCACCTGAAGG
MRPL40	reference gene	NA	R: GTTAATGCTGCCACCCTCTCAC
			P: ACAACAACATCACCA

Appendix D

Supplementary table to Chapter 2

Table D.1: Table of pairwise comparisons from Dunn's tests of netscore for sockeye salmon captured by beach seine at three locations in the Fraser River watershed. The Z statistic and *p* value (with Benjamini-Hochberg FDR adjustment) are given for each pair. Group abbreviations are created as follows: the first two letters represent beach seine (BS) or gillnet (G), the third letter represents sex, and the following letters represent location where McM = McMillan Island, Hop = Peters Road in 2014, Sav = Savona, and 2015 = Peters Rd in 2015.

	BSF2015	BSFHop	BSFMcM	BSFSav	BSM2015	BSMHop	BSMMcM	BSMSav	GNF2015	GNFHop	GNFMcM	GNFSav	GNM2015	GNMHop	GNMMcM
BSFHop	3.399869 0.0007*														
BSFMcM	1.999906 0.0359	-0.590461 0.3083													
BSFSav	4.081032 0.0001*	1.004595 0.1891	1.404318 0.1092												
BSM2015	0.881729 0.2223	-2.359760 0.0155*	-1.291986 0.1309	-3.146637 0.0017*											
BSMHop	1.222638 0.1399	-1.113038 0.1661	-0.497957 0.3313	-1.847727 0.0504	0.613715 0.3025										
BSMMcM	1.671011 0.0693	0.029948 0.4881	0.391983 0.3626	-0.558754 0.3172	1.253244 0.1370	0.742751 0.2666									
BSMSav	2.993326 0.0025*	0.646947 0.2930	1.046898 0.1807	-0.162174 0.4430	2.341999 0.0156*	1.457630 0.1011	0.401867 0.3620								
GNF2015	-9.726355 0.0000*	-11.14569 0.0000*	-8.421995 0.0000*	-10.92986 0.0000*	-9.483210 0.0000*	-7.230882 0.0000*	-5.832665 0.0000*	-8.592377 0.0000*							
GNFHop	-6.329836 0.0000*	-8.229465 0.0000*	-6.353545 0.0000*	-8.419404 0.0000*	-6.509397 0.0000*	-5.411548 0.0000*	-4.677477 0.0000*	-6.802397 0.0000*	1.791462 0.0556						
GNFMcM	-6.258892 0.0000*	-8.132522 0.0000*	-6.357148 0.0000*	-8.349494 0.0000*	-6.457307 0.0000*	-5.445390 0.0000*	-4.735188 0.0000*	-6.811758 0.0000*	1.517242 0.0923	-0.185457 0.4374					
GNFSav	-4.480680 0.0000*	-6.521302 0.0000*	-5.053306 0.0000*	-6.886874 0.0000*	-4.810051 0.0000*	-4.244684 0.0000*	-3.888320 0.0001*	-5.642061 0.0000*	2.861173 0.0038*	1.100089 0.1678	1.243412 0.1379				
GNM2015	-6.007538 0.0000*	-7.817010 0.0000*	-6.274079 0.0000*	-8.093107 0.0000*	-6.243560 0.0000*	-5.437855 0.0000*	-4.810160 0.0000*	-6.748180 0.0000*	1.011155 0.1891	-0.512503 0.3288	-0.332251 0.3826	-1.482620 0.0975			
GNMHop	-2.712596 0.0059*	-4.515099 0.0000*	-3.647415 0.0003*	-5.005113 0.0000*	-3.090209 0.0020*	-3.067042 0.0021*	-3.123877 0.0018*	-4.315316 0.0000*	2.763930 0.0051*	1.417683 0.1078	1.531726 0.0908	0.527153 0.3262	1.727972 0.0630		
GNMMcM	-1.842611 0.0503	-4.238352 0.0000*	-3.059356 0.0021*	-4.796439 0.0000*	-2.361846 0.0156*	-2.349664 0.0154*	-2.494777 0.0110*	-3.852248 0.0001*	5.521202 0.0000*	3.470993 0.0005*	3.546181 0.0004*	2.209369 0.0217*	3.624940 0.0003*	1.237648 0.1378	
GNMSav	1.255550 0.1380	-1.001095 0.1882	-0.418083 0.3589	-1.723286 0.0628	0.669175 0.2876	0.063688 0.4786	-0.683176 0.2853	-1.363564 0.1164	7.050395 0.0000*	5.314696 0.0000*	5.353176 0.0000*	4.192239 0.0000*	5.358916 0.0000*	3.063108 0.0021*	2.350527 0.0156*

