THE INFLUENCE OF SMOLT AGE AND PHYSIOLOGICAL CONDITION ON SURVIVAL AND BEHAVIOUR OF WILD MIGRATING JUVENILE SOCKEYE SALMON (ONCORHYNCHUS NERKA) IN BRITISH COLUMBIA

by

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis/dissertation entitled:

THE INFLUENCE OF AGE AND PHYSIOLOGICAL CONDITION ON SURVIVAL AND BEHAVIOUR OF WILD MIGRATING SOCKEYE SALMON SMOLTS (ONCORHYNCHUS NERKA) IN BRITISH COLUMBIA

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Abstract

Sockeye salmon (*Oncorhynchus nerka*) smolts typically experience high mortality during outmigration through freshwater and into the marine environment, yet factors influencing survival remain poorly understood. Telemetry studies investigating migration survival have largely focused on tracking large hatchery smolts or larger individuals within wild populations, which may not be representative of the majority of migrants. I used recently developed miniaturized acoustic transmitters (VEMCO V4) to track age-1 sockeye smolts (*n* = 200) for the first time over ~950 km from Chilko Lake, British Columbia, Canada through the coastal marine environment using large-scale receiver arrays. I compared their survival with concomitantly tagged and tracked age-2 smolts (*n* = 100). I paired acoustic telemetry with non-lethal gill biopsies and used high-throughput quantitative polymerase chain reaction to assess how infectious agents and host gene expression profiles influence smolt migration survival. Cumulative freshwater survival of age-1 smolts was double that of age-2 survival (56% vs 28%), potentially due to higher proportional tag burdens of age-2 smolts. Although survival between the age classes differed, trends in landscape-specific survival were similar, with the poorest survival occurring the in the upper river tributaries (76% and 53% for age-1 and age-2 smolts respectively over the first 14 km from release), and after ocean entry for age-1 smolts (36%). Three infectious agents (*Flavobacterium psychrophilum*, ‘*Candidatus Branchiomonas cisticola*’, and infectious hematopoietic necrosis virus) were most prevalent in tagged smolts, and *F. psychrophilum* was related to migration survival. Gene expression profiles differed between age groups and were strongly associated with migration survival. Smolts that died earlier in the river had a significantly higher expression of inflammatory (IL-11 and IL-17D) and stress (HSC70 and JUN) response genes than smolts that survived migration. These genes were also more highly expressed in age-2 smolts than age-1 smolts. My work highlights the importance of expanding research to include smaller age-1 smolts and provides important survival estimates for an indicator population of Fraser River sockeye salmon. My work also provides important and novel links between infectious agents and gene expression profiles with migration survival of age-1 and co-migrating age-2 smolts for the first time, highlighting mechanisms contributing to sockeye smolt mortality during migration.
Lay Summary

Pacific salmon (*Oncorhynchus spp*) smolts typically experience high mortality during migration through freshwater and into the marine environment. I tagged and tracked co-migrating wild one- and two-year-old Chilko Lake sockeye smolts over the first ~950 km of their migration through freshwater and the coastal marine environment. Survival of one-year-old smolts was higher than two-year-olds, but both age groups experienced the lowest survival in the upper river tributaries. Through pairing survival data of tagged smolts with genomic techniques, I identified an important bacterium that was related to migration survival. I also identified genes associated with inflammation and stress responses that were more highly expressed in individuals that died during early seaward migration. My thesis identifies important environmental and physiological factors influencing the survival of one of the largest sockeye populations in the Fraser River watershed.
Preface

This research was carried out as one component of the Salish Sea Marine Survival Project, which is an international initiative to determine the primary factors affecting the survival of juvenile salmonids in the Salish Sea. This research was also part of the Ocean Tracking Network Canada. I held primary responsibility for the study designs, collection and analysis of the data, as well as preparation of manuscripts for submission. Throughout the process I received supervision and guidance from my supervisor, Dr. Scott Hinch and my supervisory committee member Dr. Nathan B. Furey. I also received support from my colleague Arthur L. Bass. David Welch, Aswea Porter and Erin Rechisky from Kintama Research Services provided support by deploying and recovering acoustic receivers in the Fraser River and Salish Sea, and were substantially involved in statistical analysis modelling survival (Chapter 2). Dr. Kristi Miller and her staff at the Molecular Genetics Lab (Pacific Biological Station, Nanaimo) were considerably involved in genomic laboratory work (Chapter 3). I received logistical support at the Chilko Lake field camp and counting fence from Fisheries and Oceans Canada and the Xeni Gwet'in First Nation. Individuals who were essential contributors to the conceptualization, development, or preparation of the manuscripts below are listed as coauthors. All capture, tagging and handling procedures were approved by the University of British Columbia Animal Care Committee (Animal Use Protocol #A15-0205).
Chapter 2: The influence of smolt age on freshwater and early marine behaviour and survival of migrating juvenile sockeye salmon (*Oncorhynchus nerka*)

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Chapter 3: Sockeye salmon (*Oncorhynchus nerka*) smolt migrations: The role of infectious agents and transcriptome profiles on freshwater and early marine survival

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Chapter 1: Introduction

Anadromous Pacific salmon (*Oncorhynchus spp.*) are central to the fabric of ecosystems, economies, and cultures on North America’s Pacific coast. Over the last few centuries, however, nearly 30% of historical populations have been lost from this region with certain populations experiencing particularly severe declines in recent decades (Gustafson et al. 2007, Irvine and Akenhead 2013). Many potential factors may be contributing to these declines, including shifts in climate regimes, interstock competition (Beamish and Bouillon 1993, Irvine and Akenhead 2013), harvest rates, habitat loss, exposure to farmed salmon (Connors et al. 2012), and disease (Miller et al. 2014). Though a number of studies have focused on determining the specific reasons for recent decreases in Pacific salmon abundance and productivity, there is still no clear consensus. Decreasing population productivity is of serious concern and research aimed at understanding the factors influencing patterns in survival is needed to understand and predict adult returns.

Sockeye salmon (*Oncorhynchus nerka*) are one of the most valuable species of Pacific salmon and have exhibited some of the largest declines. Nearly half of historical populations are estimated to be extinct from the Pacific Northwest (Gustafson et al. 2007). Sockeye have also experienced a disproportionally higher loss of genetic diversity compared to other Pacific salmon species, likely due to a decrease in required lacustrine rearing habitats due to human development (Gustafson et al. 2007). In British Columbia, Canada, Fraser River sockeye populations have experienced particularly drastic declines with the lowest adult returns in 2009 since 1947. This event led to fisheries closures and the Canadian government launching a judicial inquiry (Cohen 2012). Furthermore, the 2016 Fraser River sockeye run was the lowest since records began in 1893 (Chandler et al. 2017). However, sockeye returns can be extremely
variable, as 2010 had the highest returns of Fraser River sockeye since 1913 (PSC 2015). Understanding mortality across life stages is necessary to better understand the extreme variations and overall declines in sockeye populations.

1.1 Smolt migration survival

The juvenile life stage as smolts leave natal freshwater habitats and migrate to the open ocean is often associated with high mortality (Groot and Margolis 1991, Wood et al. 1993, Welch et al. 2009, 2011, Jeffries et al. 2014, Clark et al. 2016) and may be critical to salmon population productivity (Beamish et al. 2004, Farley et al. 2007, Irvine & Akenhead 2013). External factors such as environmental and biotic conditions can contribute to mortality during migration. Predation (Ruggerone and Rogers 1984, Collis et al. 2001, Beamish et al. 2004, Evans et al. 2012, Hostetter et al. 2012, Cavallo et al. 2013) and competition for food (Beamish et al. 2004, Beamish and Mahnken 2001, Hertz et al. 2016) are considered to be a key drivers of migration survival in freshwater and the marine environment. Small size and slower growth rates are also often associated with increased smolt mortality rates, particularly in the early period of ocean residence (Beckman et al. 1999, Beamish et al. 2004, Farley et al. 2007). Poor marine conditions at the time of ocean entry such as inadequate levels of food (Beamish et al. 2012) and the presence of harmful algae blooms (Rensel et al. 2010) can also influence the growth and/or survival of smolts. These factors and interactions among them can vary across migration regions and have varying impacts on smolt behaviour and survival. Because the smolt life stage is particularly critical for the overall productivity of salmon populations, investigating landscape-specific migratory survival and behaviour is essential.
Smolt physiological condition can also influence smolt migration survival (Hostetter et al. 2011; Jeffries et al. 2014, Healy et al. 2018). As juveniles undergo the demanding process of smoltification in preparation for transition from freshwater into the ocean they may experience osmoregulatory failure, resulting in mortality either directly or through increased risk of predation (Franklin et al. 1992, Kennedy et al. 2007). Infectious agents (e.g. viruses, bacteria, protozoans, myxozoans, and some fungi) and immune responses may also predict downstream smolt survival (Hostetter et al. 2011, Van Gaest et al. 2011, Ferguson et al. 2012, Jeffries et al. 2014, Miller et al. 2014). Infectious disease can impact smolt swim performance, feeding ability, growth rates, osmotic function, and predator avoidance, which can reduce the ability to sustain migration and/or increase susceptibility to predation or starvation (Webb and Brett 1973, Wedemeyer et al. 1990, Dieperink et al. 2002, Miller et al. 2014). Furthermore, infectious agents can increase vulnerability to additional pathogens and stressors (Miller et al. 2014). However, studies determining direct links between smolt condition and migration survival are limited (but see Jeffries et al. 2014, Healy et al. 2018). Further research is needed to elucidate the mechanisms contributing to smolt migration mortality both in freshwater and the early marine environment.

1.2 Tracking smolt migration survival

The freshwater and marine survival and behaviour of migrating smolts can be examined using acoustic telemetry techniques. Studies in recent years have used acoustic telemetry to determine survival estimates of sockeye salmon smolts during migration from freshwater rearing areas through the Fraser River watershed and into the Salish Sea (e.g., Welch et al. 2009, 2011,
Clark et al. 2016, Rechisky et al. 2018). Collectively, these studies documented significant mortality (up to 90%) during downstream migration and into the ocean. However, they focused on hatchery smolts or larger individuals within wild populations, which can differ behaviourally, physiologically, and morphologically may not accurately represent the wild population (e.g. Piggins and Mills 1985, Huntingford 2004). Continued miniaturization of tags can allow for research incorporating a larger size distribution within wild smolt populations.

Acoustic telemetry can be paired with transcriptomics technology to investigate the influence of smolt health and condition on survival. The presence and load of infectious agents can be assessed in biopsy samples along with host biomarkers indicative of immune and stress responses, and then be related to individual migration fate (Miller et al. 2014). Continued advancements of these methods have also made it possible to distinguish fish in an active viral disease state from those carrying a latent viral infection (Miller et al. 2017). However, many studies have used lethal sampling techniques, limiting the ability to link the roles of these stressors with individual smolt out-migration success or behaviour. Understanding how infectious agents and gene expression profiles influence smolt survival is essential because disease has been linked with smolt mortality during the period of freshwater and early marine migration (Van Gaest et al. 2011, Ferguson et al. 2012, Hostetter et al. 2012, Miller et al. 2014). However, studies using this combined approach to make direct connections between these factors in wild populations are currently limited (but see Jefferies et al. 2014).

1.3 Chilko Lake sockeye salmon smolts

The Fraser River watershed is one of the largest salmon producing systems in the world,
particularly for sockeye because of its great abundance of accessible lake rearing areas (Groot and Margolis 1991). The Chilko Lake sockeye salmon population in the Fraser River watershed are an ideal model for studying factors influencing smolt behaviour and survival because it is one of the four largest populations in the system, with 10-70 million smolts migrating out to sea each spring. It is also an indicator stock for salmon management in British Columbia (Cass 1989, Irvine and Akenhead 2013). On average, ~96% of migrants are age-1, with the remaining 4% consisting of age-2 smolts that spent an extra winter in the lake. Chilko sockeye have shown similar trends of declining productively as many sockeye stocks throughout the North Pacific (Irvine and Akenhead 2013).

In order to investigate smolt survival, telemetry studies have tracked the outmigration of Chilko sockeye smolts from 2010-2014 (Clark et al. 2016, Rechisky et al. 2018). Over the 5-year period, ~2000 age-2 smolts were tagged and tracked during the first 1044 km of their freshwater and marine migration. Overall, Clark et al. (2016) and Rechisky et al. (2018) found that cumulative survival ranged from 4-14%, with particularly low survival (57–78%) in the clear waters of the Chilko and Chilcotin Rivers (80 km). Through pairing telemetry with non-lethal gill biopsies and transcriptomics techniques, Jeffries et al. (2014) found that smolts that died immediately after release possessed gene expressions reflective of an immune response to one or more viral pathogens compared with smolts that successfully completed freshwater migration. Infectious haematopoietic necrosis virus (IHNV) was the most prevalent pathogen observed and 82% of smolts positive for IHNV as well as 100% carrying high loads experienced mortality before reaching the ocean. These studies characterized smolt behaviour, highlighted high-risk landscapes, and identified potential mechanisms for smolt mortality, but only focused on age-2 smolts which make up < 5% of the outmigrant population (Irvine and Akenhead 2013). Thus a
need remains to understand if the collective findings from these studies are applicable to the vast majority of this important population.

### 1.4 Thesis overview and research objectives

My thesis examines the influence of age and physiological condition on individual behaviour and survival of Chilko Lake sockeye smolts in freshwater and marine environments. My first objective was to use acoustic telemetry to investigate survival and movement of age-1 and age-2 Chilko Lake sockeye smolts to identify particular areas of high mortality during migration, along with the influence of smolt age on migration rates and survival. I predicted that age-1 smolts would experience similar trends in survival as age-2 smolts in previous telemetry studies reported in Clark et al. (2016) and Rechisky et al. (2018), with the upper freshwater tributaries and early marine regions being landscapes of particularly poor survival. My second objective was to combine acoustic telemetry survival estimates with non-lethal gill biopsies and sophisticated transcriptomics technology to directly link the role of infectious agents and physiological condition to individual migration success of age-1 and age-2 smolts. I predicted that smolts with high numbers and loads of infectious agents and gene expressions consistent with stress and immune responses and/or indicating osmotic dysfunction would experience poor migration survival regardless of age.

In Chapter 2, I determine and compare migration rates and survival estimates of acoustically tagged age-1 and age-2 Chilko Lake sockeye smolts over the first ~950-1050 km of their migration in 2016. Modified mark-recapture models were used estimate survival, highlight critical regions, and identify differences between age classes. I place migration rates and survival
estimates from 2016 into context of previous telemetry studies of age-2 Chilko Lake sockeye smolts tracked in the same region from 2012-2014. In Chapter 3, I compare the presence and load of infectious agents, as well as gene expression profiles (i.e. immune and stress responses, and osmoregulatory preparedness) between age classes. I also make important and novel connections between infectious agents and immune and stress responses and how they relate to migration survival of both age classes. The survival estimate results from Chapter 2 along with the transcriptomics results from Chapter 3 increases our understanding of factors influencing smolt migration survival of one of the largest sockeye populations in Canada and can help to inform conservation and management practices. In my 4th and concluding chapter, I synthesize my results from these studies, highlighting how my results have expanded the understanding of smolt migrations and potential factors contributing to mortality. I outline some of the limitations of my research and provide suggestions for possible future directions for continued research. Lastly, I discuss how my results can be used to improve management and conservation of salmon populations on the coast of British Columbia.
Chapter 2: The influence of smolt age on freshwater and early marine behaviour and survival of migrating juvenile sockeye salmon (*Oncorhynchus nerka*)

2.1 Introduction

Anadromous Pacific Salmon *Oncorhynchus spp.* are of cultural, ecological, and economic importance throughout the Pacific Northwest, yet in the past few centuries, an estimated 29% of historical populations have been lost from this region (Gustafson et al. 2007). Among Pacific salmon species, Sockeye Salmon *Oncorhynchus nerka* are one of the most valued and have exhibited some of the most dramatic declines (Irvine and Akenhead 2013), with almost half (47%) of historical populations estimated to be extinct from the Pacific Northwest (Gustafson et al. 2007). In particular, sockeye salmon stocks throughout the Fraser River watershed in British Columbia, Canada (one of the largest salmon producing systems in the world) have experienced considerable declines, with exceptionally low adult returns in 2009 (the lowest since 1947) leading to the Canadian government launching a judicial inquiry (Cohen 2012). Moreover, adult sockeye salmon returns in 2016 were well below forecast levels and the lowest on record (<1 million; Chandler et al. 2017). Decreasing sockeye salmon population productivity is of serious concern and research aimed at understanding the trends in survival across life stages is necessary to better understand and predict adult returns.

Among life stages, the period when juvenile Fraser River sockeye salmon smolts undergo an impressive annual migration in which populations of millions of fish travel thousands of
kilometres from their freshwater rearing grounds to the open ocean is often associated with low survival (Groot and Margolis 1991, Welch et al. 2009, 2011, Clark et al. 2016, Rechisky et al. 2018). Throughout their migration, smolts are exposed to a number of challenges including intensive physiological and morphological changes, exposure to novel predators, competition, pathogens and immune responses, and changing environmental conditions (Beamish et al. 2004, Farley et al. 2007, Beamish et al. 2012, Irvine & Akenhead 2013, Jeffries et al. 2014). Surviving these challenges during migration is considered to be particularly critical for the overall productivity of salmon populations. Investigating migratory survival and behaviour of smolts is therefore a key component in understanding the long-term declines in adult sockeye salmon returns.