Appendix E

Supplementary material to Chapter 4

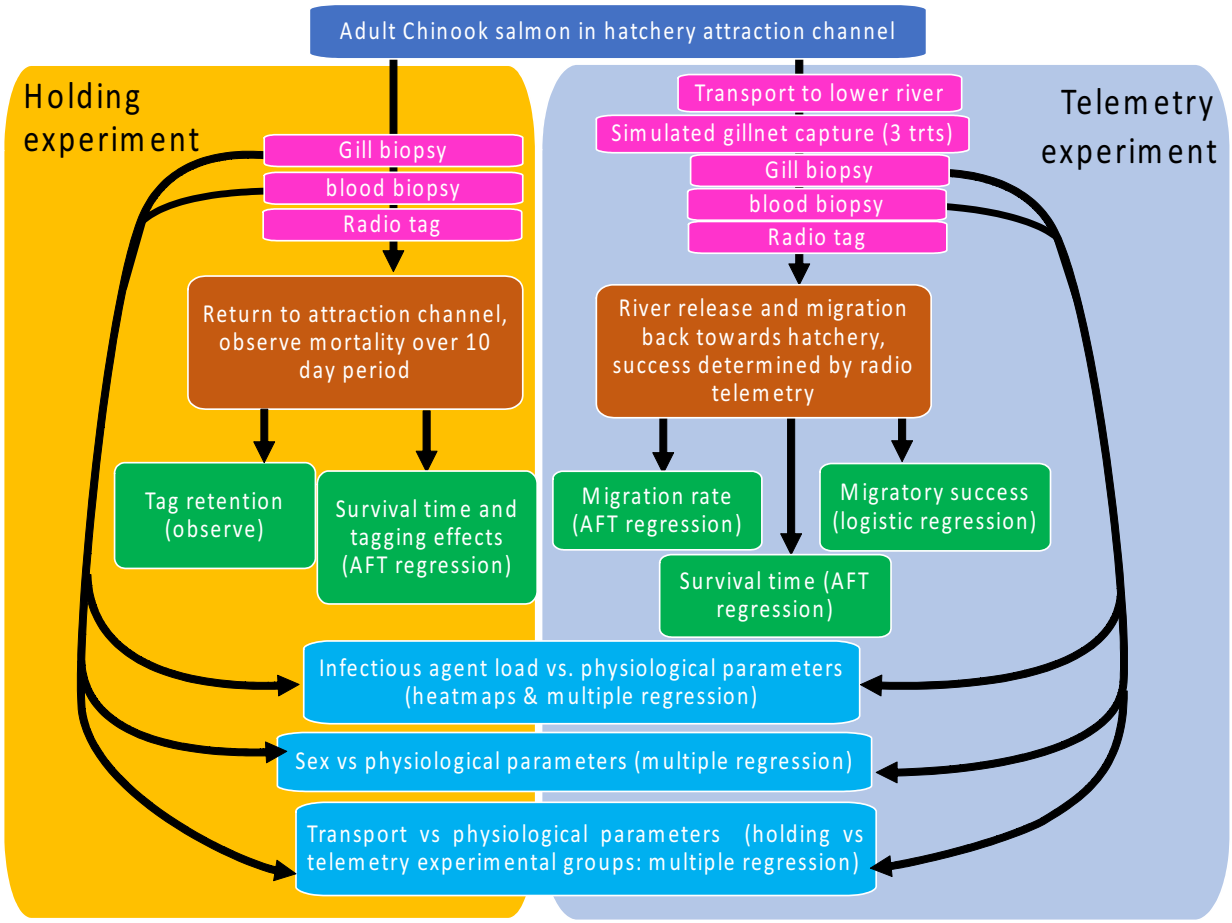

Figure E.1: Schematic of the experimental design for this study, which consisted of both a holding experiment and a telemetry experiment. Chinook salmon were collected from the hatchery attraction channel for both experiments. Pink boxes represent the handling, experimental manipulation, and biopsy steps. Brown boxes represent where salmon were released and subsequently monitored following handling. Green boxes represent the primary response variables and, in parentheses, the statistical analyses that allowed the testing of hypotheses. Blue boxes represent the variables that were compared via statistical analyses (parentheses) to gain further understanding of model results.

Table E.1: Taqman assays run for 15 infectious agents in 2013 Chinook salmon mixed-tissue samples using the Fluidigm Biomark HT-qRT-PCR platform (DFO Pacific Biological Station, Nanaimo, BC). infectious agents are categorized here based on the most current taxonomic assignment.

Scientific Name	Type	Efficiency	Forward Primer Sequence (5'-3') Reverse Primer Sequence (5'-3') Probe Sequence (FAM-5'-3'-MGB)
Aeromonas salmonicida	bacteria	2.03	F: TAAAGCACTGTCTGTTACC R: GCTACTTCACCCTGATTGG P: ACATCAGCAGGCTTCAGAGTCACTG
'*Candidatus* Branchiomonas cysticola'	bacteria	1.92	F: AATACATCGGAACGTGTCTAGTG R: GCCATCAGCCGCTCATGTG P: CTCGGTCCCAGGCTTTCCTCTCCCA
Flavobacterium psychrophilum	bacteria	1.84	F: GATCCTTATTCTCACAGTACCGTCAA R: TGTAAACTGCTTTTGCACAGGAA P: AAACACTCGGTCGTGACC
Rickettsia-like organism	bacteria	1.97	F: GGCTCAACCCAAGAACTGCTT R: GTGCAACAGCGTCAGTGACT P: CCCAGATAACCGCCTTCGCCTCCG
Dermocystidium salmonis	mesomycetozoean	1.92	F: CAGCCAATCCTTTCGCTTCT R: GACGGACGCACACCACAGT P: AAGCGGCGTGTGCC
Sphaerothecum destruens	mesomycetozoean	1.93	F: GGGTATCCTTCCTCTCGAAATTG R: CCCAAACTCGACGCACACT P: CGTGTGCGCTTAAT
Loma salmonae	microsporidian	1.95	F: GGAGTCGCAGCGAAGATAGC R: CTTTTCCTCCCTTTACTCATATGCTT P: TGCCTGAAATCACGAGAGTGAGACTACCC
Ceratonova shasta	myxozoan	1.94	F: CCAGCTTGAGATTAGCTCGGTAA R: CCCCGGAACCCGAAAG P: CGAGCCAAGTTGGTCTCTCCGTGAAAAC
Kudoa thyrsites	myxozoan	1.94	F: TGGCGGCCAAATCTAGGTT R: GACCGCACACAAGAAGTTAATCC P: TATCGCGAGAGCCGC
Parvicapsula minibicornis	myxozoan	1.90	F: AATAGTTGTTTGTCGTGCACTCTGT R: CCGATAGGCTATCCAGTACCTAGTAAG P: TGTCCACCTAGTAAGGC
Tetracapsuloides bryosalmonae	myxozoan	1.93	F: GCGAGATTTGTTGCATTTAAAAAG R: GCACATGCAGTGTCCAATCG P: CAAAATTGTGGAACCGTCCGACTACGA
Cryptobia salmositica	protozoan	1.93	F: TCAGTGCCTTTCAGGACATC R: GAGGCATCCACTCCAATAGAC P: AGGAGGACATGGCAGCCTTTGTAT
Ichthyophthirius multifiliis	protozoan	1.93	F: AAATGGGCATACGTTTGCAAA R: AACCTGCCTGAAACACTCTAATTTTT P: ACTCGGCCTTCACTGGTTCGACTTGG
Piscine reovirus	virus	1.94	F: TGCTAACACTCCAGGAGTCATTG R: TGAATCCGCTGCAGATGAGTA P: CGCCGGTAGCTCT
Viral erythrocytic necrosis virus	virus	1.94	F: CGTAGGGCCCCAATAGTTTCT R: GGAGGAAATGCAGACAAGATTTG P: TCTTGCCGTTATTTCCAGCACCCG

Table E.2: Taqman assays run for 35 host genes including 3 reference genes in 2013 Chinook salmon gill samples using the Fluidigm Biomark HT-qRT-PCR platform (DFO Pacific Biological Station, Nanaimo, BC).