Continued advances in telemetry technology have provided opportunities to investigate in-situ behaviour and survival of smolts during seaward migration. Numerous studies in recent years have used acoustic telemetry to determine survival estimates of sockeye salmon smolts as they migrate across hundreds of kilometres through the Fraser River watershed and the Salish Sea (e.g., Welch et al. 2009, 2011; Clark et al. 2016, Rechisky et al. 2018). These studies collectively documented low survival during parts of the downstream migration and early ocean phase. However, due to the size of the tags then available, these studies focused on large hatchery smolts or larger individuals within wild populations. Consequently, they may not be representative of wild populations because they may differ behaviourally, physiologically, and morphologically from smaller individuals that often make up the majority of wild migrants (e.g., Piggins and Mills 1985, Huntingford 2004). Continued miniaturization of tags can allow for research to incorporate a larger size distribution within wild smolt populations.
Chilko Lake sockeye salmon are an ideal model for studying factors influencing smolt behaviour and survival because they are one of the four largest populations in the Fraser River watershed, with 10 - 70 million smolts migrating out to sea each spring, and form a key indicator stock for salmon management in British Columbia (Cass 1989; Irvine and Akenhead 2013). Telemetry estimates of Chilko smolt survival are generally low (4 – 14%) during downstream migration and through the Salish Sea (1044 km), with low survival particularly evident in the clear upper tributaries of the Chilko and Chilcotin Rivers (34 – 49% over 178 km; Clark et al. 2016, Rechisky et al. 2018). Clark et al. (2016) and Rechisky et al. (2018) focused on the larger age-2 smolts within the Chilko population, which represents only a small fraction of outmigrants. In most years, ~96% of migrating Chilko smolts are age-1, with the remaining ~4% consisting of age-2 individuals that spent an additional winter in the lake before migrating (Irvine and Akenhead 2013). Consequently, telemetry estimates of behaviour and survival of these older and larger smolts may not be representative of the smaller age-1 smolts that make up the majority of the population. With recent advancements in acoustic tag miniaturization, age-1 sockeye salmon smolts can now be tagged and tracked. I used new, smaller transmitters to investigate the migration behaviour and survival of both wild age-1 and age-2 Chilko Lake sockeye salmon smolts for the first time in 2016. I hypothesized that age-1 smolts would experience similar landscape-specific trends in survival as the age-2 smolts reported in Clark et al. (2016) and Rechisky et al. (2018), with the upper freshwater tributaries and early marine regions being particularly critical landscapes of poor survival. This study is the first to not only track smaller age-1 Chilko Lake sockeye salmon smolts, but also provide direct comparisons to larger co-migrating age-2 smolts.
2.2 Methods

2.2.1 Study system

Chilko Lake is located in the Fraser River watershed 654 km upstream from the Pacific Ocean (Figure 2.1) and is one of the largest lakes in British Columbia. Each spring, smolts migrate downstream from the lake into the Chilko and Chilcotin Rivers before entering the Fraser River and then the Salish Sea. Smolts then largely travel northeast through the Discovery Islands, Johnstone Strait, and Queen Charlotte Strait before moving offshore into the open Pacific Ocean. Since the 1950’s, Fisheries and Oceans Canada (DFO) has estimated daily smolt abundances at an enumeration fence located at the lake outlet throughout the period of outmigration.

2.2.2 Smolt collection and surgery

Two-hundred age-1 and 100 age-2 smolts were collected from the DFO enumeration fence from April 17-23 in 2016 and surgically implanted with acoustic transmitters. As Chilko smolts primarily migrate at night, smolts were collected between 22:00 and 2:30 and were held until morning in a covered ~200-L flow-through tank. Age-1 smolts were implanted with VEMCO V4 transmitters (180 kHz, 3.6 mm height, 5.7 mm width, 11 mm length, 0.42 g in air) and age-2 smolts with VEMCO V7 transmitters (69 kHz, 7 mm diameter, 18 mm length, 1.4 g in air). Prior to surgery, smolts were anaesthetized in 100 mg/L Tricaine methanesulphonate (MS-222) mixed with 200 mg/L NaHCO₃ buffer for ~1-2 minutes. Weight (g) and fork length (FL; mm) were measured before smolts were transferred into a V-shaped trough on a surgery table. A maintenance dose of MS-222 (50 mg/L) buffered with NaHCO₃ (100 mg/L) flowed continuously
over the gills to maintain sedation during surgery. All water baths were continuously aerated and temperatures were maintained within 4°C of the river temperature.

Tags were inserted into the abdominal cavity through a ~6-mm (V4 tag in age-1) or ~7-8-mm (V7 tags in age-2) incision on the midventral line. Incisions were closed using one suture (Ethicon Monocryl 5-0 monofilament with a 3/8 circle reverse cutting 13 mm [P-3] needle) on age-1 smolts, and two interrupted sutures for the larger incision on age-2 smolts. Using epoxy coated carbon steel bone cutting forceps, non-lethal biopsies of ~1 mm of tissue from the ends of 2-3 gill filaments were collected from half of the tagged age-1 smolts (n = 100) and from nearly all tagged age-2 smolts (n = 89). The gill biopsies will be used in a separate study to examine the association between infectious agents and transcriptome patterns and individual migration success. Recent work (Jeffries et al. 2014) found this biopsy procedure had negligible effects on survival of Chilko sockeye smolts. Surgeries and biopsy procedures took on average ca. three minutes per fish.

All tagged age-1 smolts were ≤ 100 mm fork length (FL) in an effort to ensure they were indeed age-1 (Irvine and Akenhead 2013; Table 1). A minimum size of 85 mm FL was chosen to limit tag burdens. To help ensure that “age-2 smolts” were from the older age class, smolts ≥120 mm FL were selected, with the exception of 6 slightly smaller smolts (117-119 mm FL). Post-surgery, smolts were transferred into an in-river release pen located below the counting fence and near the centre of the river. Smolts were allowed to recover for an average of 10.5 hours before being released at night between 23:30 and 1:30 along with ~200 untagged smolts to provide a small school. Tagged age-1 and age-2 smolts were released together in an approximately 2:1 ratio.
2.2.3 Tag programming

To help ensure all age-1 smolts would be detected at the final arrays within the projected life span of the VEMCO V4 transmitters (which have an especially limited battery life), tag programming was designed based on previous experience with travel times of age-2 smolts (Clark et al. 2016). V4 tags were programmed to transmit with a randomized time interval of 45-105 seconds for 4 days, followed by 30-60 seconds for 7 days, then a 10-day silent period where acoustic transmission was temporarily terminated to reserve power. At day 10 the V4 tags reactivated transmission with a time interval of 30-60 seconds for the remainder of the battery lifespan. This strategy was designed so that the 10-day silent period occurred while fish were expected to be transiting the Strait of Georgia after passing the Fraser River mouth acoustic arrays and begin transmitting again before reaching the 180 kHz capable receiver arrays in the Discovery Islands. This specific tag programming resulted in an estimated tag lifespan of 52 days. VEMCO V7 tags were programmed to transmit every 20-40 seconds for the first 14 days of the migration period, followed by 40-80 seconds for the remainder of the projected tag life span (123 days total), similar to what Clark et al. (2016) and Rechisky et al. (2018) used. The V7 tags (rather than V4) were used in age-2 smolts because of their stronger battery, their ability to be detected at the final array (Array K, 1044 km), and to allow direct comparisons with previous telemetry studies. For both tag types, the initial shorter transmission interval was used to boost the probability of detection during the initial migration phase, when smolts migrated rapidly past individual receivers in relatively narrow rivers.
2.2.4 Acoustic receiver arrays

Groups of VEMCO VR2W, VR3, and VR4 (69 kHz and 180 kHz) acoustic receivers (referred to as arrays) were deployed throughout the migration route (Figure 2.1). Freshwater array locations were the same as those used in previous studies (Clark et al. 2016). Array A was located by the release pen (i.e., 0 km from release) and Array B was 14 km downstream in the Chilko River. Arrays C (80 km) and D (178 km) were located in the Chilcotin River. Arrays E (599 km), F (627 km), and G (657 km) were located in the lower Fraser River into the estuary. Marine arrays H (804 km; Northern Strait of Georgia), I (882 km; Discovery Islands), J (951 km; Johnstone Strait), and K (1044 km; Queen Charlotte Strait) were maintained by the Ocean Tracking Network (OTN) and Kintama Research Ltd., and were used to detect smolts as they moved north in the Salish Sea. Both age-1 and age-2 smolts could be detected on all arrays, with the exception of Array K (the final array), which was equipped to only detect 69 kHz tags in the age-2 fish. Thus, Array J was the last array along the northern migration route able to detect age-1 smolts. Eight receivers on Array H were able to detect both 69 kHz and 180 kHz tags, but the majority could only detect 69 kHz tags. A southern marine array with 69 kHz receivers exists in Juan de Fuca Strait, but did not detect any smolts.

2.2.5 Laboratory holding study

To determine tag retention and potential tagging effects on survival, I implanted age-1 (n = 38, FL = 82-98 mm) and age-2 (n = 20, FL = 115-129 mm) smolts with dummy tags that had the same dimensions and weight as the transmitting V4 and V7 tags. All surgical procedures were conducted in the same manner as for smolts that were released. Dummy-tagged and non-
tagged (control) age-1 (n = 43) and age-2 (n = 20) smolts, were held in a ~1000-L flow-through tank on shore next to our release location for 12 days to simulate the longest potential duration spent in freshwater during outmigration (Clark et al. 2016). The tank was checked at least twice a day for water quality and smolt mortalities. The size of dummy-tagged age-1 smolts (FL = 90 ± 4 mm) did not differ significantly from age-1 controls (FL = 89 ± 4 mm; ANOVA, P = 0.19). Dummy-tagged age-2 smolts (124 ± 3 mm FL) were significantly larger than age-2 controls (119 ± 3 mm FL; ANOVA, P < 0.001).

2.2.6 Survival analysis

A spatial mark-recapture approach was used to estimate survival of acoustic-tagged smolts where detection at each acoustic receiver array along the migration path was interpreted as ‘recapture’. Estimates of survival (φ), detection probability (p), and their associated variances were calculated using the Cormack-Jolly-Seber (CJS) model (and special cases of the CJS model) for live-recaptured animals (Cormack 1964, Jolly 1965, Seber 1965). This model jointly estimates survival and detection probability within a maximum likelihood framework. The R (R Core Team 2015) with the package RMark (Laake 2015) was used to construct CJS models using Program MARK (White and Burnham 1999). CJS model assumptions apply for all analyses: equal survival probability, equal probability of detection, and instantaneous sampling.

The analysis followed a series of steps described below. First, in preparation for analysis the detection data were screened for false detections which could occur because of environmental conditions or collisions between acoustic-tag transmissions (for full details on screening methods see Clark et al. 2016), forming detection histories for each tagged individual,
and assessing goodness of fit (GOF) of the data to the model. None of the 3,259 detections of smolts recorded were considered false. Second, I assessed if fork length and tag burden affected survival for age-1 and age-2 smolts separately. I then estimated survival for each migration segment and used the resulting segment-specific survival estimates to calculate cumulative survival from release, and survival rates per unit time and distance. Finally, the age-1 and age-2 groups were combined to evaluate the effect of age on survival.

A capture history (CH) is a sequence of 1s and 0s that indicates whether each individual smolt was detected at each acoustic array during their migration. The CH sequence differed by age group because the detection efficiency of the arrays differed by tag type (and thus by age group). Arrays that detected few fish were excluded (Table A1). CJS models confound $\phi$ and $p$ at the final detection site. For age-1 smolts, Array J was divided into two subarrays (East and West) in order to obtain survival estimates to arrival at Array J. For age-2 smolts and for the combined age analysis, Array G was divided into two subarrays (Upper and Lower; Figure 2.1) in order to obtain survival estimates to the river mouth (~ 25 km from the ocean). I note that the close spacing between these subarrays (~25 km and 10 km) means they may not function independently.

Goodness of fit (GOF) of the data was assessed to a fully parameterized CJS model ($\phi$ and $p$ estimated at each applicable subarray) using the bootstrapped GOF test within Program MARK with 1000 simulations. Each age group was tested separately, and then a third test was run for the age groups combined. After bootstrapping, there are two approaches to estimating the over-dispersion parameter $\hat{c}$: one approach is based on the deviance and the second is based on $\hat{c}$. To be conservative, the higher of the two values was used. For age-1, $\hat{c}$ was 1.51. For age-2 and for the combined age group, $\hat{c}$ was 0.81 and 0.92 respectively which was increased to 1 (Cooch
and White, 2016). I adjusted the Akaike’s Information Criteria (AIC) values and inflated (multiplied) the standard errors on the estimates by the $\hat{c}$ value to account for overdispersion in the data.

I used four candidate hypotheses to estimate survival ($\phi$) and detection probability ($p$) for age-1 and age-2 Chilko River sockeye salmon. First, for each age group separately, a base model was built where $\phi$ and $p$ were estimated independently in each migration segment and on each array ($\phi$(segment) $p$(array)). Next, I assessed if fork length, tag burden, or gill clipping affected survival. I hypothesized that these factors might cause a consistent shift in survival without changing the relative mortality between migration segments (i.e. an additive effect). I also considered that any tag burden effects might manifest most strongly shortly after release as the result of the tagging process so I included a hypothesis where the burden effect was limited to the first 80 km migration segment between Array A (release) and C. The tag burden parameter was intended to represent the potential effects of the acoustic tags; however, for a given tag size it was correlated with fork length (Spearman's rho = -0.97 for age-1 and -0.93 for age-2; $P < 0.001$) so any effect on survival could be due to either parameter. I used AIC corrected for overdispersion and low sample size (QAICc) to compare the relative performance of these four models. Since there was weak relative evidence that length, burden, or gill clipping affected survival (Table 4), I extracted the final estimates of survival and detection probability for each age group from their base model. To calculate cumulative survival estimates from release to each applicable array, I multiplied survival probabilities for the consecutive migration segments. The variance for the cumulative survival estimates was derived using the Delta Method.
2.2.7 Survival rates and migration timing

In order to better compare survival between migration segments, I converted the survival estimates to survival rates per day and per km as:

$$S^{1/d}$$

where $S =$ estimated survival, and $d =$ the mean travel time (days) or mean distance travelled (km). Segment travel time (days) was calculated for each fish from release to arrival on the first array, and then from departure from one array until arrival at the next along the migratory path. Distances were measured for each fish as the shortest in-water distance between the central point of each array. For Array I (Discovery Islands) that spans multiple channels, I measured the distance to the central point of each channel (i.e. each component subarray).

Since both survival and travel time are random variables with associated error, bootstrapping was used to calculate the variance around the estimates of daily survival rate. First, fish were sampled 1000 times with replacement and survival was calculated using the CJS model as described above for each sample. It was not possible to calculate the travel times for all samples because not all fish that survive have travel times (individual fish have to be detected on both ends of the segment in order to have a travel time calculation). Rather than discard samples without fully corresponding travel times, the travel times were calculated for each segment from separate samples that were drawn only from fish with travel times in that segment. Survival rates were then calculated by applying all 1000 travel time samples to each survival sample to generate the rate. The mean of these estimates was used as the final estimate of survival rate per day, and the distribution to calculate standard deviations and confidence intervals on the mean.
For consistency, bootstrapping was also used to estimate survival rate per km; however, error in the distance estimates was of less concern because there were only a few alternate routes, and because the true distance swam is unknown. I simply adjusted the survival estimates for each sample by the average migration distance in each segment. Although the bootstrapped results accounted for error in both survival and travel time estimates, this method also underestimates the error on survival because it does not include the variance inflation factor ($c^\hat{}$). Currently, there is no clear method for handling over dispersed data when employing bootstrap techniques. Because $c^\hat{}$ was only 1.5 for age-1 smolts and 1 for age-2 smolts, this effect was likely minimal.

Migration rates (km/day) between each pair of arrays were determined using the segment travel time and the shortest in-water distance travelled. To investigate the differences in migration rate between age classes and between migration segments I used a pairwise comparison (ANCOVA) using Bonferroni adjusted $P$-values, with only ecologically relevant comparisons included. To assess patterns in diel timing of migration, I used one-sample proportions tests to compare the proportion of smolts arriving to each array during the night to the proportion of night-time hours in a 24-hour period. Night and day-time hours were calculated using the average sunrise and sunset standard times over the migration period of the tagged smolts (National Research Council Canada 2016, https://www.nrc-cnrc.gc.ca/eng/services/sunrise/advanced.html).

**2.2.8 Survival between age classes**

As a final step, I evaluated the effect of age on survival. For this comparison, I modeled $\phi$ both with and without effects for age and used AIC to compare the performance of the resulting
model set. I hypothesized both that age might cause a constant shift in survival across all migration segments ($\phi(\text{segment}+\text{age})$), or that age might have a different effect in different segments ($\phi(\text{segment}^{\ast}\text{age})$). In all models, detection probability was modeled independently for each tag type ($p(\text{array}^{\ast}\text{tag type})$). I note that this comparison is confounded because the age-2 smolts were both physically larger and carried a larger tag burden than age-1 smolts (Table 2.1). Thus, any difference in survival between the groups could also be attributed to these differing characteristics. Although there was little evidence that fork length or tag burden affected the survival of each age group separately (Table 2.4), these results should be interpreted carefully since they apply to the more limited data range encompassed within each group.

### 2.2.9 Marine route selection and survival

Since some smolts were detected on more than one of the three channels across Array I, route selection was assigned using the last detection site across Array I. Given that the sample sizes were small when divided by route, I estimated route-specific survival as a proportion: the number of smolts detected at Array J (Johnstone Strait) and thus known to have survived, divided by the number of smolts assigned to each migration route. This method underestimates survival because it does not account for smolts that survived but were not detected. I assumed the detection efficiency for each route was equivalent because the array configuration was similar across routes (receiver spacing and depth). Only one smolt was detected on the eastern-most route (Desolation Sound) and so this route was excluded from further route analysis. I converted the route-specific survival estimates to survival rate rates per day and per km using the method
described above for the main migration path, but without the use of bootstrapping. Average smolt size, tag burden, and travel times and rates were determined and compared between routes.

2.3 Results

2.3.1 Survival estimates

Cumulative survival of age-1 smolts from Array A (release) to the last array able to detect V4 tags (Array J, 951 km) was 16% (Figure 2.2, Table 2.2; age-1 fish could not be detected on Array K). Cumulative survival for age-2 smolts from release to the marine arrays (Array H-K) could not be formally estimated within a CJS framework because there were too few detections (only two individuals were detected on these arrays). However, the minimum cumulative survival of age-2 smolts from Array A – H was 2%, and remained 2% to the final array (Array K, 1044 km). Freshwater survival (Array A – G, 657 km) was 56% for age-1 smolts, twice that of age-2 smolts (28%). Model weights provided further evidence that age-1 smolts experienced higher survival than age-2 smolts throughout their migration (combined \( w_1 \) of models including age = 1.0, Table 2.4). Age-2 survival was consistently poorer than age-1 survival in all migratory segments assessed, indicating an additive effect of age (\( w_1 = 0.89 \)).