Gene Abbreviation	Gene Name	Function	Efficiency	Forward Primer Sequence (5'-3') Reverse Primer Sequence (5'-3') Probe Sequence (FAM-5'-3'-MGB)
ATP5G3-C	ATP synthase complex subunit 3	energy metabolism (MRS)	1.81	F: GGAACGCCACCATGAGACA R: CGCCATCCTGGGCTTTG P: AGCCCCATTGCCTC
B2M	Beta-2-microglobulin	MHCI component	1.91	F: TTTACAGCGCGGTGGAGTC R: TGCCAGGGTTACGGCTGTAC P: AAAGAATCTCCCCCCAAGGTGCAGG
C3	complement component 3	complement system	2.17	F: ATTGGCCTGTCCAAAACACA R: AGCTTCAGATCAAGGAAGAAGTTC P: TGGAATCTGTGTGTCTGAACCCC
C4B	complement component 4B	complement system	1.78	F: TCCAACCACATCGCATTATCC R: ATCTCTGACACCACTGACCACAA P: ATAGACAGGCTTCCC
C7	complement component 7	complement system	1.83	F: ACCTCTGTCCAGCTCTGTGTC R: GATGCTGACCACATCAAACTGC P: AACTACCAGACAGTGCTG
CD4	cluster of differentiation 4	MHC class II antigen presentation	1.94	F: CATTAGCCTGGGTGGTCAAT R: CCCTTTCTTTGACAGGGAGA P: CAGAAGAGAGAGCTGGATGTCTCCG
CD83	cluster of differentiation 83	cell mediated immunity	1.86	F: GATGCACCCCTTGAGAAGAA R: GAACCCTGTCTCGACCAGTT P: AATGTTGATTTACACTCTGGGGCCA
CIRP	cold inducble RNA-binding protein	cellular stress response (MRS)	NA	F: AAGCTGTGATTGTGCTCTAAAGAC R: TCCCACTTAGCATTCCATCCTTG P: CTCCTTCAGTTCTGTAATGC
EIF4E	eukaryotic translation initiation factor 4E	transcription factor (MRS)	NA	F: TCTGGAAACCCACACACAAAGA R: GCGTTTTGAGGTTTGCATGTT P: CCTGCCATAGCCACAC
GR-2	glucocorticoid receptor 2	stress response	1.90	F: TCCAGCAGCTATGCCAGTTCT R: TTGCCCTGGGTTGTACATGA P: AAGCTTGGTGGTGGCGCTG
HEP	hepcidin antimicrobial protein	innate immunity, iron homeostasis	1.91	F: GAGGAGGTTGGAAGCATTGA R: TGACGCTTGAACCTGAAATG P: AGTCCAGTTGGGGAACATCAACAG
HSC70	heat shock cognate 70	stress response	1.72	F: GGGTCACACAGAAGCCAAAAG R: GCGCTCTATAGCGTTGATTGGT P: AGACCAAGCCTAAACTA
HSP90	heat shock protein 90	stress response	1.82	F: TGGGCTACATGGCTGCCAAG R: TCCAAGGTGAACCCAGAGGAC P: AGCACCTGGAGATCAA
IFNA	interferon alpha	cytokine, innate viral response	2.00	F: CGTCATCTGCAAAGATTGGA R: GGGCGTAGCTTCTGAAATGA P: TGCAGCACAGATGTACTGATCATCCA
IgMs	immunoglobulin M	antibody, humoral defense	1.92	F: CTTGGCTTGTTGACGATGAG R: GGCTAGTGGTGTTGAATTGG P: TGGAGAGAACGAGCAGTTCAGCA
				F: GCAATCTCTTGCCTCCACTC

IL-11	interleukin 11	cytokine, immune modulation	1.89	R: TTGTCACGTGCTCCAGTTTC P: TCGCGGAGTGTGAAAGGCAGA F: TTGGATTTTGCCCTAACTGC
IL-15	interleukin 15	cytokine, innate viral response	2.01	R: CTGCGCTCCAATAAACGAAT P: CGAACAACGCTGATGACAGGTTTTT F: ATCATCCTGTCAGCCCAGAG
IL-1R	interleukin 1 receptor	cytokine receptor, pro-inflammatory response	1.89	R: TCTGGTGCAGTGGTAACTGG P: TGCATCC CCTCTACACCCCAAA F: CAAACCGCAAGAGTTCCTCATT
IRF1	interferon regulatory factor 1	immune related transcription factor	1.78	R: AGTTTGGTTGTGTTTTTGCATGTAG P: CTGGCGCAGCAGATA F: TTGTTGCTGGTGAGAAAACTCAGT
JUN	activator protein 1	gene transcription factor (MRS)	1.81	R: CCTGTTGCCCTATGAATTGTCTAGT P: AGACTTGGGCTATTTAC F: TGTTTGTTAAAAGGGGACACAGTG
KCTD1	K channel tetradmerization domain 1	transcription factor (MRS)	1.96	R: GTGAAGTGTTATCTGGGCTGAAAG P: CTCCAAGGCTGAAAT F: GCTCTCTCAATCCTTGGCTTTAC
MCSF	macrophage colony stimulating factor	macrophage proliferation (MRS)	1.84	R: ACCAGCATAATTGAAAACCAGAGG P: CTCAATGTCCTCAATGCT F: GCGACAGGTTTCTACCCCAGT
MHCI	major histocompatability complex I	intracellular antigen presentation	1.95	R: TGTCAGGTGGGAGCTTTTCTG P: TGGTGTCCTGGCAGAAAGACGG F: TGCCATGCTGATGTGCAG
MHCII-B	major histocompatability complex II beta	extracellular antigen presentation	1.89	R: GTCCCTCAGCCAGGTCACT P: CGCCTATGACTTCTACCCCAAACAAAT F: GCCAGCGGAGCAGGAA
MMP13	matrix metalloproteinase 13	breakdown of extracellular matrix, inflammatory response	1.84	R: AGTCACCTGGAGGCCAAAGA P: TCAGCGAGATGCAAAG F: AGATGATGCTGCACCTCAAGTC
Mx	interferon induced GTP-binding protein	innate antiviral immunity	1.90	R: CTGCAGCTGGGAAGCAAAC P: ATTCCCATGGTGATCCGCTACCTGG F: ACAGCTGTTACACAGACGACATCA
RIG-I	retinoic acid inducible gene I	innate antiviral immunity	1.93	R: TTTAGGGTGAGGTTCTGTCCGA P: TCGTGTTGGACCCCACTCTGTTCTCTC F: GCGGTAGTGGAGTCAGTTGGA
SHOP21	hyperosmotic protein 21	saltwater adaptation (MRS)	1.95	R: GCTGCTGACGTCTCACATCAC P: CCTGTTGATGCTCAAGG F: TTCACTGCTGGAAAATGTGG
TF	transferrin	innate immunity, macrophage activation	1.91	R: GCTGCACTGAACTGCATCAT P: TGGTCCCTGTCATGGTGGAGCA F: GTCAAGACTGGAGGCTCAGAG
78d16.1	(reference gene)		1.93	R: GATCAAGCCCCAGAAGTGTTTG P: AAGGTGATTCCCTCGCCGTCCGA F: GCTCATTTGAGGAGAAGGAGGATG
COIL-P84-2	coilin (reference gene)	mitosis	1.81	R: CTGGCGATGCTGTTCCTGAG P: TTATCAAGCAGCAAGCC F: CCCAGTATGAGGCACCTGAAGG
MRPL40	mitochondrial ribosomal protein (reference gene)	protein synthesis	1.72	R: GTTAATGCTGCCACCCTCTCAC P: ACAACAACATCACCA

Table E.3: Multiple linear regression models of physiological parameters (blood plasma indices and gene expression) measured in gill and blood biopsies from adult Chinook salmon, Chilliwack, BC. *P* values ≤ 0.01 and ≤ 0.001 are indicated by bold text and bold text accompanied by an asterisk, respectively.