There was no evidence of an effect of gill clip on survival (Table 2.4).

Segment-specific survival was the lowest through the first phase of freshwater migration in the Chilko River (Array A – B; 14 km) and was 76% and 53% for age-1 and age-2 smolts respectively (Figure 2.3, Table 2.3). Survival for age-1 and age-2 smolts remained low through this segment when accounting for either time (survival per day; 78% and 63% respectively) or distance (survival per 100 km; 13% and 1% respectively, Table 2.3). Survival from Array B – C
(66 km) was 89% for age-1 smolts compared to 77% for age-2 smolts, whereas survival rates per day were 85% and 69%, and survival rates per 100 km were 85% and 69% respectively. Age-1 survival was 83% from Array C – D (98 km). This segment had the lowest age-1 survival per day (76%) whereas survival per 100 km was 84%. Age-1 survival was highest (100%) through the Fraser River (Array D – G; 479 km), and remained the highest (100%) when accounting for time (survival per day; 100%) or distance (survival per 100 km; 100%). Survival of age-2 smolts could not be determined for this same segment because there were too few detections at Array D. However, age-2 smolt survival was the highest (83%) over a similar segment through the Chilcotin and Fraser Rivers (Array C – E; 519 km), and remained highest when accounting for time (survival per day; 93%) or distance (survival per 100 km; 96%). Freshwater detection efficiencies for the arrays used in the analysis ranged from 10 – 98% for V4 tags in age-1 smolts, and from 27 – 97% for V7 tags in age-2 smolts (Table 2.2).

Marine survival of age-1 smolts migrating through the Strait of Georgia (Array G – I; 225 km) was 36% (Table 2.3). Marine survival rate per day was high in this segment (96%) but survival rate per 100 km was only 63%. Marine segment-specific survival was highest (79%) through the Discovery Islands (Array I – J; 69 km; Table 2.3). This segment also had a high survival rate per day (95%) and the highest marine survival rate per 100 km (73%). Overall marine survival from the mouth of the Fraser River to Johnstone Strait (Array G – J; 294 km) was 28%. Daily marine survival was 95%, whereas survival per 100 km was 64%. Marine survival is only reported for age-1 smolts because there were too few detections of age-2 smolts on the marine arrays for formal analysis. The detection efficiency for V4 tags in age-1 smolts was 50% at Array I and 22% at Array J (Table 2.2).
Among age-1 smolts, I found weak evidence that fork length and/or tag burden may have affected age-1 smolt survival given that the null model had approximately half as much relative weight \(w_i = 0.15\) as models with effects for fork length \(w_i = 0.35\) or tag burden \(w_i = 0.29\); Table 2.4). It is unlikely this possible effect of tag burden was limited to the first migration segment (Array A – C; \(w_i = 0.13\)). Larger fish were estimated to have higher survival \((\beta = 0.07 [lcl= 0.008; ucl= 0.132]\), which corresponded to a 50% increase in the odds of survival for each 5 mm increase in length), while greater tag burdens reduced survival \((\beta = -0.270 [lcl= -0.517; ucl= -0.024]\), which corresponds to a 24% decrease in the odds of survival for each 1% increase in tag burden). Within the age-2 group, the null model was the best candidate model, suggesting that fork length and tag burden did not significantly affect survival despite higher tag burdens.

### 2.3.2 Migration rates and timing

In freshwater, smolts from both age classes spent on average ~5 days migrating between the release site and the mouth of the Fraser River (Array A – G; Figure 2.4, Table 2.5). The migration rate of age-1 \((25.2 \text{ km/day})\) and age-2 \((18.8 \text{ km/day})\) smolts was significantly slower in the first 14 km (Array A – B) than in other freshwater segments (ANCOVA, \(P < 0.001\). Migration rates then significantly increased to 108.6 km/day and 105.4 km/day for age-1 and age-2 smolts respectively in the remainder of the Chilko River (Array B-C), and to 160-200 km/day (mean 187 km/day) through the Chilcotin and Fraser rivers (Array C – G) for both age classes (ANOVA, \(P < 0.001\). Travel rates of age-1 smolts did not differ significantly from age-2 smolts through any freshwater segment (ANCOVA, all \(P\)-values > 0.05). Age-1 smolts migrated
significantly faster through freshwater segments (Array B – C and Array C – G) than through marine segments (Array G – I and Array I – J; ANCOVA, \(P < 0.001\)).

Age-1 smolts began migration from the release site (Array A) during the night, and arrived at Arrays B and C primarily during night time hours (proportions tests, all \(P\)-values < 0.001; Figure 2.5). Coupled with their slow travel times (mean 1.4 days and 2.1 days from release to Array B and C respectively) this arrival timing suggests smolts were stopping migration during the day in headwater regions and migrating largely at night. Age-2 smolts arrived at Array B primarily during night-time hours (proportions test, \(P < 0.001\)); however, this did not hold true for their arrival times to Array C (proportions test, \(P = 0.90\)). For the remainder of migration to the estuary and into the nearshore environment, arrival times of both age groups suggest that smolts were migrating during both day and night periods (proportions test, all \(P\)-values > 0.05).

In the marine environment, age-1 smolts took on average 28.7 days from the mouth of the Fraser River (Array G) to reach Johnstone Strait (Array J), which was the last array where 180 kHz tags could be detected. Smolts spent an average of 24.2 days migrating through the Strait of Georgia travelling at 9.4 km/day (Array G – I; 225 km). They spent an additional 7.6 days on average in the Discovery Islands to Johnstone Strait region (Array I – J; 69 km) and travelled faster (14.5 km/day) through this segment (Table 2.5). Overall, age-1 smolts took on average 35 days from release to reach the last array they could be detected at (Array J). Travel times in the marine environment could not be determined for age-2 fish because only two fish were detected at any of the subsequent arrays.
2.3.3 Marine route selection

Nearly equal numbers of age-1 smolts took the western (Discovery Passage; \( n = 10 \)) and central (Sutil; \( n = 9 \)) channels at Array I. Only 1 smolt took the eastern channel (Desolation Sound). I was unable to determine route selection for 7 of the smolts that reached Array J because they were not first detected on Array I. Although not formally assessed within a modelling framework, survival through Discovery Passage (40%) was greater than through Sutil Channel (22%), but when accounting for distance (27% per 100 km in Discovery Passage vs 24% in Sutil) or time (83% per day in Discovery Passage vs 86% in Sutil), survival was similar between the two channels. Mean FL and tag burden also did not appear to differ between smolts that took Discovery Passage (97 mm and 6.5% respectively), or Sutil Channel (96 mm and 6.4%, respectively). Travel times and rates were faster through Discovery Passage (4.6 days and 17.4 km/day respectively) than through Sutil Channel (10 days and 11.5 km/day respectively).

2.3.4 Holding study

Survival was significantly lower for age-2 dummy-tagged smolts (85%) than for controls (100%) after 12 days (Chi-squared test, \( P = 0.04 \); Table 2.6). Although survival was also lower for age-1 dummy-tagged fish after 12 days (85%) relative to the control group (95%), the difference was not significant (Chi-squared test, \( P = 0.09 \)). The average tag burden of age-2 mortalities was slightly higher (12.8% ± 0.7) than that of age-2 smolts that survived (11.7% ± 0.9); however, tag burden did not have a significant impact on survival of dummy-tagged age-2 smolts (ANOVA, \( P = 0.09 \)). These holding study results suggest that tagging had nominal effects
on tagged age-1 migrants and a small to modest effect on survival of tagged age-2 migrants relative to losses due to other factors in the wild.

2.4 Discussion

My study is the first to use telemetry to estimate survival, travel times and rates, and migration routes for wild age-1 sockeye salmon smolts that generally comprise > 95% of the migratory population. This study is also the first to provide a direct telemetry comparison of behaviour and survival between two age classes of sockeye smolt migrants. Differences in survival between age classes began immediately after release in freshwater and persisted throughout the migration into the marine environment. Freshwater migration (Array A – G; 657 km) occurred over a 5-day period for both age classes, however, age-1 smolts surprisingly had two-fold higher freshwater survival (56%) than age-2 smolts (28%). Although I cannot directly compare survival of the two age classes in the marine environment because few age-2 smolts reached the marine arrays in 2016, it appears that this age-specific difference persisted; 16% of age-1 smolts survived to Array J (951 km) while only two age-2 smolts (2% of those released) reached Array J and Array K (1044 km).

The higher survival of the age-1 smolts compared to the older age-2 smolts was unexpected given that fish body size is often positively correlated with survival (McGurk 1996, 1999), and age-1 smolts were generally 35 mm shorter and 9.4 g smaller than age-2 smolts. For smolts in particular, larger body size can reduce predation risk during migration (Koenings et al. 1993, Furey et al. 2015a, Tucker et al. 2016). Yet many telemetry studies have found no significant effect of smolt size on migration survival (Furey 2016, Rechisky et al. 2018). These
studies, however, focused on narrow size ranges of a single age class, limiting their ability to observe size-specific differences. In fact, I found limited evidence for an influence of size on survival within each age class, particularly the age-2 smolts.

Variation in tag burden between age classes may have contributed to the observed age-specific differences in survival. Age-1 smolts, which had higher survival, also had a lower average tag burden (7.3%) than age-2 smolts (10.7%). My laboratory and field study results indicate that tag burden had a negligible impact on age-1 survival, but may have had a modest effect on age-2 smolts. Previous studies on this population found tagging to have minimal or no impact on age-2 smolt survival (Jeffries et al. 2014, Clark et al. 2016, Furey et al. 2016) or swim performance (Clark et al. 2016). However, Rechisky et al. (2018) demonstrated that in 2012, migrating age-2 smolts with higher tag burden had lower survival, particularly in the upper Chilko River. Furthermore, Brown et al. (2006) found that wild sockeye salmon smolts with tag burdens exceeding 10.3% experienced reduced critical swimming speeds compared to untagged smolts. Collins et al. (2013) demonstrated that hatchery sockeye salmon smolts with tag burdens exceeding 8% had shorter swimming durations than smolts with lower tag burdens. Lastly, Furey et al. (2016) found that acoustic tagged age-2 Chilko sockeye salmon smolts experienced reduced migration rates compared to PIT-tagged age-2 smolts in the upper Chilko River. In my study, the size of available age-2 smolts resulted in some tag burdens exceeding the suggested ~8-10% maximum needed to avoid adverse effects on swim performance, which could impact survival. Consequently, my survival estimates of age-2 smolts may underestimate the migration survival of the age-2 smolt population. More research is needed over a wide size range of smolts to determine if, and the degree that, tag burden influences telemetry-based survival estimates of smolts.
Age-specific differences in survival may also be influenced by the great disparity between the proportions of each age class in the outmigrant population. Prey that appear obviously different in a group may be more conspicuous to predators and therefore at greater risk of predation (Pielowski 1959, Mueller 1973, 1975, Ohguchi 1978, Visser 1982, Theodorakis 1989, Rutz 2012). One example of how fish may be especially conspicuous and targeted by predators is to be of a distinctly different size than the majority of others within the school (Theodorakis 1989). Given that the tagged age-2 smolts were 40% longer and 160% heavier than the tagged age-1 smolts on average (and are historically 40% longer and 300% heavier than age-1 smolts in the population on average; Irvine and Akenhead 2013) and only make up ~4% of the out-migrating population, it is possible that predators target them in preference to the age-1 smolts, particularly in the clear and relatively slower flowing Chilko and Chilcotin rivers where high age-2 mortality occurs consistently across years (Clark et al. 2016, Furey et al. 2016). The wide range of predators such as bull trout (Salvelinus confluentus), rainbow trout (Oncorhynchus mykiss), mergansers (Mergus spp), gulls (Larus and Chroicocephalus spp), and river otters (Lontra canadensis; Clark et al. 2016) that gather near the outlet of Chilko Lake to feed on smolts are visual foragers, such that larger smolts could stand out. Birds in particular often preferentially select for relatively large size classes of smolts and other juvenile fish (Britton and Moser 1982; Trexler et al. 1994, Hostetter et al. 2012, Osterback et al. 2014). Although many studies have suggested that smolt size is often positively associated with survival, particularly in the early period of ocean residence (Beckman et al. 1999, Beamish et al. 2004, Farley et al. 2007), it is possible that these processes operate on different scales.

My results agree with previous work in demonstrating that survival is landscape-dependent for Chilko sockeye salmon smolts. Both age-1 and age-2 smolts experienced poor
survival in the first 14 km of the Chilko River, a difference highlighted by the markedly lower survival rates per 100 km travelled (13% and 1% for age-1 and age-2s respectively). Although a large portion of overall mortality occurred during early migration, my results for age-1 smolt survival through this segment (76%) are similar to age-2 smolts in previous years in this system (68 – 90%; Clark et al. 2016, Furey et al. 2016, Rechisky et al. 2018). However, age-2 smolts in 2016 had lower survival through this region (53%) than previously observed. My results for age-1 smolts illustrate that, similar to age-2 smolts in earlier studies, the upper freshwater landscape of the Chilko and Chilcotin Rivers is high risk for all migrating smolts—despite the essentially pristine nature of the landscape. The increase in survival rates to nearly 100% once smolts entered the Fraser River is also consistent with previous years (Clark et al. 2016), and is attributed to reduced predation risk because of the highly turbid water and fast discharge resulting in rapid migration rates (up to ~240 km/day; Wood et al. 1993, Clark et al. 2016).

In the marine environment, the general patterns of survival for age-1 smolts were similar to those of age-2 smolts in previous years (Clark et al. 2016). Age-1 smolts experienced a decrease in survival after they entered the Strait of Georgia, potentially because the transition to saltwater is a physiologically stressful process and considered to be a highly vulnerable period for smolts (Hinch et al. 2006). Survival increased as smolts moved from the Strait of Georgia into the Discovery Islands region towards Johnstone Strait. However, age-1 smolts only took approximately one week to migrate through the Discovery Islands to the Johnstone Strait array compared to nearly a month to travel the much longer distance through the Strait of Georgia, and daily survival rate was virtually the same (96% and 95%) for these segments. Consequently, marine survival rates per day in 2016 appear to have been essentially constant and the survival
differences evident were simply the result of the different length of time smolts spent travelling through each segment.

Predation is widely assumed to be the primary cause of smolt mortality in many systems (Ruggerone and Rogers 1984, Heggenes and Borgstrom 1988, Mesa 1994, Collis et al. 2001, Evans et al. 2012, Hostetter et al. 2012, Cavallo et al. 2013), and is likely a large influence in the Chilko and Chilcotin rivers as well as the Strait of Georgia. Similar to when smolts exit Chilko Lake, smolts are likely met with an additional abundance of predators known to target salmonids as they leave the Fraser River and enter the Strait of Georgia. For example, highly abundant harbour seals (*Phoca vitulina*) in this region consume a considerable amount of salmon smolts each spring, with sockeye salmon being among their top preferred juvenile salmon prey species (2.5%; Thomas et al. 2016). Although sockeye smolts are a relatively small proportion of harbor seal diets, this translates into a considerable number of fish given the large number of harbor seals present in the Strait of Georgia (~40,000; Olesiuk 2009). Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) are abundant throughout the Salish Sea and are also known to consume salmon smolts (Kajimura et al. 1980). Other marine mammals that are likely predators that smolts encounter include California sea lions (*Zalophus californianus*), Steller sea lions (*Eumetopias jubatus*), Dall’s porpoise (*Phocoenoides dalli*), and harbour porpoises (*Phocoena phocoena*) which are all highly abundant in the Strait of Georgia, especially during the spring as smolts are migrating out from the Fraser River mouth (Keple 2002, Chasco et al. 2017). Additionally, a number of bird species (e.g. double-crested cormorants (*Phalacrocorax auritus* auritus), great blue herons (*Ardea herodias fannini*), and common mergansers (*Mergus merganser*) are present in the region and known to consume smolts (Butler et al. 2015).
The general freshwater movement patterns of both age-1 and age-2 smolts were similar to those reported for age-2 smolts in previous years (Clark et al. 2016). Migration rates of age-1 smolts were the slowest though the upper Chilko River in 2016 (2.6 BL/s) but comparable to age-2 smolt rates through this region in most previous years (2.2 – 3.9 BL/s; 2011-2014), with the exception of age-2 smolts 2010 (1.3 BL/s). However, migration rates of age-2 smolts through this region in 2016 (1.7 BL/s) were slower than most previous years. Migration rates were likely the slowest through the upper Chilko River compared to the subsequent freshwater segments across years because the water velocity is much slower in this segment compared to the Chilcotin and Fraser rivers. Average freshwater migration rates from Array B onward were more rapid (9.6 BL/s – 23.6 BL/s) for both age groups in 2016 and were faster than age-2 smolts in previous years (3.2 BL/s – 14 BL/s), potentially due to higher water levels and faster flows in 2016. Although maximum absolute swim speed generally increases with fish size (Weihs and Webb 1983), I presume migration rates do not appreciably differ between age classes in these landscapes due to the rapidly flowing Chilcotin and Fraser rivers dominating overall movement patterns, and smolts may adopt a passive migration strategy at this time (Fried 1978, Melnychuk et al. 2010, Clark et al. 2016).

In the marine environment, age-1 migration rates slowed dramatically and became more variable (0.3 BL/s – 3.8 BL/s). Age-1 marine migration rates were similar to both prior telemetry estimates (Clark et al. 2016, Welch et al 2009) and to migration estimates using otolith microstructure techniques (Freshwater et al. 2016). Although my marine migration rate estimates were determined by only 14 smolts, they are also comparable with the optimal swimming speeds (0.8 – 2 BL/s) for small sockeye salmon smolts (Hinch et al. 2006). Slowed migration rates are
likely due to a shift from passive travel down the fast rivers to active swimming in marine waters where smolts may also stop to feed and routes may not be strictly linear (Furey et al. 2015b).

The time smolts spend migrating through the different regions of the nearshore environment is important to consider given that it directly influences their exposure to predators (Christensen and Trites 2011), pathogens (Miller et al. 2014), food resources (Mackas et al. 2004, McKinnell et al. 2014), and other marine conditions. Age-1 smolts spent the majority of time in the Strait of Georgia (~24 days) and traveled more slowly (~1 BL/s; ~9 km/day) compared to the much shorter region between the Discovery Islands and Johnstone Strait (~8 days; 1.7 BL/s; ~15 km/day). Age-2 smolts in previous years travelled at ~1 BL/s (10 – 20 km/day) through the Strait of Georgia and increased their rate to 1.7 BL/s (7.7 – 46 km/day) as they moved through the Discovery Islands and Johnstone Strait to Queen Charlotte Strait (Clark et al. 2016, Rechisky et al. 2018). Although marine migration rates were measured over different distances in prior years, migration rates were generally faster through final marine segment compared to the initial segment in the Strait of Georgia. Smolts may travel faster as they move north because of the strong tidal surface currents from Discovery Islands to Johnstone Strait (surpassing ~4m/s in some areas; Foreman et al. 2012), which could enable rapid migration if smolts modify their behaviour to shelter from the tidal currents in bays or near bottom when the tide runs against them, and then only actively migrate when the tide runs with them (Metcalf et al. 1990, Metcalfe & Arnold 1997). Smolts may travel more slowly through the Strait of Georgia due to the potentially higher prey densities compared to the Discovery Islands and Johnstone Strait. Neville et al. (2016) found that in 2014, twice as many sockeye salmon smolts had empty stomachs in the Discovery Islands (58%) compared to those in the Strait of Georgia (29%). Thus, smolts may migrate slower in the Strait of Georgia to feed before reaching a region of lower prey.
availability. Conversely, Price et al. (2013) found few empty stomachs in sockeye salmon smolts throughout these regions (0-3%) during 2009 and 2010, suggesting that prey availability and distribution may vary among years. Regardless, if rapid growth occurs while feeding in the Strait of Georgia, it could contribute to the higher swim rates once smolts reach the Discovery Islands.

The variance in selected migration routes through the Discovery Islands provides a unique insight into the potential of route-specific survival for smolts through this region. It appears that smolts preferred Discovery Passage and Sutil Channel, as only one smolt was detected in Desolation Sound. Though I could not formally test whether survival differed by route, survival to Johnstone Strait was nearly double through Discovery Passage (4 of 10; 40%) compared to Sutil Channel (2 of 9; 22%). However, when distance or time was accounted for, survival was similar between the two channels. Healy et al. (2017) found that acoustically tagged and tracked migrating steelhead smolts (Oncorhynchus mykiss) using Discovery Passage experienced over twice as high survival to Johnstone Strait as those that used Sutil Channel. The ecological factors driving potential differences in sockeye salmon smolt route selection and route-specific survival remains poorly understood and future research should focus on tracking increased sample sizes of smolts through this important seascape.

2.5 Conclusion

My study provides the first survival estimates for age-1 smolts during their freshwater and early marine life phase. Age-1 smolts form >95% of the Chilko Lake smolt run, which is one of the most important populations of Fraser River sockeye salmon. My successful use of miniaturized transmitters in age-1 smolts highlights the ability to tag and track smaller
individuals and provide more representative information on this important age class. I confirmed that the upper tributaries of the Chilko and Chilcotin rivers are regions of low survival for age-1 smolts, as previously identified for age-2 smolts tracked from 2010-2014 (Clark et al. 2016, Rechisky et al. 2018). I also demonstrated that age-1 survival was fairly low in the Strait of Georgia compared to age-2 smolts in previous years. I provided evidence that although spatial trends were similar, survival of age-1 smolts may be higher than the less abundant age-2 smolts. Because smolt size is usually assumed to influence adult returns of sockeye salmon and population productivity, future work should seek to understand if there are true ecological differences in survival between age classes. I suggest that smolt selection of specific migratory corridors through the Discovery Islands region may be important, and future studies should focus on sockeye salmon smolt movements and environmental conditions through this region.
2.6 Chapter 2 tables

Table 2.1: Summary of tagged and released Chilko Lake sockeye salmon smolts in 2016.

<table>
<thead>
<tr>
<th>Age Class</th>
<th>( n )</th>
<th>( n ) Gill Clip</th>
<th>Fork Length (mm)</th>
<th>Mass (g)</th>
<th>Tag Burden (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean (± SD)</td>
<td>Range</td>
<td>Mean (± SD)</td>
</tr>
<tr>
<td>Age-1</td>
<td>200</td>
<td>100</td>
<td>93 (± 3.8)</td>
<td>85 - 100</td>
<td>5.9 (± 0.7)</td>
</tr>
<tr>
<td>Age-2</td>
<td>99</td>
<td>89</td>
<td>128 (± 5.7)</td>
<td>117 - 143</td>
<td>15.3 (± 2.2)</td>
</tr>
</tbody>
</table>

Table 2.2: Cormack-Jolly-Seber model estimates of cumulative survival (SE) for age-1 and age-2 Chilko Lake sockeye salmon smolts, and detection efficiencies (SE) of each receiver array. na = not applicable because too few fish were detected for an estimate to be determined.

<table>
<thead>
<tr>
<th>Cumulative Survival ( \Phi ) (SE)</th>
<th>Detection Efficiency ( p ) (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 1</td>
<td>Age 2</td>
</tr>
<tr>
<td>Age 1</td>
<td>Age 2</td>
</tr>
<tr>
<td>Array B (14 km)</td>
<td>0.76 (0.04) 0.53 (0.05)</td>
</tr>
<tr>
<td>Array C (80 km)</td>
<td>0.68 (0.06) 0.41 (0.06)</td>
</tr>
<tr>
<td>Array D (178 km)</td>
<td>0.56 (0.13) na</td>
</tr>
<tr>
<td>Array E (599 km)</td>
<td>na</td>
</tr>
<tr>
<td>Array G (657 km)</td>
<td>0.56 (0.13) 0.28 (0.05)</td>
</tr>
<tr>
<td>Array I (882 km)</td>
<td>0.20 (0.06) na</td>
</tr>
<tr>
<td>Array J (951 km)</td>
<td>0.16 (0.11) na</td>
</tr>
</tbody>
</table>
Table 2.3: Cormack-Jolly-Seber model estimates of segment-specific survival (SE), survival rates per day (SE), and survival rates per 100 km (SE) for age-1 and age-2 Chilko Lake sockeye salmon smolts through the migration route. na = not applicable due to too few detections for an estimate to be determined.

<table>
<thead>
<tr>
<th>Migration Segment</th>
<th>Segment Survival</th>
<th>Survival Rate per day (SE)</th>
<th>Survival Rate per 100 km (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Segment Survival</td>
<td>Age 1</td>
<td>Age 2</td>
</tr>
<tr>
<td></td>
<td>Φ (SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A – B (14 km)</td>
<td>0.76 (0.04)</td>
<td>0.78 (0.03)</td>
<td>0.13 (0.04)</td>
</tr>
<tr>
<td></td>
<td>0.53 (0.05)</td>
<td>0.63 (0.05)</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td>B – C (66 km)</td>
<td>0.89 (0.06)</td>
<td>0.85 (0.06)</td>
<td>0.84 (0.07)</td>
</tr>
<tr>
<td></td>
<td>0.77 (0.09)</td>
<td>0.69 (0.12)</td>
<td>0.69 (0.12)</td>
</tr>
<tr>
<td>C – D (98 km)</td>
<td>0.83 (0.17)</td>
<td>0.76 (0.17)</td>
<td>0.84 (0.12)</td>
</tr>
<tr>
<td></td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>D – G (479 km)</td>
<td>1.00 (0.00)</td>
<td>1.00 (0.02)</td>
<td>1.00 (0.01)</td>
</tr>
<tr>
<td></td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>C – E (519 km)</td>
<td>na</td>
<td>0.93 (0.06)</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>0.83 (0.16)</td>
<td>na</td>
<td>0.96 (0.03)</td>
</tr>
<tr>
<td>E – G (58 km)</td>
<td>na</td>
<td>0.64 (0.26)</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>0.83 (0.15)</td>
<td>na</td>
<td>0.74 (0.19)</td>
</tr>
<tr>
<td>C – G (577 km)</td>
<td>0.83 (0.17)</td>
<td>0.94 (0.04)</td>
<td>0.97 (0.02)</td>
</tr>
<tr>
<td></td>
<td>0.69 (0.11)</td>
<td>0.89 (0.04)</td>
<td>0.94 (0.02)</td>
</tr>
<tr>
<td>G – I (225 km)</td>
<td>0.36 (0.13)</td>
<td>0.96 (0.01)</td>
<td>0.63 (0.09)</td>
</tr>
<tr>
<td>I – J (69 km)</td>
<td>0.79 (0.55)</td>
<td>0.95 (0.05)</td>
<td>0.73 (0.27)</td>
</tr>
</tbody>
</table>
Table 2.4: Cormack-Jolly-Seber model selection results to assess the effects of age, fork length, tag burden, and gill clipping on the survival of Chilko Lake sockeye smolts during freshwater migration in 2016. Detection efficiency was allowed to vary freely by array in all models $p(array)$. FL=fork length; QAICc= Quasi-likelihood Akaike Information Criterion adjusted for small sample sizes; K= number of parameters.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Model</th>
<th>n</th>
<th>K</th>
<th>QAICc</th>
<th>ΔQAICc</th>
<th>$w_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age-1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fork Length</td>
<td>$\phi(\text{segment}^a + \text{FL})$</td>
<td>200</td>
<td>14</td>
<td>641.33</td>
<td>0.00</td>
<td>0.35</td>
</tr>
<tr>
<td>Tag Burden</td>
<td>$\phi(\text{segment} + \text{burden})$</td>
<td>200</td>
<td>14</td>
<td>641.69</td>
<td>0.36</td>
<td>0.29</td>
</tr>
<tr>
<td>Null</td>
<td>$\phi(\text{segment})$</td>
<td>200</td>
<td>13</td>
<td>643.01</td>
<td>1.68</td>
<td>0.15</td>
</tr>
<tr>
<td>Tag Burden to Array C</td>
<td>$\phi(\text{segment} + \text{burden}_{\text{Array A-C}})$</td>
<td>200</td>
<td>14</td>
<td>643.38</td>
<td>2.05</td>
<td>0.13</td>
</tr>
<tr>
<td>Gill Clip</td>
<td>$\phi(\text{segment} + \text{clip})$</td>
<td>200</td>
<td>14</td>
<td>644.28</td>
<td>2.95</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Age-2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>$\phi(\text{segment})$</td>
<td>99</td>
<td>9</td>
<td>398.56</td>
<td>0.00</td>
<td>0.44</td>
</tr>
<tr>
<td>Fork Length</td>
<td>$\phi(\text{segment} + \text{FL})$</td>
<td>99</td>
<td>10</td>
<td>399.79</td>
<td>1.23</td>
<td>0.24</td>
</tr>
<tr>
<td>Tag Burden to Array C</td>
<td>$\phi(\text{segment} + \text{burden}_{\text{Array A-C}})$</td>
<td>99</td>
<td>10</td>
<td>400.50</td>
<td>1.95</td>
<td>0.17</td>
</tr>
<tr>
<td>Tag Burden</td>
<td>$\phi(\text{segment} + \text{burden})$</td>
<td>99</td>
<td>10</td>
<td>400.70</td>
<td>2.14</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Both Age Classes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (additive)</td>
<td>$\phi(\text{segment}+\text{age})$</td>
<td>299</td>
<td>12</td>
<td>1105.27</td>
<td>0.00</td>
<td>0.89</td>
</tr>
<tr>
<td>Age (varying)</td>
<td>$\phi(\text{segment}*\text{age})$</td>
<td>299</td>
<td>14</td>
<td>1109.43</td>
<td>4.16</td>
<td>0.11</td>
</tr>
<tr>
<td>Null</td>
<td>$\phi(\text{segment})$</td>
<td>299</td>
<td>11</td>
<td>1122.64</td>
<td>17.38</td>
<td>0.00</td>
</tr>
</tbody>
</table>

$a$ Notation indicates that estimates differ for each migration segment
Table 2.5: Mean segment-specific migration times (days; SE) and rates (km/day and BL/s; SE) for age-1 and age-2 Chilko Lake sockeye salmon smolts though the migration route. BL/s = body lengths per second. na = not applicable due to too few detections to determine travel times or rates.

<table>
<thead>
<tr>
<th>Migration Segment</th>
<th>Travel Time (days)</th>
<th>Migration Rate (km/day)</th>
<th>Migration Rate (BL/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age 1</td>
<td>Age 2</td>
<td>Age 1</td>
</tr>
<tr>
<td>A – B (14 km)</td>
<td>1.1 (0.09)</td>
<td>1.4 (0.16)</td>
<td>25.2 (20.10)</td>
</tr>
<tr>
<td>B – C (66 km)</td>
<td>0.7 (0.03)</td>
<td>0.7 (0.04)</td>
<td>108.6 (38.02)</td>
</tr>
<tr>
<td>C – D (98 km)</td>
<td>0.6 (0.03)</td>
<td>na</td>
<td>159.5 (20.74)</td>
</tr>
<tr>
<td>D – G (479 km)</td>
<td>2.7 (0.05)</td>
<td>na</td>
<td>185.6 (9.42)</td>
</tr>
<tr>
<td>C – E (519 km)</td>
<td>2.6 (0.07)</td>
<td>2.6 (0.07)</td>
<td>na</td>
</tr>
<tr>
<td>E – G (58 km)</td>
<td>0.4 (0.09)</td>
<td>0.3 (0.05)</td>
<td>na</td>
</tr>
<tr>
<td>C – G (577 km)</td>
<td>3.1 (0.04)</td>
<td>3.1 (0.07)</td>
<td>187.6 (14.25)</td>
</tr>
<tr>
<td>A – G (657 km)</td>
<td>5.1 (1.9)</td>
<td>5.4 (1.9)</td>
<td>135.4 (27.89)</td>
</tr>
<tr>
<td>G – I (225 km)</td>
<td>24.2 (3.67)</td>
<td>na</td>
<td>9.4 (2.54)</td>
</tr>
<tr>
<td>I – J (69 km)</td>
<td>7.6 (1.7)</td>
<td>na</td>
<td>14.5 (7.07)</td>
</tr>
<tr>
<td>G – J (294 km)</td>
<td>28.7 (3.61)</td>
<td>na</td>
<td>10.7 (2.73)</td>
</tr>
<tr>
<td>A – J (1044 km)</td>
<td>35.0 (1.5)</td>
<td>30.9 (1.0)</td>
<td>27.8 (4.34)</td>
</tr>
</tbody>
</table>
Table 2.6: Summary of dummy tagged Chilko Lake sockeye salmon smolts held and monitored for 12 days. Survival to day 5 is reported as this was the average time it took smolts to reach the ocean from the release site.

<table>
<thead>
<tr>
<th>Tag</th>
<th>n</th>
<th>Mean fork length (mm; SD)</th>
<th>Mean tag burden in air (%; SD)</th>
<th>Survival to day 5</th>
<th>Survival to day 12 (end)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V4</td>
<td>38</td>
<td>90 ± 3.8</td>
<td>8.2 ± 1.1</td>
<td>34 (89%)</td>
<td>32 (85%)</td>
</tr>
<tr>
<td>Control</td>
<td>43</td>
<td>89 ± 3.6</td>
<td>0</td>
<td>42 (98%)</td>
<td>41 (95%)</td>
</tr>
<tr>
<td>Age-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V7</td>
<td>20</td>
<td>124 ± 2.8</td>
<td>11.9 ± 0.9</td>
<td>17 (85%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>119 ± 3.1</td>
<td>0</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>
Figure 2.1: Study area for Chilko Lake sockeye salmon smolts (*Oncorhynchus nerka*) in 2016. Blue star represents the release site. Yellow circles and lines represent either individual receivers or receiver arrays able to detect V7 (69 kHz) tags. Red circles and lines represent individual receivers or receiver arrays able to detect V4 (180 kHz) and V7 tags. Smolts were collected and tagged between April 17 – 23 in 2016.
Figure 2.2: Cumulative survival estimates (± SE) of wild acoustic tagged age-1 (implanted with V4 tags) and age-2 (implanted with V7 tags) Chilko Lake sockeye salmon smolts from release near the outlet of Chilko Lake to the Johnstone Strait array (JS; Array J). Cumulative marine survival estimates for age-2 smolts could not be formally determined beyond the Johnstone Strait array due to only two fish being detected. Error bars represent 95% confidence intervals. SOG = Strait of Georgia, DP/JS = Discovery Passage/Johnstone Strait.
**Figure 2.3:** Model-averaged segment specific survival (± 95% CI) estimates for acoustic tagged sockeye salmon smolts (*Oncorhynchus nerka*), (A) per migration segment, (B) per day, and (C) per 100 km for freshwater and marine regions following their release. Marine segment survival estimates for age-2 smolts could not be formally determined because only two fish were detected in the ocean.
Figure 2.4: Migration times (days; A) and rates (km/day; B) of acoustic tagged age-1 and age-2 sockeye salmon smolts (*Oncorhynchus nerka*) per migration segment for freshwater and marine regions following their release. Marine migration times and rates for age-2 smolts could not be formally determined due to only two fish being detected.
Figure 2.5: Beanplots of diel arrival times of age-1 and age-2 sockeye salmon smolts (Oncorhynchus nerka) to each array, with the exception of the release site where departure times (last detections) were used. Grey shaded area represents night-time hours calculated using the average sunrise and sunset standard times over the migration period of the tagged smolts. Solid lines represent individual smolt arrival times to each array. Dotted lines represent the median arrival times for each array. Note that y-axis begins and ends immediately prior to sunset.
Chapter 3: Sockeye salmon (*Oncorhynchus nerka*) smolt migrations: The role of infectious agents and transcriptome profiles on freshwater and early marine survival

3.1 Introduction

The seaward migration of anadromous Pacific salmon smolts (*Oncorhynchus spp*) is an impressive annual event in which millions of fish travel thousands of kilometres from freshwater rearing areas to marine environments. During this period, smolts undergo metabolically and physiologically demanding processes in preparation for the transition to saltwater, and mortality rates can often be high (Groot and Margolis 1991, Welch et al. 2009, 2011, Clark et al. 2016). Survival during out-migration may be particularly important for the productivity of salmon populations (Beamish et al. 2004, Farley et al. 2007, Irvine & Akenhead 2013), thus it is imperative to investigate potential causes of mortality during this critical life stage.