			Explanatory Variables			
Response Variable	adjusted R^2	Model Parameter	Relative Infectious Agent Burden	Sex (female)	Release Location (river)	Date Tagged
		β	-0.20	-3.13	11.48	1.14
hematocrit (%)	0.25	SE	0.49	1.48	1.64	0.46
		p	0.69	0.04	**<0.001***	**0.01**
		β	0.004	0.07	-0.71	-0.12
leucocrit (%)	0.08	SE	0.06	0.18	0.19	0.05
		p	0.94	0.69	**<0.001***	0.03
		β	-0.16	-0.44	0.30	0.14
glucose (mmol/L)	0.09	SE	0.07	0.20	0.22	0.06
		p	0.02	0.03	0.18	0.02
		β	0.16	0.55	0.53	-0.28
lactate (mmol/L)	0.02	SE	0.20	0.59	0.66	0.18
		p	0.41	0.36	0.42	0.12
		β	-1.23	0.06	-1.67	0.45
chloride (mmol/L)	0.10	SE	0.32	0.97	1.07	0.30
		p	**<0.001***	0.95	0.12	0.13
		β	-2.48	-2.61	9.12	0.41
osmolality (mOsm/kg)	0.16	SE	0.75	2.24	2.48	0.69
		p	**0.001***	0.24	**<0.001***	0.55
		β	-0.81	-2.70	0.28	1.29
sodium (mmol/L)	0.11	SE	0.42	1.27	1.41	0.39
		p	0.06	0.04	0.84	**0.001***
		β	0.005	-0.98	0.74	-0.33
potassium (mmol/L)	0.16	SE	0.11	0.32	0.35	0.10
		p	0.96	0.003	0.04	**0.001**
		β	0.04	0.77	-0.04	0.11
cortisol (log ng/mL)	0.17	SE	0.05	0.15	0.16	0.05
		p	0.47	**<0.001***	0.79	0.02
		β	-0.16	2.62	0.27	-0.09
17β-estradiol (log ng/mL)	0.62	SE	0.06	0.19	0.20	0.06
		p	**0.01**	**<0.001***	0.18	0.10
		β	-0.19	1.13	0.14	0.13
testosterone (log ng/mL)	0.24	SE	0.06	0.18	0.19	0.05
		p	**0.002**	**<0.001***	0.45	**0.01**
		β	-0.21	0.76	0.11	
ATP5G3	0.29	SE	0.03	0.09	0.10	0.03
		p	**0.002**	0.03	**<0.001***	**<0.001***
		β	0.005	-0.25	0.99	0.001
B2M	0.17	SE	0.06	0.17	0.19	0.05
		p	0.93	0.15	**<0.001***	0.98
		β	-0.01	-0.26	0.79	-0.08
CD4	0.30	SE	0.04	0.12	0.13	0.04
		p	0.78	0.02	**<0.001***	0.03
		β	0.04	0.11	0.86	-0.07
CD83	0.28	SE	0.04	0.13	0.14	0.04
		p	0.31	0.39	**<0.001***	0.08
		β	0.05	-0.23	0.35	-0.17
GR-2	0.20	SE	0.04	0.13	0.14	0.04
		p	0.19	0.07	**0.01**	**<0.001***

HEP	0.23	β	0.43	1.19	-0.20	0.08
		SE	0.10	0.29	0.32	0.09
		p	**<0.001***	**<0.001***	0.52	0.38
Hsp90	0.09	β	0.11	0.21	-0.22	-0.02
		SE	0.04	0.11	0.12	0.03
		p	**0.003**	0.06	0.08	0.53
IFNa	0.13	β	0.04	0.25	0.39	-0.03
		SE	0.04	0.11	0.12	0.03
		p	0.23	0.02	**0.002**	0.32
IGMs	0.04	β	0.15	0.23	0.04	-0.11
		SE	0.07	0.21	0.24	0.07
		p	0.04	0.28	0.87	0.11
IL-11	0.47	β	0.12	0.85	0.56	-0.32
		SE	0.05	0.16	0.18	0.05
		p	0.03	**<0.001***	**0.002**	**<0.001***
IL-15	0.45	β	-0.003	-0.06	0.80	-0.11
		SE	0.03	0.09	0.10	0.03
		p	0.92	0.50	**<0.001***	**<0.001***
IL-1R	0.30	β	0.08	-0.09	0.04	-0.23
		SE	0.03	0.10	0.11	0.03
		p	0.02	0.40	0.72	**<0.001***
JUN	0.37	β	-0.03	0.26	1.89	0.52
		SE	0.07	0.22	0.24	0.07
		p	0.65	0.22	**<0.001***	**<0.001***
MHCI	0.15	β	-0.01	0.03	0.42	-0.21
		SE	0.06	0.18	0.20	0.06
		p	0.92	0.87	0.04	**<0.001***
MHCII- B	0.32	β	-0.06	-0.19	0.55	-0.27
		SE	0.05	0.15	0.17	0.05
		p	0.28	0.23	**0.001***	**<0.001***
MMP13	0.13	β	0.17	0.27	0.15	-0.07
		SE	0.04	0.13	0.15	0.04
		p	**<0.001***	0.05	0.31	0.09
Mx	0.29	β	0.22	0.20	0.48	-0.26
		SE	0.06	0.17	0.19	0.05
		p	**<0.001***	0.23	**0.01**	**<0.001***
RIG-I	0.07	β	0.12	-0.12	0.66	-0.01
		SE	0.06	0.18	0.20	0.06
		p	0.04	0.51	**0.001***	0.91

Table E.4: Model parameters for infectious agents as explanatory variables in multiple linear regression models of physiological parameters (blood plasma indices and gene expression). Physiological parameters were measured in gill and blood biopsies from adult Chinook salmon, Chilliwack, BC. Other variables included in these models but not presented here include sex, release location, and date tagged. *P* values ≤ 0.01 and ≤ 0.001 are indicated by bold text and bold text accompanied by an asterisk, respectively.