The large number of factors that can influence survival of migrating smolts makes it challenging to establish and understand the specific causes of recent declines in smolt survival and salmon population productivity. Predation (Ruggerone and Rogers 1984, Collis et al. 2001, Evans et al. 2012, Hostetter et al. 2012, Cavallo et al. 2013), prey availability (Beamish and Mahnken 2001, Hertz et al. 2016), and variable abiotic conditions (Beamish et al. 2000; Friedland et al. 2014) have been considered to be primary influences on smolt survival. Small size and slower growth rates are often associated with increased smolt mortality, particularly in the early period of ocean residence (Beckman et al. 1999, Beamish et al. 2004, Farley et al. 2007). In addition, before leaving freshwater, juvenile salmon smolts must undergo intensive
changes to ion regulation in the gills (smoltification) to prepare for high salinity environments, and those that are inadequately prepared may experience osmoregulatory failure, which can affect feeding capacity and result in mortality directly or through increased risk of predation (Franklin et al. 1992, Kennedy et al. 2007). Other aspects of salmon condition, including infectious agent profiles (e.g. viruses, bacteria, fungi, protozoans, myxozoans) and stress and immune activation can also influence smolt survival (Hostetter et al. 2011, Van Gaest et al. 2011, Ferguson et al. 2012, Jeffries et al. 2014, Miller et al. 2014), as well as increasing vulnerability to additional stressors (Miller et al. 2014). However, linking fish health and condition with migration success is difficult because mortalities are rarely observable in the wild (Miller et al. 2014). Recent developments in molecular biology and telemetry have opened new avenues to investigate smolt health and condition. High-throughput real-time quantitative polymerase chain reaction (HT-qRT-PCR) allows for simultaneous assessment of infectious agent presence and load as well as host biomarkers associated with immune and stress responses on a large number of samples (Miller et al. 2014). Latest advancements of these methods also include biomarker panels that can recognize fish in an active viral disease state (viral disease development [VDD]; Miller et al. 2017). Recent studies have used acoustic telemetry to estimate survival of sockeye salmon smolts (Onchorhynchus nerka) as they migrate across hundreds of kilometres through the Fraser River watershed and the Salish Sea (e.g., Welch et al. 2009, 2011; Clark et al. 2016). Combining telemetry with transcriptomic assessments of non-lethal gill biopsies of have effectively revealed links among infection, expression of immune response genes, and survival in migrant smolts (Jeffries et al. 2014), but this approach has rarely been applied.
My study aimed to identify links between presence and load of infectious agents, physiological state (i.e., immune and stress responses, and osmoregulatory preparedness) and survival of out-migrating Chilko Lake sockeye smolts in freshwater and marine environments. Chilko Lake sockeye are one of the largest populations in the Fraser River watershed and are a key indicator stock for salmon management in British Columbia (Cass 1989; Irvine and Akenhead 2013). Recent telemetry-based survival estimates for Chilko smolts are generally low during downstream migration and through the Salish Sea (4-14% survival over 1044 km migration to open ocean; Clark et al. 2016, Rechisky et al. 2018). Survival is particularly low in the upper tributaries of the Chilko River (74 – 90% over 14 km migration; Clark et al. 2016, Rechisky et al. 2018). Furthermore, Jeffries et al. (2012) found that smolts with infectious haematopoietic necrosis virus (IHNV) experienced poor survival within the first 14 km of their out-migration. IHNV is a cold-water pathogen naturally present in Chilko Lake and can cause significant mortality in juvenile salmonids (LaPatra 1998). These current telemetry studies, however, only included age-2 smolts, which make up < 5% of the out-migrating Chilko Lake population (Irvine and Akenhead 2013). With new, miniaturized acoustic transmitters, I can determine if the findings from these prior studies are applicable to the smaller age-1 smolts that make up vast majority of migrants. I collected non-lethal gill biopsies from tagged wild age-1 Chilko Lake sockeye smolts and tracked them over the first >1000 km of their migration. I also gill-clipped and tagged co-migrating larger age-2 smolts in order to make direct comparisons between age classes for the first time. I predicted that smolts with higher presence and load of infectious agents, along with gene expression indicating immune and/or stress responses would experience poor survival in both freshwater and the early marine environment.
3.2 Methods

3.2.1 Study system

Chilko Lake is one of the largest lakes in British Columbia, Canada and is located in the Fraser River watershed 654 km upstream from the Pacific Ocean (Figure 3.1). Up to 70 million sockeye salmon smolts exit the lake during spring each year and migrate downstream into the Chilko and Chilcotin Rivers, through the Fraser River, and into the Salish Sea. Smolts then predominantly travel northeast through the Discovery Islands, Johnstone Strait, and Queen Charlotte Strait before moving offshore into the open Pacific Ocean (Figure 3.1). Fisheries and Oceans Canada (DFO) have estimated daily smolt abundances during the outmigration period since the 1950’s using an enumeration fence located at the outlet of the lake.

3.2.2 Tagging and gill biopsies

Tagging and gill biopsy methods were performed following procedures outlined by Jefferies et al. (2014) and Healy et al. (2018; see for further details). Two-hundred age-1 (85 – 100 mm fork length [FL]) and 100 age-2 smolts (117 – 143 mm FL) were collected from the DFO enumeration fence from April 17-23 in 2016 and surgically implanted with acoustic transmitters. As Chilko smolts primarily migrate at night, smolts were collected between 22:00 and 2:30 and were held until morning in a covered ~200-L flow-through tank. Age-1 smolts were implanted with VEMCO V4 transmitters (180 kHz, 3.6 mm height, 5.7 mm width, 11 mm length, 0.42 g in air) and age-2 smolts with VEMCO V7 transmitters (69 kHz, 7 mm diameter, 18 mm length, 1.4 g in air). Prior to surgery, smolts were anaesthetized in 100 mg/L Tricaine methanesulphonate (MS-222) mixed with 200 mg/L NaHCO₃ buffer for ~1-2 minutes. Weight
(g) and fork length (FL; mm) were measured before smolts were transferred into a V-shaped trough on a surgery table. A maintenance dose of MS-222 (50 mg/L) buffered with NaHCO₃ (100 mg/L) flowed continuously over the gills to maintain sedation during surgery. All water baths were continuously aerated and temperatures were maintained within 4° of the river temperature. Tags were inserted into the abdominal cavity through a ~6-mm (V4 tag in age-1) or 7-mm (V7 tags in age-2) incision on the midventral line. Incisions were closed using one suture (Ethicon Monocryl 5-0 monofilament with a 3/8 circle reverse cutting 13 mm [P-3] needle) for age-1 smolts, and two interrupted sutures for the larger incision on age-2 smolts.

Using epoxy-coated carbon steel bone cutting forceps, non-lethal biopsies of ~1 mm of tissue from the ends of 2-3 gill filaments were collected from half of the tagged age-1 smolts (n = 100) and from nearly all tagged age-2 smolts (n = 89). Forceps were sterilized between each smolt. I used gill tissue because it can be removed without affecting smolt survival, and is sufficient for identifying infectious agents and changes in gene expression (Jeffries et al. 2014, Healy et al. 2018). Gill biopsies were transferred into RNAlater (Life Technologies, Grand Island, NY) and stored at -20 °C for two weeks before being frozen at -80 °C prior to laboratory work. Post-surgery, smolts were transferred into an in-river release pen located below the counting fence and near the centre of the river. Smolts were allowed to recover for an average of ~11 hours before being released. Surgeries and biopsy procedures took on average of three minutes per fish.
3.2.3 Telemetry array framework

Migrating tagged smolts were detected by groups of VEMCO VR2W, VR3, and VR4 (69 kHz and 180 kHz) acoustic receivers (referred to as arrays) deployed throughout the migration route (Figure 3.1). Array A was located near the release pen (i.e. 0 km from release) and Array B was 14 km downstream in the Chilko River. Array C (80 km) and D (178 km) were in the Chilcotin River. Array E (599 km), F (627 km), and G (657 km) were in the lower Fraser River and into the estuary. Marine arrays H (804 km; Northern Strait of Georgia), I (882 km; Discovery Islands), J (951 km; Johnstone Strait), and K (1044 km; Queen Charlotte Strait) were implemented and maintained by the Ocean Tracking Network (OTN) and Kintama Research Ltd., and were used to detect smolts as they moved north in the Salish Sea. Both age-1 and age-2 smolts could be detected on all arrays, with the exception of Array K (the final array), which was equipped to only detect 69-kHz tags in the age-2 fish. Therefore, Array J was the last array along the migration route able to detect age-1 smolts. In addition, only a limited number of receivers on Array H were able to detect both tag types, while the majority could only detect 69 kHz tags. Consequently, no age-1 smolts (180 kHz tags) were detected on Array H. A southern marine array with 69 kHz receivers exists in the Juan de Fuca Strait, but did not detect any smolts.

3.2.4 Infectious agent detection and gene expression

Gill tissue biopsies were analyzed using HT-qRT-PCR on the Fluidigm BioMark™ HD platform (Fluidigm, San Francisco, CA, USA) at the Molecular Genetics Laboratory at the DFO Pacific Biological Station, Nanaimo, BC. I screened for the presence of 17 salmon infectious agents known from previous studies to infect gill tissue of adult salmon migrating in freshwater.
(Table 3.1) and ran the microbe assays in duplicate. The infectious agents selected are suspected or known to cause disease in Pacific salmon (Jefferies et al. 2014, Miller et al. 2014, 2016). I also assessed 59 host genes including individual genes predictive of general stress response, involved in various immune pathways of defense, and associated with osmoregulatory preparedness, as well as gene panels predictive of viral disease or associated with premature mortality across multiple studies. These were run singularly on cDNA from gill tissue biopsies, along with 3 reference genes run in duplicate (Table 3.1; Miller et al. 2016, Teffer et al. 2017).

Total RNA (0.1 µg) was extracted from gill samples using the ‘spin method’ for MagMAX™-96 for Microarrays Total RNA Isolation Kits (Ambion Inc., Austin, TX; as described in Miller et al. (2011) and Jeffries et al. (2014). First, gill tissue samples were homogenized using TRI-reagent™ (Ambion Inc., Austin, TX) and 1-bromo-3-chloropropane was added to the homogenate. Aliquots of the aqueous phase (100 µL) were then dispensed into 96-well plates. Additional RNAse treatment was used to prevent RNA contamination and Biomek FXP liquid handling instrument (Beckman-Coulter, Mississauga, ON, Canada) was used to measure the A$_{260}$/A$_{280}$ to assess RNA quality. RNA was normalized and then used to synthesize cDNA (SuperScript VILO MasterMix; Life Technologies).

The cDNA (1.25 µL) from each gill sample was pre-amplified with primer pairs corresponding to all 76 assays in a 5-µL reaction mix using TaqMan Preamp Master Mix (Life Technologies), following the BioMark protocol, in order to increase sensitivity of the small assay volume (7 nL) used on the BioMark platform. Following specific target amplification (STA), unincorporated primers were eliminated using ExoSAP-IT PCR Product Clean Up (MJS BioLynx Inc, ON, Canada). Gill samples were then diluted 1:5 in DNA Suspension Buffer (TEKnova, Hollister, CA). An assay mix containing 10 µM primers and 3 µM probes was
prepared for the TaqMan assays. Positive and negative controls were included at each step in the protocol to monitor the potential for false negative and false positive results. A serial dilution of artificial positive constructs (APC clones) containing all infectious agent assays was run as six samples on each dynamic array. The APC clones contained an additional probe (VIC) that allowed for the detection contamination. For biomarker assays, 5 3-X serial dilutions of host DNA were run on each dynamic array during the final qPCR using 1 µL from each pooled sample (Miller et al. 2016). Infectious agent and host gene assay efficiencies were calculated using these serial dilutions.

The qPCR assays and individual samples were loaded onto 96.96 dynamic arrays and run on the BioMark platform, enabling simultaneous quantitation of 9,216 independently measured assays at once (96 samples by 96 assays). The same distribution of assays was used for each array and smolt samples were randomly stratified among arrays. 2x Assay Loading Reagent (Fluidigm) was used to dilute the amplified cDNA 1:2. A 5-µL reaction mix was prepared using 2x TaqMan Mastermix (Life Technologies), GE Sample Loading Reagent, nuclease-free water and 2.7 µL of amplified cDNA. The reaction mix was added to each assay inlet of the dynamic array as per manufacturer’s protocols. After loading the assays and samples into the chip by 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 (Ct) for each assay was determined using the BioMark Real-Time PCR analysis software (Fluidigm, www.fluidigm.com). For infectious agent assays, samples with detection in only one of the duplicates were removed, and duplicates were averaged. Contaminated samples (indicated by VIC positives) were removed. Amplification curves of all assays were visually assessed for any irregularities, consistency between replicates, and
contamination. R statistical software (R Core Team 2015) was used to calculate the efficiencies for each assay using the slope of a regression between Ct values and serial dilutions. I removed values that were not distinctly within the linear relationship, often either the lowest or highest RNA concentrations, in order to improve accuracy of assay efficiency estimates and/or R² values. Only assays with an amplification factor of 1.80 – 2.20 (i.e. efficiencies of 80 – 120%), an R² ≥0.98, and with normally shaped amplification curves were used in analyses. Consequently, all 17 infectious agents, and 46 of the 59 biomarkers were included (Table 3.1). Minimum average Ct values indicating infectious agent detection with high statistical certainty (95% of the time; limit of detection [LOD]) were determined for each specific infectious agent assay as defined by Miller et al. (2016). Host genes were normalized against all assays and relative expression was calculated using the 2−ΔΔCt method (Livak and Schmittgen 2001). RNA copy numbers of infectious agents were calculated from Ct values measured in samples using the standard curves created using the APC clone dilutions. Samples were visually assessed and outliers (samples with low expression of reference genes) were removed. Thus, 154 (77 age-1 and 77 age-2) samples were included in subsequent analyses (Table 3.2).

3.2.5 Statistical analysis

To examine differences in infectious agents between age classes, prevalence of each microbe was determined and compared between age groups using a Fisher’s exact test along with the odds ratio. RNA copy number (relative load) of each infectious agent was log-transformed (to improve normality) and was compared between age groups using ANOVA tests. Infectious agent richness was calculated as the number of detected infectious agents per individual. The relative
infection burden (RIB), which includes infectious agent presence and relative load was also calculated for each smolt using the following equation (Bass et al. In Review):

$$RIB = \sum_{i=1}^{m} \frac{L_i}{L_{max_i}}$$

where the relative load of the $i^{th}$ infectious agent ($L_i$) is divided by the maximum load within the population for the $i^{th}$ microbe ($L_{max_i}$) and then summed across all infectious agents ($m$) present in a given smolt.

Survival of gill-biopsied smolts was estimated in a concurrent study using a spatial mark-recapture approach where detection at each acoustic receiver array along the migration path was interpreted as ‘recapture’ (Chapter 2). To determine the influence of infectious agents on migration survival, I divided smolts into 4 survival groups based on their last detection location: (1) Imminent Mortality (IM; smolts that were not detected post release), (2) River Mortality (RM; smolts that were detected in freshwater, but not at the Fraser River mouth array or beyond), (3) Ocean Mortality (OM; smolts that were detected at the Fraser River mouth, but not at the last array at Johnstone Strait), and (4) Migration Survivor (MS; smolts that were detected at the final array at Johnstone Strait). I examined the relative load of infectious agents between survival groups and compared infectious agent prevalence using a Fisher’s exact test along with the odds ratio.

I used an unsupervised principle component analysis (PCA) to examine the main trends in the stress and immune profiles between age groups and between survival groups. The PCA ranks host biomarker expression of individual smolts and portrays their contribution (in terms of variance) to each principal component (PC; i.e. the loading). To test for differences in stress and
immune profiles between groups, I used a permutational multivariate analysis of variance using distance matrices (PERMANOVA) based on Euclidean distance metric (Anderson 2001). This analysis partitions distance matrices among sources of variation while fitting linear models. My linear predictors were either age or survival group and the response matrix was the gene expression matrix of all smolt samples. The relevant centroids were identified and the squared deviations from these points were calculated. The permutation test is based on sequential sums of squares from permutations of the raw data. The PERMANOVA was run using the vegan package in R statistical software (Oksanen et al. 2008). To further investigate the relationship between gene expression and age I compared the first 3 principle components (which explained 50% of the variation in the data; see Results) to smolt age and tested for differences using ANOVAs. To further examine how stress and immune profiles influenced smolt survival I used a paired t-test with Bonferroni adjusted p-values to separately compare the first 3 PCs against the 4 survival groups. Stripcharts were generated to visually assess the degree and direction of differential expression between age and survival groups. I used hierarchical clustering (heatmaps) to visualize overall relationships between important gene expression groupings and age, survival, and infectious agent load (Miller et al. 2017).

3.3 Results

3.3.1 Migration survival

Gill biopsies did not influence survival for either age group (Chapter 2). Survival to the first array post release (Array B; 14 km) in the Chilko River was poor for both age groups (76% and 53% for age-1 and age-2 smolts respectively; Table 3.3). Cumulative survival to the mouth of the
Fraser River (Array G; 657 km) was 56% and 28% for age-1 and age-2 smolts respectively (Chapter 2). Cumulative survival to Johnstone Strait (Array J; 951 km), the final array along the migration route able to detect both tag types and therefore age classes, was 16% and 2% for age-1 and age-2 smolts respectively (Chapter 2).