Variable	Model Parameter	'*Ca.* Branchiomonas cysticola'	*Ceratonova shasta*	*Cryptobia salmositica*	*Flavobacterium psychrophilum*	*Ichthyophthirius multifiliis*	*Tetracapsuloides bryosalmonae*	Relative Infectious Agent Burden
	β	0.04	−0.46	−2.02	−0.47	−4.30	0.24	−0.20
hematocrit	SE	0.45	0.20	0.38	0.39	1.44	0.33	0.49
(%)	*p*	0.92	0.02	**<0.001***	0.24	**0.01**	0.47	0.69
	β	−0.01	0.01	0.20	0.10	−0.07	−0.07	0.004
leucocrit	SE	0.05	0.03	0.06	0.05	0.12	0.04	0.06
(%)	*p*	0.81	0.80	**0.001***	0.03	0.55	0.08	0.94
	β	0.02	-0.06	-0.24	-0.05	-0.21	-0.03	-0.16
glucose	SE	0.06	0.03	0.04	0.05	0.20	0.04	0.07
(mmol/L)	*p*	0.70	0.04	**<0.001***	0.36	0.30	0.51	0.02
	β	-0.31	0.17	0.41	0.08	0.03	-0.002	0.16
lactate	SE	0.18	0.08	0.19	0.16	0.63	0.12	0.20
(mmol/L)	*p*	0.09	0.05	0.03	0.60	0.96	0.99	0.41
	β	-0.98	-0.08	-0.71	-0.55	-1.21	0.21	-1.23
chloride	SE	0.30	0.14	0.31	0.26	0.78	0.19	0.32
(mmol/L)	*p*	**0.001***	0.60	0.03	0.04	0.13	0.28	**<0.001***
	β	-2.83	0.09	-1.00	-1.41	-2.74	0.58	-2.48
osmolality	SE	0.66	0.33	0.50	0.58	1.50	0.42	0.75
(mOsm/kg)	*p*	**<0.001***	0.79	0.05	0.02	0.08	0.17	**0.001***
	β	-1.14	-0.18	-0.14	-0.88	-0.90	0.23	-0.81
sodium	SE	0.38	0.18	0.39	0.33	0.99	0.25	0.42
(mmol/L)	*p*	**0.003**	0.32	0.73	**0.01**	0.37	0.35	0.06
	β	-0.0004	0.05	-0.14	-0.05	0.27	0.04	0.005
potassium	SE	0.10	0.04	0.08	0.08	0.21	0.07	0.11
(mmol/L)	*p*	1.00	0.25	0.08	0.55	0.21	0.61	0.96
	β	0.01	0.05	0.11	0.05	0.09	0.003	0.04
cortisol	SE	0.05	0.02	0.04	0.04	0.13	0.03	0.05
(log ng/mL)	*p*	0.85	0.03	**0.01**	0.18	0.48	0.94	0.47
	β	-0.24	-0.06	-0.11	-0.14	-0.28	0.04	-0.16
17β-estradiol	SE	0.06	0.03	0.06	0.05	0.20	0.04	0.06
(log ng/mL)	*p*	**<0.001***	0.03	0.08	**0.01**	0.19	0.36	**0.01**
	β	-0.17	-0.08	-0.13	-0.08	-0.25	-0.005	-0.19
testosterone	SE	0.06	0.03	0.05	0.05	0.16	0.04	0.06
(log ng/mL)	*p*	**0.002**	**0.01**	**0.01**	0.13	0.14	0.91	**0.002**
	β	0.05	-0.01	0.05	0.01	-0.04	-0.01	0.10
ATP5G3	SE	0.03	0.01	0.03	0.02	0.06	0.02	0.03

	p	0.09	0.69	0.08	0.58	0.51	0.74	**0.002**
B2M	β	-0.01	-0.02	0.03	-0.06	-0.13	0.005	0.005
	SE	0.05	0.02	0.04	0.04	0.19	0.03	0.06
	p	0.82	0.35	0.44	0.10	0.50	0.89	0.93
CD4	β	-0.03	-0.004	-0.04	-0.12	0.11	0.02	-0.01
	SE	0.03	0.02	0.04	0.03	0.13	0.03	0.04
	p	0.37	0.81	0.25	**<0.001***	0.41	0.42	0.78
CD83	β	0.07	-0.04	-0.07	-0.08	-0.06	0.03	0.04
	SE	0.04	0.02	0.03	0.03	0.11	0.03	0.04
	p	0.07	0.05	0.03	**0.01**	0.63	0.35	0.31
GR-2	β	0.04	0.04	-0.04	-0.06	0.01	-0.01	0.05
	SE	0.04	0.02	0.04	0.03	0.12	0.03	0.04
	p	0.35	0.04	0.26	0.05	0.92	0.69	0.19
HEP	β	0.15	-0.01	0.52	0.16	0.43	-0.04	0.43
	SE	0.09	0.04	0.05	0.08	0.28	0.06	0.10
	p	0.10	0.87	**<0.001***	0.04	0.14	0.49	**<0.001***
Hsp90	β	0.08	0.01	0.04	0.06	0.16	0.001	0.11
	SE	0.03	0.02	0.03	0.03	0.09	0.02	0.04
	p	0.02	0.58	0.21	0.03	0.07	0.97	**0.003**
IFNa	β	0.06	-0.002	0.001	0.02	-0.12	0.01	0.04
	SE	0.03	0.02	0.04	0.03	0.09	0.02	0.04
	p	0.07	0.92	0.98	0.45	0.20	0.68	0.23
IGMs	β	0.10	-0.03	0.27	0.05	0.40	-0.06	0.15
	SE	0.07	0.03	0.06	0.06	0.17	0.04	0.07
	p	0.12	0.42	**<0.001***	0.43	0.03	0.14	0.04
IL-11	β	0.15	0.04	0.04	0.11	-0.26	-0.02	0.12
	SE	0.05	0.02	0.05	0.04	0.15	0.04	0.05
	p	**0.002**	0.09	0.42	**0.01**	0.10	0.57	0.03
IL-15	β	0.05	-0.001	-0.04	-0.06	-0.06	-0.01	-0.003
	SE	0.03	0.01	0.03	0.02	0.07	0.02	0.03
	p	0.07	0.92	0.14	**0.01**	0.42	0.77	0.92
IL-1R	β	0.15	-0.002	0.01	-0.01	0.14	-0.04	0.08
	SE	0.03	0.01	0.02	0.03	0.08	0.02	0.03
	p	**<0.001***	0.92	0.57	0.85	0.09	0.10	0.02
JUN	β	0.04	-0.07	0.07	0.09	-0.27	0.03	-0.03
	SE	0.07	0.03	0.07	0.06	0.19	0.05	0.07
	p	0.57	0.03	0.33	0.13	0.17	0.52	0.65
MHCI	β	0.09	-0.004	-0.04	-0.11	0.12	-0.04	-0.01
	SE	0.06	0.03	0.04	0.05	0.12	0.05	0.06
	p	0.12	0.87	0.36	0.02	0.32	0.44	0.92

MHCII-	β	0.04	-0.05	-0.05	-0.12	0.01	-0.02	-0.06
	SE	0.05	0.02	0.04	0.04	0.14	0.03	0.05
	p	0.38	0.02	0.28	**0.002**	0.97	0.61	0.28
MMP13	β	0.11	-0.01	0.16	0.01	0.26	-0.05	0.17
	SE	0.04	0.02	0.03	0.04	0.11	0.03	0.04
	p	**0.01**	0.69	**<0.001***	0.71	0.03	0.05	**<0.001***
Mx	β	0.26	0.04	0.05	0.09	0.40	-0.02	0.22
	SE	0.05	0.03	0.06	0.05	0.15	0.04	0.06
	p	**<0.001***	0.14	0.35	0.07	**0.01**	0.64	**<0.001***
RIG-I	β	0.14	-0.01	0.07	0.04	0.09	-0.0001	0.12
	SE	0.05	0.03	0.05	0.05	0.19	0.04	0.06
	p	**0.01**	0.83	0.23	0.45	0.63	1.00	0.04