### 3.3.2 Infectious agent presence and immune profiles

Four of the 17 infectious agents assessed were detected in the gill samples. Two of the infectious agents were the bacteria ‘Candidatus Branchiomonas cysticola’ and Flavobacterium psychrophilum. ‘Ca. B. cysticola’ was found in 90% of samples and was the most commonly detected infectious agent, whereas F. psychrophilum was present in 10% of samples. IHNV was found in 10% of samples and Ichthyophthirius multifiliis was only found in one smolt (0.6%). Of the infectious agents detected, age-2 smolts had significantly higher prevalence (Fisher’s exact test, $P = 0.005$; Figure 3.2) and relative load (ANOVA, $P = 0.001$; Figure 3.3) of ‘Ca. B. cysticola’ compared to age-1 smolts. The other two prevalent infectious agents were not significantly different in presence or load between the age groups. Mean infectious agent richness was 1.1 per individual (SE ± 0.04). Overall, mean RIB was 0.61, with age-2 smolts having a significantly higher RIB (mean = 0.70, SE = 0.04) than in age-1 (mean = 0.53, SE = 0.05) smolts (ANOVA, $P = 0.004$).

Of the infectious agents detected, F. psychrophilum was correlated with migration survival. Within the survival groups, the IM group contained 60% of the smolts positive for F. psychrophilum, compared to 26.7% in the RM group, 13.3% in the OM group, and 0% in the MS group (Figure 3.4B). All smolts with F. psychrophilum were within either the IM, RM, or OM
group, and were 3-times more likely to experience mortality during early migration, while none of the smolts in the MS group had the bacterium (Fisher’s exact test, P > 0.05). The other two prevalent infectious agents did not appear to negatively influence migration survival.

The first principle component explained 27% of the total variation in the gene expression data and was associated with several viral immune response genes (VDD genes from Miller et al. 2017; i.e. NFX, VHSV.P10, DEXH, IFI44A, SRK2, IFIT5, STAT1, MX, VHSVIP4, RSAD, 52RO, and GAL3) loading positively together along the PC1 axis (Figure 3.5A). Smolts with the highest relative loads of IHNV were clustered together on the positive end of the PC1 axis, indicating these individuals were showing an antiviral immune response to the IHN virus (Figure 3.7A). PC2 explained 12% of the variation and was most driven by genes associated with an inflammatory immune response (IL-17D and IL-11) and stress response (HSC70 and JUN) positively loading together along the PC2 axis (Figure 3.5B); the co-expressed panel of genes on the positive end of PC2 represent the mortality-related signature [MRS] defined in Miller et al. 2011). PC3 explained 11% of the variation and was most associated with genes related to osmoregulatory preparedness (NKAa1-b and NKAa1-a) loading positively together on the PC3 axis.

Age groups appeared to be separated on the overall PCA, with age-1 located more towards the negative end of the PC2 axis and age-2 smolts located more towards the positive end of the PC2 axis (Figure 3.6). The PERMANOVA revealed that gene expression profiles indeed differed between age groups (P = 0.002). Further examination of the first 3 PCs against the age groups revealed that age-2 smolts had significantly higher expressions of inflammatory and stress response genes associated with the PC2 axis compared to age-1 smolts (ANOVA, P <
There was no significant difference between age groups on PC1 or PC3 (ANOVA, P-values > 0.05).

There was no immediately clear distinction among the survival groups on the overall PCA (Figure 3.7), but the PERMANOVA demonstrated that gene expression profiles differed among the 4 groups (P = 0.03). Further analysis of the first 3 PCs against the 4 survival groups revealed that PC2 was correlated with migration survival. Fish in the IM group had significantly higher expressions of inflammatory and stress response genes associated with PC2 than smolts that died later in the river and smolts that survived to the last arrays in the ocean (ANOVA, P-values < 0.05; Figure 3.8B). There was no significant difference between survival groups on PC1 or PC3 (ANOVA, P-values > 0.05).

The hierarchical heatmap revealed that groups of host biomarkers defining the MRS and indicative of inflammatory and stress responses were clustered and up-regulated together in both age-1 and age-2 smolts primarily in the IM or RM survival groups, and none from the MS group (Appendix, Figure A1). The heatmap also demonstrated that groups host biomarkers indicative of an RNA viral disease state (Miller et al. 2017) were clustered and up-regulated together but with no clear pattern or relation with among age or survival groups.

3.4 Discussion

Age-1 smolts comprise > 95% of this stock’s migratory population and my study provides a direct comparison of infectious agents, stress and immune profiles, and survival between two age classes of migrants. Through the combination HT-qRT-PCR of non-lethal gill biopsies with acoustic telemetry, I found that *F. psychrophilum*, ‘*Ca. B.cysticola*’, and IHNV
were most prevalent in the out-migrating population, and *F. psychrophilum* was related to early migration survival. I also identified the same immune and stress profile as documented in association with premature mortality of adult migrants (Miller et al. 2011) was related to early migration survival, and more highly expressed in age-2 smolts. Previous work also identified infectious agents and gene expression signatures associated with immune responses in acoustically-tagged migrating age-2 Chilko Lake sockeye smolts associated with early migration survival through this same region (Jeffries et al. 2014), but that differed from the results herein.

Presence and load of *F. psychrophilum* did not differ between age classes, but appeared to be associated with migration survival given that none of smolts that reached the final array (Johnstone Strait; 951 km) had the bacterium and were 3-times more likely to die early in migration. *F. psychrophilum* is a pathogen that can cause bacterial cold water disease (BCWD) and rainbow trout fry syndrome in nearly all salmonid species worldwide, including sockeye salmon (Nematollahi et al. 2003). Transmission can occur both vertically and horizontally via waterborne and contact exposure, with the potential to enter the host through a combination of routes. *F. psychrophilum* can be present in skin mucus, and connective tissue of the fins, gills and operculum (Nematollahi et al. 2003). Juvenile salmonids are particularly susceptible to the disease and for some species mortality can occur < 10 days post infection (Holt et al. 1989) with rates up to 90% (Barnes and Brown 2011), but can vary depending on environmental conditions. *F. psychrophilum* has been detected in Chilko Lake sockeye smolts in previous years (Furey 2016). The water in Chilko Lake is generally 2 – 10 °C during smolt migration in the spring and *F. psychrophilum* is a cold-water pathogen that is most harmful when water temperatures range between 4 – 10 °C (Borg 1960), but it can cause mortality at water up to 18 °C (Cipriano and Holt 2005).
Smolts infected with *F. psychrophilum* also had immune profiles consistent with activated inflammatory and stress responses and subsequently experienced poor early migration success. *F. psychrophilum* excretes a psychrophilic protease causing lesions and necrosis in affected tissues (Barnes and Brown 2011), which may explain gene expression results. Smolts in the IM and RM groups had a significantly higher expression of IL-17D, IL-11, HSC70 and JUN (associated with PC2) compared to the MS group. Interleukin-11 (IL-11) and interleukin-17D (IL-17D) are pro-inflammatory cytokines indicative of an inflammatory response and may indicate chronic inflammation at the gills (Castro et al. 2011, Tadiso et al. 2011, Krasnov et al. 2012, Zou and Secombes 2016). Heat shock cognate 70 protein (HSC70) is a molecular chaperone crucial for protein functioning, that is expressed in response to a variety of stressors (Boone and Vijayan 2002). Transcription factor (JUN) is linked to cell apoptosis and elevated expression can be induced by various forms of cellular stress including inflammation. Interestingly, age-2 smolts showed a higher expression of these immune response genes (PC2) compared to age-1 smolts. Immune profiles may have differed between age groups because age-2 smolts had different parents and spent an additional year in Chilko Lake prior to age-1s, and thus were potentially exposed to varying microbe communities and conditions. Previous work has also found that immune profiles consistent with elevated immune and stress responses (including IL-11 and IL-17D) were associated with poor early migration smolt survival (Jeffries et al. 2014, Healy et al. 2018). Similar associations between *F. psychrophilum*, immune genes JUN and IL-11, and post-release mortality have been determined for adult sockeye salmon (Teffer et al. 2017). While my results do not explain all out-migration mortality, they suggest infectious disease may be a partial contributing factor.
Smolts with *F. psychrophilum* and gene expression profiles indicative of an inflammatory and stress responses may have experienced poor migration survival either due to direct mortality, or through potentially increased vulnerability to predation. Predation is likely a primary factor contributing to smolt migration mortality, especially in the clear upper waters of the Chilko River (Clark et al. 2016, Furey et al. 2015a). As smolts exit Chilko Lake, they are met with an abundance of predators such as bull trout (*Salvelinus confluentus*), rainbow trout (*Oncorhynchus mykiss*), mergansers (*Mergus spp*), gulls (*Larus and Chroicocephalus spp*), and river otters (*Lontra canadensis*; Clark et al. 2016). Bull trout in particular have been shown to binge-feed almost exclusively on migrating Chilko smolts at the lake outlet (Furey et al. 2015a, Furey et al. 2016). Smolts in poor physiological condition may have compromised swim performance, growth rates, or altered behaviours such as schooling, foraging, and predator avoidance (Handeland et al. 1996, Dieperink et al. 2002, Miller et al. 2014), which could increase their risk to predation. For example, Furey (2016) found that smolts infected with *F. psychrophilum* were more susceptible to bull trout predation at the outlet of Chilko Lake such that *F. psychrophilum* was 3-times more prevalent in predated smolts than non-predated. Furthermore, IHNV-infected Chilko smolts were 34-times more likely to be consumed by bull trout (Furey 2016). Infection status can also influence the probability of smolt predation by avian predators in the ocean (Miller et al. 2014). Consequently, the interactions between infectious agents, elevated immune and stress responses, and predation are likely a contributing factor to smolt mortality during out-migration.

My results highlight an important link between the presence of IHNV and expression of VDD genes identified by Miller et al. (2017). Smolts with IHNV also had elevated expression of
viral immune response genes such as NFX, VHSV-P10, DEXH, IFI44A, SRK2, IFIT5, STAT1, MX, VHSVIP4, RSAD, 52RO, and GAL3. Individuals with the highest loads of IHNV were clustered together on the positive end of the PC1 axis. These antiviral genes driving PC1 have been confirmed to be capable of distinguishing fish in an active viral disease state from those carrying a latent viral infection (Miller et al. 2017), indicating that smolts with this antiviral type signature may have in fact been in a viral disease state. There were also a small number of smolts without IHNV located on the positive end of the PC1 axis. This suggests that IHNV could have been present but undetected in these fish, or that these individuals may have been responding to another viral pathogen that I didn’t screen for.

Although IHNV was associated with a gene expression signature indicative of a viral disease state, the pathogen and antiviral signature surprisingly did not significantly impact migration survival. IHNV is primarily a cold-water pathogen that can cause significant mortality in juvenile salmonids (LaPatra 1998). It is naturally present in Chilko Lake (Williams & Amend 1976) and was correlated with migration survival of age-2 Chilko smolts in 2012 (Jeffries et al. 2014, Furey 2016). It is possible IHNV did not influence survival in 2016 because loads were lower than previously observed. Although IHNV prevalence in 2016 (10%) was similar to 2012 (13%), migration mortality in 2012 was primarily driven by smolts considered to have high loads of the virus (Ct < 20; Jeffries et al 2014). Approximately 7% of smolts sampled in 2012 were considered to have levels of infection high enough to cause adverse symptoms in fish (Jefferies et al. 2014), whereas none of my samples were above this threshold. Furthermore, IHNV has been absent from Chilko smolt samples in some years (Jeffries et al. 2014, Furey 2016), highlighting the variation in its presence, prevalence, and influence in this system among years.
While ‘Ca. B. cysticola’ was present in nearly all samples and was significantly more prevalent and in higher loads in age-2 smolts compared to age-1s, it was not related to migration survival. Although ‘Ca. B. cysticola’ can be an agent of gill epitheliocystis (Toenshoff et al. 2012), it may not always result in mortality for fish (Bass et al. 2017, Gunnarsson et al. 2017, Teffer et al. 2017). Some studies suggest that this bacterium may occur as a result of a secondary infection (Tengs and Rimstad 2017), however, others have provided evidence that it is a member of the normal gill microbiota of healthy fish (Steinum et al. 2009, Toenshoff et al. 2012). It has also been found in high prevalence and load in Chilko sockeye smolts in previous years (Furey 2016), as well as in migrating steelhead smolts (Healy et al. 2018) with no impact on migration survival. However, further research may be necessary to better understand the association between the presence of this bacterium and disease in salmonids.

My ability to detect all possible infectious agents associated with smolt mortality may have been limited by using exclusively gill tissue. Although all of the infectious agents I assessed can be detected in gill, some have a higher tendency to be detected in other tissues. For instance, IHNV is more likely to infect kidney tissues (e.g., Purcell et al. 2011), and Pacific salmon parvovirus is more likely to be present in liver (Miller et al. 2014). Furthermore, due to the small size of my gill tissue biopsies, I normalized RNA to a much lower concentration than standard procedures used for larger samples (Miller et al. 2016). Consequently, this may have increased the possibility of false negatives for infectious agents. Additionally, although I screened for 17 key infectious agents, there are others that can affect smolt survival that were not included in my study (e.g., Rhodes et al. 2006, Jacobson et al. 2008). Furey (2016) assessed gill, brain, kidney, liver, and heart tissues of Chilko smolts in 2014 and used a larger quantity of RNA to screen for 44 microbes and found nine to be present. Due to the constraints of non-lethal gill sampling my
study likely underestimates the presence and load of infectious agents in the out-migrating population. The inclusion of additional infectious agent assays and a concurrent assessment of whole body tissue samples from the population may provide further insight to the role of infectious agents on smolt migration survival.

3.5 Conclusion

My study is the first to link large-scale acoustic telemetry with infectious agents and immune profiles of migrating age-1 smolts which make up the vast majority of the Chilko Lake sockeye smolt run. I also provide a direct comparison of infectious agent and immune profiles between age-1 and age-2 smolts. Identifying both the direct and indirect impacts of infectious agents and disease on migration survival is challenging, but is a crucial factor in understanding impacts of smolt condition on migratory success. I provide evidence that *F. psychrophilum* and immune profiles consistent with elevated inflammatory and stress responses influence migration survival for both age groups, particularly in the early high-risk landscape. I also provide evidence that age-2 smolts experienced higher inflammatory and stress response than the more abundant age-1 smolts, which may have partly contributed to their lower survival. Because survival during this life stage may be particularly important for the overall productivity of salmon populations (Beamish et al. 2004, Farley et al. 2007, Irvine & Akenhead 2013), determining the links between smolt physiology and mortality is key for management of this important species. These results can be incorporated into population models to help improve predictions of adult returns. Increased accuracy in predictive models can in turn help to improve management plans and result in enhanced conservation of salmonids.
### Table 3.1: Summary of the primer and probe sequences for the 17 infectious agents and 59 genes involved in immune, stress and osmoregulatory responses along with the three reference genes used in the used in HT-qRT-PCR analyses on age-1 and age-2 Chilko Lake sockeye salmon (*Oncorhynchus nerka*) collected in 2016.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Infectious agent/ host gene name</th>
<th>Assay Class</th>
<th>Type/ Function</th>
<th>Forward Primer Sequence (5'-3’),</th>
<th>Reverse Primer Sequence (5'-3’),</th>
<th>Probe Sequence (FAM-5’-3'-MGB)</th>
<th>Amplification Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>52RO</td>
<td>52 kDa Ro protein-2</td>
<td>Host Gene</td>
<td>Viral</td>
<td>F: TGCACTATTGCCCAGTAACCAT</td>
<td>R: TGCAAGAGGAGATGCCAACA</td>
<td>P: AGTAGGATTCACAGAGGT</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Immune Response</td>
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<td></td>
</tr>
<tr>
<td>ACTB_v1</td>
<td>Beta actin</td>
<td>Host Gene</td>
<td>Osmoregulation</td>
<td>F: GAAAATCGCCGCACTGGGT</td>
<td>R: CGGCGAATCCGGCTTT</td>
<td>P: TGACAACGGATCCGGT</td>
<td>1.92</td>
</tr>
<tr>
<td>C1Qc</td>
<td>Complement C1q C chain</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: CGCCGGTGAGTGGAATCTA</td>
<td>R: CTTCTCCATCATGTGTGTGCTA</td>
<td>P: ACCTCCAAACATAGAAGAG</td>
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</tr>
<tr>
<td>C3_onmy</td>
<td>Complement factor C3</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: ATTTGCCCTGTCCAAAACACA</td>
<td>R: AGCTTCAGATCAAGGAAGATGCTC</td>
<td>P: TGGAATCTGTGTGTCTGAACCCC</td>
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</tr>
<tr>
<td>C7</td>
<td>Complement component C7 precursor</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: ACCTCTGTCCAGCTGTGCTC</td>
<td>R: GATGCTGACCACATCAAACTGC</td>
<td>P: AACTACCAGACAGTGCTG</td>
<td>1.95</td>
</tr>
<tr>
<td>CA054694</td>
<td>Mitochondrial ribosomal protein (VAR1)</td>
<td>Host Gene</td>
<td>Viral</td>
<td>F: CCACCTGAAGTACTGAAGATAAGACA</td>
<td>R: TTAAGTCCCTCTTCTCATGCTTGA</td>
<td>P: TCTACCAGGCTTTAAG</td>
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<tr>
<td>CA4_v1</td>
<td>Carbonic anhydrase 4</td>
<td></td>
<td></td>
<td>F: GGTCAATTTTGGTTTGTACAGTCT</td>
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<td>1.84</td>
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<td>Symbol</td>
<td>Infectious agent/ host gene name</td>
<td>Assay Class</td>
<td>Type/Function</td>
<td>Forward Primer Sequence (5'-3’),</td>
<td>Reverse Primer Sequence (5'-3’),</td>
<td>Probe Sequence (FAM-5’-3’-MGB)</td>
<td>Amplification Factor</td>
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<tr>
<td></td>
<td></td>
<td>Host Gene</td>
<td>Osmoregulation</td>
<td>R: CCTAGATATAGCTATCCACGTACTCACCTA</td>
<td>P: TGATACGTGGTATAGAAAAAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL4_v1</td>
<td>CC chemokine 4</td>
<td>Host Gene</td>
<td>Osmoregulation</td>
<td>F: TCTCTTCATTGCAACAATCTGCTT</td>
<td>R: ACAGCAGTCCACGGGTACCT</td>
<td>P: CTACGCAGCAGCATT</td>
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<td>Immune</td>
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<td>R: CCCCCCTTTGACAGGGAAG</td>
<td>P: CAGAAGAGAGAGCTGGATGTCTCCG</td>
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<td></td>
<td></td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: CAAAGCCAGTATGAGACTGTTCAG</td>
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<td>P: ACCTGATCGCCAGTGATGAGCATGTAC</td>
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<td></td>
<td></td>
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<td>F: CCATAAGGAGGGGTTGCTACAATAAGAT</td>
<td>R: CTCTCCCCCTTCAGCTTCTGT</td>
<td>P: TGGCGCGCTACGTG</td>
<td>2.04</td>
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<tr>
<td>DEXH</td>
<td>ATP-dependent RNA helicase</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: GCTGGTCAAGACGTAGCCAAA</td>
<td>R: CATCGTTGAGCCTGTAGACAAACA</td>
<td>P: TTGCCCTCCTCGTTCTGCAGATG</td>
<td>1.90</td>
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<tr>
<td>FYB</td>
<td>FYN-T-binding protein</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: TTGTAGCGCCTGTGGTAATCATAT</td>
<td>R: TACACTGCTGGCGCCATGGGA</td>
<td>R: CTTGGCGTGGTCGG</td>
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</tr>
<tr>
<td>GAL3</td>
<td>Galectin-3-binding protein precursor</td>
<td>Host Gene</td>
<td>Viral Immune Response</td>
<td>F: TTGTAGCGCCTGTGTAATCATATC</td>
<td>R: TACACTGCTGGCGCCATGGGA</td>
<td>R: CTTGGCGTGGTCGG</td>
<td>1.85</td>
</tr>
<tr>
<td>HBA_v1</td>
<td>Hemoglobin subunit α</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: GCCCTGGCTGCAAAATACAGA</td>
<td>R: GAGCAGGAACTGAAGCTCAATG</td>
<td>P: ACCATCATGAAAGTCC</td>
<td>2.12</td>
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<tr>
<td>hep</td>
<td>Hepcidin</td>
<td>Host Gene</td>
<td>Immune</td>
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<td>R: TGACGCTTGAACCTGAAATG</td>
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<td>Symbol</td>
<td>Infectious agent/ host gene name</td>
<td>Assay Class</td>
<td>Type/ Function</td>
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<td>Amplification Factor</td>
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<tr>
<td>HSC70</td>
<td>Heat shock cognate 70 protein</td>
<td>Host Gene</td>
<td>Stress</td>
<td>F: GGGTCAACACAGAAGCACAAG  R: GCGCTCTATAGCGTTGATTG  P: AGACCAAGCCTAAAATCA</td>
<td>2.06</td>
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<td>HTA</td>
<td>HIV-1 Tat interactive protein</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: CTTTGTAACAGTTCGACATGGCTTATT  R: TGGTGAAAGCATTCTGTATGCTA  P: TCTGTACTGAGCATCCCGCACATTACA</td>
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<tr>
<td>IFI44A</td>
<td>IFN-induced protein 44-1</td>
<td>Host Gene</td>
<td>Viral Immune Response</td>
<td>F: CCGAGTCCAGAAGCAGCTACT  R: TCCAGTGGTCATCCTC        P: CGCTGTTCCGTTGTA</td>
<td>2.07</td>
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<td>IFIT5</td>
<td>Interferon-induced protein with tetratricopeptide repeats 5</td>
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<td>Viral Immune Response</td>
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<td>1.85</td>
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<td>IgMs</td>
<td>Immunoglobulin</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: CTTGGCTTGTGACAGATG  R: GGCTAGTGTTGGAATTGG  P: TGGAGAGAAAGCTGTCACGCA</td>
<td>2.09</td>
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<td>IL-11</td>
<td>Interleukin 11</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: GCAATCTCTTGCTCCAACCTC  R: TTGTCAAGTGCACGTTTTC  P: TCGCGGAGTGGAAAGGCAGA</td>
<td>2.09</td>
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<td>IL-15</td>
<td>Interleukin 15</td>
<td>Host Gene</td>
<td>Immune</td>
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<td>Host Gene</td>
<td>Immune</td>
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<td>2.14</td>
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<tr>
<td>IL-1B</td>
<td>Interleukin 1 beta</td>
<td>Immune</td>
<td></td>
<td>F: AGGACAAGGACCTGCTCAACT</td>
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<td>Symbol</td>
<td>Infectious agent/ host gene name</td>
<td>Assay Class</td>
<td>Type/ Function</td>
<td>Forward Primer Sequence (5’-3’),</td>
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<td>Probe Sequence (FAM-5’-3’-MGB)</td>
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<tr>
<td>R: CCGACCTCCAACTCCAACACTA</td>
<td>P: TTGCTGGAGAGTGTGTGGAAGAA</td>
<td>IL-8</td>
<td>Interleukin 8</td>
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<td>Immune</td>
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<td>P: CTGGCCSACGAGATA</td>
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<td>IRF1</td>
<td>interferon regulatory factor 1</td>
<td>Host Gene</td>
<td>Viral Immune Response</td>
<td>F: CAAACCGCAAGAGATTTTCCTCATTT</td>
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<tr>
<td>R: TTGGCCAGCATCTTCTCAAT</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P: ATGTACGCGCTCCGGTGTGT</td>
<td></td>
<td>JUN</td>
<td>Transcription factor</td>
<td>Host Gene</td>
<td>Stress Response</td>
<td>F: TTGTGCTGGTGAGAAAACCTCAT</td>
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</tr>
<tr>
<td>R: CCTGTGCTTCTATGAAATGCTTAG</td>
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<td></td>
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<td>P: AGACTTTGGGCTATT</td>
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<td>KRT8</td>
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<td>Host Gene</td>
<td>Immune</td>
<td>F: CGATTTGACGGCTTGGATAA</td>
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<tr>
<td>R: GATTTTGGTGTGTTTTTGTGATGAT</td>
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<td>Immune</td>
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<td>R: AGTCACCTGGAGGCAAAAGA</td>
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<td>R: CGTAACTGCCAGAGTGGTCAAT</td>
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<td>NFX</td>
<td>Zinc finger NFX1-type</td>
<td>Host Gene</td>
<td></td>
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<td>1.87</td>
</tr>
<tr>
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<td>Amplification Factor</td>
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</tr>
<tr>
<td>NKA-a3</td>
<td>Na+/K+ ATPase a3 subunit</td>
<td>Host Gene</td>
<td>Osmoregulation</td>
<td>F: GGAGACCAGCAGAGGAACAG R: CCCTACCAGCCCTCTGAGT P: AAGACCCAGCCTGAAATG</td>
<td>2.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKAa1-a_v2</td>
<td>Na/K ATPase α-1a subunit</td>
<td>Host Gene</td>
<td>Osmoregulation</td>
<td>F: TGGATCAAGGTTATCACGTTGACT R: CCCACACCTTGGCAATG P: ATCATCCATCAGTGGCA</td>
<td>1.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKAa1-b_v2</td>
<td>Na/K ATPase α-1b subunit</td>
<td>Host Gene</td>
<td>Osmoregulation</td>
<td>F: GCCTGGTGAAGAATCTTGACT R: GAGTCAGGTTCCGGTCTTG P: CCTCCACCATTGGCTCA</td>
<td>1.88</td>
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<tr>
<td>PCBL</td>
<td>precerebellin</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: TGTTGGTTCGTTGCTGTTG R: GCCACTTTTGGTTGCTCTC P: ATGGTGAGACTGAGACTG</td>
<td>1.95</td>
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<td></td>
</tr>
<tr>
<td>PRAS</td>
<td>G-protein</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: GCAGGATGAGCAGAGGAAGAA R: GCCCTGGGCAATGTAACACT P: CCCCCTAAAGATGCA</td>
<td>1.96</td>
<td></td>
<td></td>
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<tr>
<td>RSAD_M GB2</td>
<td>Radical S-adenosyl methionine domain-containing protein 2</td>
<td>Host Gene</td>
<td>Viral Immune Response</td>
<td>F: GGGAAATTAGTCCAAATCAGTCAAAC R: GCCATGGTCGACAAACTGACACT P: CGACCCCTGACTGCA</td>
<td>1.90</td>
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<tr>
<td>SAA</td>
<td>Serum amyloid protein a</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: GGAGATGATTCCAGGGTTCCA R: TTACGTCCCAGTGTTAGC P: TCAGGACACGGGACTGCA</td>
<td>1.86</td>
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<tr>
<td>SCG</td>
<td>Secretogranin II [Ctenopharyngodon idella]</td>
<td>Host Gene</td>
<td>Immune</td>
<td>F: GGATGGAAGAATCACAAGACTGAT R: ACACCCTTAAACTAGCCATACAT P: CGGCTGATGTCGACTG</td>
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</tr>
<tr>
<td>SRK2_MG B3</td>
<td>Tyrosine-protein kinase FRK</td>
<td>Host Gene</td>
<td>Viral Immune Response</td>
<td>F: CCAACGAGAAGTCACCATCAA R: TCATGATCTCACAGCAAGATTCC P: TGTGACGTGGTCCT</td>
<td>1.77</td>
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<tr>
<td>STAT1</td>
<td>Signal transducer and activator of transcription 1-alpha/beta</td>
<td>Host Gene</td>
<td>Viral Immune Response</td>
<td>F: TGTACCGTGTCAGACAGATCTG R: TGGTGCTGTCTGTAAGGAACGT P: AGTTGCTGAAAAACCGG</td>
<td>1.81</td>
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<tr>
<td>TCRb</td>
<td>T-cell receptor beta</td>
<td>Host Gene</td>
<td>Immune Response</td>
<td>F: TCACCAGCAGACTGAGATCC  R: AAGCTGACATGCAGGTGATC P: CCAATGAATGGCACAAACCAGAGA</td>
<td>1.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF</td>
<td>Transferrin</td>
<td>Host Gene</td>
<td>Immune Response</td>
<td>F: TTCAGCTGGAAAAATGTGG R: GCTGCAGCTGACTGCATCAT P: TGGTGCTGAGTGGGACTGAGA</td>
<td>1.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VHSV-P10</td>
<td>VHSV-induced protein-10</td>
<td>Host Gene</td>
<td>Viral Immune Response</td>
<td>F: GCAAACGTAAGAAACCATCAAGA R: CCGTCAGCTCCCTCTCGCAT P: TGGTGAGAAATGTTGAGA</td>
<td>2.12</td>
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<tr>
<td>VHSVIP4</td>
<td>VHSV-inducible protein-4</td>
<td>Host Gene</td>
<td>Viral Immune Response</td>
<td>F: GCTCTCGTAAAGCCCCACATC R: GGGCGACTGCTCTCTGATCT P: AAACGTGACGTCGCC</td>
<td>1.95</td>
<td></td>
<td></td>
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<tr>
<td>c_b_cys</td>
<td>Candidatus Branchiomonas cysticola</td>
<td>Microbe</td>
<td>Bacteria</td>
<td>F: AATAACTCGGAACGTGCTAGTG R: GCCATCAGCCTCGTGATCT G: CTCGGCTCCAGGCTTCC</td>
<td>2.07</td>
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<td></td>
</tr>
<tr>
<td>ee_sha</td>
<td>Ceratomyxa shasta</td>
<td>Microbe</td>
<td>Bacteria</td>
<td>F: GATCCCTTTATTCTACAGTACCAGTCA R: TGGAAAACGTCTTGGACAGGA P: AAACACTCGGTCGAGA</td>
<td>2.02</td>
<td></td>
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<tr>
<td>de_sal</td>
<td>Dermocystidium salmonis</td>
<td>Microbe</td>
<td>Parasite</td>
<td>F: CAGCCAATCTCTTCCGTTCT TT R: GACGGACGCACACACCAGT</td>
<td>2.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symbol</td>
<td>Infectious agent/ host gene name</td>
<td>Assay Class</td>
<td>Type/ Function</td>
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<td>Amplification Factor</td>
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</tr>
<tr>
<td>fl_psy</td>
<td><em>Flavobacterium psychrophilum</em></td>
<td>Microbe</td>
<td>Bacteria</td>
<td>F: GATCCCTATTCTCACAGTACCGTCAA R: TGTAAGCTGCTTTGTGACAGGAA P: AACAACGCACGTGCC</td>
<td>1.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ic_mul</td>
<td><em>Ichthyophthirius multifiliis</em></td>
<td>Microbe</td>
<td>Protozoan</td>
<td>F: AAATGGGCATACGTTGCAA R: AACTGCTGAAACACTCTATTTTT P: ACTGAGCTGAGCGGCA</td>
<td>2.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ihnv</td>
<td>Infectious hematopoietic necrosis virus</td>
<td>Microbe</td>
<td>Virus</td>
<td>F: AGAGCCAAAGCGACTGTGGC R: TTCTTTGCCCCTGTTGTTGA P: TGAGACTGAGCGGCA</td>
<td>2.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lo_sal</td>
<td><em>Loma salmonae</em></td>
<td>Microbe</td>
<td>Microsporidian</td>
<td>F: GGAGTCGACCGAAGATAGC R: CTTTTCTCCCTTTACTCATATGCTT P: TGCCCTGAAATCAGCAGGTGAGACTACC</td>
<td>2.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pa_min</td>
<td>Parvicapsula minibicornis</td>
<td>Microbe</td>
<td>Myxozoan</td>
<td>F: AATAGGGTTTGTGCTGACCTGTGT P: TGCCACCTGATAGGC</td>
<td>2.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pa_pse</td>
<td>Parvicapsula pseudobranchia</td>
<td>Microbe</td>
<td>Myxozoan</td>
<td>F: CAGCTCCAGTAGTGATTTCA R: TTGACCTCCTGCTTTATTTCAA P: CGTATTGCTGTCTTTGACATGCAGT</td>
<td>2.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pa_ther</td>
<td>Paranucleospora theridion</td>
<td>Microbe</td>
<td>Microsporidian</td>
<td>F: CGGACAGGGGAGCATGGTATAG R: GGTCCAGGGTGCTTGGAG P: TGGCCAGAAATGAAA</td>
<td>2.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pch_sal</td>
<td>Piscichlamydia salmonis</td>
<td>Microbe</td>
<td>Bacteria</td>
<td>F: TACCCCCAGGGCTGCTT R: GAATTCATTTCCTCCCTCTTGG P: CAAAAGGCTGACTAGAGT</td>
<td>1.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>prv</td>
<td>Piscine reovirus</td>
<td>Microbe</td>
<td>Virus</td>
<td>F: TGCTAACACTCCAGGAGTCATTG</td>
<td>2.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symbol</td>
<td>Infectious agent/ host gene name</td>
<td>Assay Class</td>
<td>Type/ Function</td>
<td>Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)</td>
<td>Amplification Factor</td>
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</tr>
<tr>
<td>pspv</td>
<td>Pacific salmon parovirus</td>
<td>Microbe</td>
<td>Virus</td>
<td>R: TGAATCCGCTGCAGATGAGTA P: CGCCGGTAGCTCT R: CGAAGACAACATGGAGGTGACA P: CAATTGGAGGCAACTGTA</td>
<td>2.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rlo</td>
<td>Rickettsia-like organism</td>
<td>Microbe</td>
<td>Bacteria</td>
<td>F: GGCTCAACCCCAAGAAGCTGCTT R: GTGCAACAGCGTCAGTGACT P: CCCAGATAACC CGCTCCTCCG</td>
<td>2.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sch</td>
<td>Salmon (gill) chlamydia</td>
<td>Microbe</td>
<td>Bacteria</td>
<td>F: GGGTAGCCCGATATCTCAAAGT R: CCCATGAGCGCTCTCTCT P: TCCTCGGACCTTAC</td>
<td>2.17</td>
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<tr>
<td>te_bry</td>
<td>Tetracapsuloides bryosalmonae</td>
<td>Microbe</td>
<td>Myxozoan</td>
<td>F: GCAGATTTGGTGCATTTAAAAAG R: GCACATGCAGTGCTCAAATCG P: CAAAATTGTGGAACCGTGACT</td>
<td>2.05</td>
<td></td>
<td></td>
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<tr>
<td>ye_ruc</td>
<td>Yersinia ruckeri (Enteric redmouth disease)</td>
<td>Microbe</td>
<td>Bacteria</td>
<td>F: TCCAGCACCCTACGAAGG R: ACGATGCAGAAGCGAGAT P: AAGGGGCTTTCCCGGTTC</td>
<td>2.07</td>
<td></td>
<td></td>
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<tr>
<td>MrpL40</td>
<td>39S ribosomal protein L40</td>
<td>Reference Gene</td>
<td>Reference Gene</td>
<td>F: CCCAGTATGAGGCACCTGAAGG R: GTTAATGCTGCCACCTCTCAC P: ACAACACATCACC</td>
<td>2.01</td>
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</tbody>
</table>
Table 3.2: Summary of tagged and released Chilko Lake sockeye salmon smolts in 2016.

<table>
<thead>
<tr>
<th>Age Class</th>
<th>n</th>
<th>n Gill Clip</th>
<th>Fork Length (mm)</th>
<th>Mass (g)</th>
<th>Tag Burden (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean (± SD)</td>
<td>Range</td>
<td>Mean (± SD)</td>
</tr>
<tr>
<td>Age-1</td>
<td>200</td>
<td>100</td>
<td>93 (± 3.8)</td>
<td>85 - 100</td>
<td>5.9 (± 0.7)</td>
</tr>
<tr>
<td>Age-2</td>
<td>99</td>
<td>89</td>
<td>128 (± 5.7)</td>
<td>117 - 143</td>
<td>15.3 (± 2.2)</td>
</tr>
</tbody>
</table>

Table 3.3: Cormack-Jolly-Seber model estimates of cumulative survival (SE) for age-1 and age-2 Chilko Lake sockeye salmon smolts and detection efficiencies (SE) of each receiver array. na = not applicable due to too few detections for an estimate to be determined.

<table>
<thead>
<tr>
<th>Array</th>
<th>Cumulative Survival Φ (SE)</th>
<th>Detection Efficiency p (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age 1</td>
<td>Age 2</td>
</tr>
<tr>
<td>Array B (14 km)</td>
<td>0.76 (0.04)</td>
<td>0.53 (0.05)</td>
</tr>
<tr>
<td>Array C (80 km)</td>
<td>0.68 (0.06)</td>
<td>0.41 (0.06)</td>
</tr>
<tr>
<td>Array G (657 km)</td>
<td>0.56 (0.13)</td>
<td>0.28 (0.05)</td>
</tr>
<tr>
<td>Array I (882 km)</td>
<td>0.20 (0.06)</td>
<td>na</td>
</tr>
<tr>
<td>Array J (951 km)</td>
<td>0.16 (0.11)</td>
<td>na</td>
</tr>
</tbody>
</table>
Figure 3.1: Study area for Chilko Lake sockeye salmon smolts (*Oncorhynchus nerka*) in 2016. Blue star represents the release site. Yellow circles and lines represent either individual receivers or receiver arrays able to detect V7 (69 kHz) tags. Red circles and lines represent individual receivers or receiver arrays able to detect V4 (180 kHz) and V7 tags. Smolts were collected and tagged between April 17 – 23 in 2016.
Figure 3.2: Bar plots of infectious agent prevalence in tagged and gill sampled Chilko Lake sockeye smolts (Oncorhynchus nerka) in 2016. C. B. Cys. = ‘Candidatus Branchiomonas cysticola’ (n = 139), F. psy. = Flavobacterium psychrophilum (n = 15), IHNV = Infectious haematopoietic necrosis virus (n = 15), Ic. mul = Ichthyophthirius multifiliis (n = 1). Red represents age-1 smolts, blue represents age-2 smolts, and purple represents both age groups combined. Asterisk indicates significant difference in prevalence between age-1 smolts and age-2 smolts.
Figure 3.3: Relative loads of infectious agents (log[RNA copy number]) for the three most commonly detected microbes in age-1 and age-2 Chilko Lake sockeye salmon (Oncorhynchus nerka) smolts in 2016. Solid lines represent the mean relative load. Dashed lines represent the limit of detection (LOD) for the specific assay. Asterisk indicates significant difference in relative load between age-1 smolts and age-2 smolts.
**Figure 3.4:** Relative loads of microbes (log[RNA copy number]) for the three most commonly detected microbes in age-1 and age-2 Chilko Lake sockeye salmon (*Oncorhynchus nerka*) smolts in 2016. The numbers in parentheses indicate the number of smolts in each survivor group with no detection of the microbe. The Imminent Mortality group represents smolts that were not detected post release (n = 58), the River Mortality group represent smolts that were detected in freshwater but not at the Fraser River mouth array or beyond (n = 49), the Ocean Mortality group represents smolts that were detected at the Fraser River mouth, but not at the last array at Johnstone Strait (n = 39), and the Migration Survivor group represents smolts that were detected at the final array at Johnstone Strait (n = 8). Solid lines represent the mean relative load for each survival group. Dashed lines represent the limit of detection (LOD) for the specific assay. The dotted line on the infectious haematopoietic necrosis virus plot represents the threshold of high levels of infection.
Figure 3.5: Bar plot of host gene loadings on first 3 principle components (PCs). PC1 explained 27% of the variance, PC2 explained 12% of the variance, and PC3 explained 11% of the variance.
**Figure 3.6:** Position of each individual smolt (n = 155) along the first three principal component (PC) axes for the principal component analysis (PCA) of gene expression data from non-lethal gill biopsies of tagging Chilko sockeye smolts (*Oncorhynchus nerka*) in 2016. PC1 explained 27% of the variance, with PC2 and PC3 explaining 12% and 11% of the variance respectively. Red represents age-1 smolts and red represents age-2 smolts. Ellipses represent 95% confidence intervals.
Figure 3.7: Position of each individual smolt (n = 155) along the first three principal component (PC) axes for the principal component analysis (PCA) conducted using gene expression data from non-lethal gill biopsies of tagging Chilko sockeye smolts (*Oncorhynchus nerka*) in 2016. PC1 explained 27% of the variance, with PC2 and PC3 explaining 12% and 11% of the variance respectively. Point color represents survival group: IM = imminent mortality (orange), RM = river mortality (green), OM = ocean mortality (blue), MS = migration survivor (purple). Shape represents age groups (● = age-1 and ▲ = age-2). Ellipses represent 95% confidence intervals. Numbers on (A) represent relative load of infectious haematopoietic necrosis virus for smolts that had the virus.
Figure 3.8: Gene expression profiles of tagged and gill biopsied age-1 and age-2 Chilko Lake sockeye salmon (*Oncorhynchus nerka*) smolts in 2016 represented by the first 3 principle components (PCs). Sample sizes for the imminent mortality, river mortality, ocean mortality, and migration survivor groups were 58, 49, 39, and 8 respectively. Solid lines represent the means for each survival group. Asterisk indicates significant difference in gene expression profiles between survival groups.
Chapter 4: Conclusions and Implications

Pacific salmon (Oncorhynchus spp) smolt migrations from their natal rearing grounds into the ocean are an impressive annual event during which high mortality can occur (Groot and Margolis 1991, Welch et al. 2009, 2011, Rechisky et al. 2018). A number of external and environmental factors can influence survival during this period as well as smolt physiological condition (Evans et al. 2014) and disease (Jeffries et al. 2014, Miller et al. 2014). Understanding how these factors influence survival is important, as this important life stage has been linked to salmon population productivity (Beamish et al. 2004, Farley et al. 2007, Irvine & Akenhead 2013), yet current research is limited. My thesis research examined the role of age and physiological condition on Chilko Lake sockeye salmon smolt migration survival and behaviour, providing valuable new insight to the migration ecology of this important population in the Fraser River watershed. In Chapter 2, large-scale acoustic telemetry and modified Cormack-Jolly-Seber mark-recapture models were used to identify regions of low survival for migrating age-1 smolts. I also directly compared the survival and behaviour of co-migrating age-2 smolts to investigate the influence of smolt age over the first ~950 km of their migration route. In Chapter 3, I used transcriptomics technology with non-lethal gill biopsies of tagged smolts to examine how infectious agents and physiological condition impacts survival of migrating age-1 and age-2 smolts. Using used high-throughput real-time quantitative polymerase chain reaction (HT-qRT-PCR), I evaluated infectious agent presence, prevalence, and load of migrating smolts, and identified 4 infectious agents present in the sampled population. I also assessed gene expression profiles of both age groups, including host biomarkers associated with immune and stress responses, and osmoregulation. I used an unsupervised principle component analysis with
a permutational multivariate analysis of variance to investigate differences in gene expression profiles between age groups, and how gene expression profiles influenced smolt migration survival. In the following sections, I outline how my results have expanded the understanding of smolt migrations and potential factors contributing to mortality, as well as some limitations of my research. I also discuss how my results can be used to improve management and conservation of salmon populations on the coast of British Columbia.

4.1 Regions of low survival

My thesis confirms that survival is landscape-dependent for Chilko Lake sockeye smolts. Previous telemetry studies demonstrated that age-2 Chilko Lake sockeye smolt survival is particularly low (68 – 90%) in the first 14 km of migration through the Chilko and Chilcotin rivers (Clark et al. 2016, Furey et al. 2016, Rechisky et al. 2018). My thesis research illustrates that age-1 smolts experience similarly poor survival through this region, confirming that this upper freshwater landscape is high risk for all migrating smolts – despite its pristine nature.

Previous telemetry studies also demonstrated that age-2 smolt survival is nearly 100% through the highly turbid and fast flowing Fraser River, and then decreases (to 38 –77%) after smolts enter the Strait of Georgia (Clark et al. 2016, Rechiskey et al. 2018). Likewise, my thesis research demonstrates that age-1 smolts experience a similar decrease in survival after entering the Strait of Georgia. Determining survival of age-1 smolts is important as they generally consist of >95% of the outmigrating population. My thesis results suggest that the general patterns in landscape-specific survival from previous telemetry studies of age-2 smolts may be similar to age-1 smolts, despite the great disparity between the proportions of each age class.
A number of external factors can influence smolt survival during migration. I hypothesized that predation likely plays a key role in regions of low survival, as it does across many systems (Ruggerone and Rogers 1984, Heggenes and Borgstrom 1988, Mesa 1994, Collis et al. 2001, Evans et al. 2012, Hostetter et al. 2012, Cavallo et al. 2013). A wide range of avian and fish predators are abundant near the outlet of Chilko Lake during the period of smolt outmigration (Clark et al. 2016). Bull trout in particular binge-feed almost exclusively on smolts as smolts exit the lake (Furey et al. 2015a). Likewise, as smolts enter the Strait of Georgia out from the Fraser River, they are met with an additional abundance of avian and marine mammal predators known to target salmonids (Thomas et al. 2016, Kajimura et al. 1980, Keple 2002, Chasco et al. 2017, Butler et al. 2015). Additional research identifying important predators and the impacts of predation on salmon population productively would be beneficial. In addition to predation, factors such as changing environmental conditions, currents and flow (Perry et al. 2013), and food availability (Beamish and Mahnken 2001) can also influence migration survival. The presence and abundance of predators, as well as other key factors impacting survival, can vary through specific migratory corridors through the Discovery Islands, thus, I suggest future studies focus on sockeye smolt behaviour and survival through this region.

4.2 Smolt age

Fish body size can often can be an important component of smolt survival (McGurk 1996, 1999, Beamish et al. 2004), with larger individuals generally experiencing increased survival and reduced predation risk (Koenings et al. 1993, Furey et al. 2015a, Tucker et al. 2016). Previous telemetry studies have characterized the survival of age-2 Chilko Lake sockeye
smolts (Clark et al. 2016, Furey et al. 2016, Rechisky et al. 2018), but given that age-2 smolts are generally 40% longer and 300% heavier than age-1 smolts in the population (Irvine and Akenhead 2013), it is necessary to investigate the migration survival and behaviour of smaller age-1 smolts that make up the majority of the outmigrant population. In Chapter 2, I demonstrated that cumulative freshwater survival of age-1 smolts was surprisingly double that of age-2 survival in 2016. Although I could not directly compare survival of the two age classes in the marine environment (due to few age-2 smolts detected), the age-specific difference appeared to persist to the final array capable of detecting both tag types (Array J, 951 km). Interestingly, the cumulative survival estimates I determined for age-1 smolts were within the range of survival estimates of age-2 smolts from previous years (Clark et al. 2016, Rechisky et al. 2018). However, my cumulative survival estimates for age-2 smolts were lower than expected relative to previous years.

I hypothesized that potential ecological factors could be driving the difference in survival between age classes, however, my thesis research also highlights the potential important impact of high tag burdens (the ratio of tag mass in air to fish mass) on survival estimates of tagged smolts. Age-1 smolts, which had better survival, also had a lower average tag burden than age-2 smolts. In Chapter 2, I demonstrated that tag burden may have had a modest impact on age-2 smolt survival, likely because some tag burdens exceeded the suggested ~8-10% maximum needed to avoid adverse effects on swim performance (Brown et al. 2006, Collins et al 2013). This suggests that high tag burden may partly contribute to observed age-specific differences in survival in released smolts. Previous studies found tagging to have minimal or no impact on age-2 smolt survival (Jeffries et al. 2014, Clark et al. 2016, Furey at al. 2016). However, Rechisky et al. (2018) found that when there was a wide distribution of tag burdens in one year out of five,
there was an effect on survival for more heavily burdened smolts. They suggested that there may not have been enough contrast in tag burden (i.e., tag burden was overall high) in the other years to determine whether tag burden less than the recommend limits would impact survival.

Additionally, a concurrent tagging study examined the effects of tag burden on migrating age-1 and age-2 Chilko Lake sockeye smolts in 2017 and found that age-2 smolts with higher tag burdens had consistently lower survival than age-1 and age-2 smolts with lower tag burdens (Stevenson et al. In Prep). My thesis research highlights the importance of the potential impact of tag burden on telemetry survival estimates of migration smolts. Given that smolt size is usually assumed to influence adult returns of sockeye salmon and population productivity, future studies should focus on determining whether or not there is a true ecological difference in age classes.

4.3 Infectious agents and smolt condition

Fish health including the presence and load of infectious agents and gene expression profiles associated with stress and immune responses can influence smolt migration survival, especially through high-risk regions (Jeffries et al. 2014). However, research linking the role of these important factors to subsequent migration fate of wild migrating smolts is currently limited (but see Jefferies et al. 2014). In Chapter 3, I used HT-qRT-PCR with non-lethal gill biopsies of acoustically tagged age-1 and age-2 Chilko Lake sockeye smolts to demonstrate that infectious agents and stress and immune profiles can influence migration survival. I demonstrated that *F. psychrophilum* was present in 10% of the out-migrating sampled population and was related to migration fate. In addition, I demonstrated that immune profiles indicative of inflammatory and
stress responses were related to migration survival, and these genes were more highly expressed in age-2 smolts. Furthermore, smolts with the highest relative loads of IHNV were clustered together on the positive end of the PC1 axis, which was associated with elevated expression of viral immune response genes. Elevated expression of these genes indicate that individuals were in a viral disease state, highlighting an important link between the presence of IHNV and an antiviral response that has rarely been made (Miller et al. 2017). Overall, these results suggest that smolts of both age classes may have been responding to one or more infectious agent and had elevated stress levels that could have contributed to mortality shortly after release. The use of acoustic telemetry along with infectious agent and physiological profiles in my thesis provides a unique opportunity to identify key factors impacting the migratory performance of wild smolts. To provide an increased understanding of links between infectious disease and smolt migration survival, future telemetry studies could include a broader range of infectious agents. Expanding upon methods used by Miller et al. (2017) to identify host transcriptional biomarkers that can distinguish between disease states of other infectious agents such as bacteria would also improve our ability to link infectious disease and important genes indicative of active disease states to juvenile salmon migration survival.

I hypothesized that *F. psychrophilum* and gene expression profiles indicative of inflammatory and stress responses may have contributed to the poor smolt survival observed through the upper freshwater regions of migration for both age classes, either through direct mortality, or potentially through increased vulnerability to predation. Predation has been identified has a likely cause of significant mortality through this high-risk landscape, as smolts encounter a number of avian and fish predators when exiting Chilko Lake (Clark et al. 2016). Poor condition may have increased the susceptibility of smolts to predation by compromising swim
performance or altering behaviours such as schooling, foraging, and predator avoidance (Handeland et al. 1996, Dieperink et al. 2002, Miller et al. 2014). Furey (2016) found that smolts with infectious agents such as *F. psychrophilum* and IHNV were significantly more likely to be consumed by bull trout as they began migration from Chilko Lake.

4.4 Implications for conservation and management

The survival estimate results from Chapter 2 combined with the transcriptomics results from Chapter 3 can help to inform conservation and management of salmonid populations. Chilko Lake sockeye are a fundamental indicator stock used by fisheries managers for the >150 sockeye populations in the Fraser River watershed. Smolt survival is challenging to estimate for fisheries managers, which can contribute to the high levels of uncertainty in models used to predict the number of returning adults (Cohen 2012). Reducing uncertainty in estimates of adult returns is important because predictions are used to allocate potential harvest among commercial, First Nations, and recreational fisheries in Canada. Given that smolt survival estimates are used in predictive population models of adult returns, my estimates of age-1 smolt survival can help to better inform management and reduce uncertainties in run size forecasts, thus improving conservation of this important species. In addition, smolt survival is variable among years (Irvine and Akenhead 2013, Clark et al. 2016, Rechiskey et al. 2018) and our understanding of the exact causes of mortality during this critical life stage is limited. The identification of important physiological factors such as particular infectious agents and genes that contribute to smolt mortality and variability in survival can help to further inform conservation objectives, and be
incorporated by managers to further improve predictive abilities of models forecasting adult returns.

4.5 Summary

Overall, this thesis determined the influence of smolt age and physiological condition on the behaviour and survival of migrating wild juvenile sockeye. My research confirms regions of poor survival for age-1 smolts that make up the vast majority of one of the largest sockeye populations in Canada. Through linking biotelemetry survival estimates with sophisticated transcriptomics techniques, I confirmed that smolt condition can contribute to early migration mortality of both age classes. Collectively, this thesis enhances our understanding of factors potentially influencing salmon population productivity, and increases the current knowledge of the ecology of smolt migrations on the on North America’s Pacific coast.
References


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haematopoietic necrosis virus (IHNV) infection or DNA vaccination. *Fish & Shellfish Immunology*, 17(5), 447–462.


Appendix

A.1 Appendix tables

**Table A1:** Detection subarrays used to form capture history sequences for Chilko Lake sockeye smolts in 2016. Numbers indicate the subarrays that were included in the sequence. Each ‘x’ indicates a subarray that was operational in 2016, but was not included in the capture history sequence. All smolts were released from the DFO counting fence at the outflow of Chilko Lake. Distances between release and each detection site are provided. JDF = Juan de Fuca Strait.

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>Array A (Release)</th>
<th>Array B 13 km</th>
<th>Array C 79 km</th>
<th>Array D 178 km</th>
<th>Array E 599 km</th>
<th>Array F 658 km</th>
<th>Array H* 800 km</th>
<th>Array I 875 km</th>
<th>Array J 944 km</th>
<th>Array K* 1041 km</th>
<th>JDF* 858 km</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>x</td>
<td>5</td>
<td>x</td>
<td>6</td>
<td>7</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Age 2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>x</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>x</td>
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<td>1</td>
<td>2</td>
<td>3</td>
<td>x</td>
<td>x</td>
<td>4</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

* Subarray could detect the 69 kHz tags used in the Age 2 smolts, but not the 180 kHz tags used in the Age 1 smolts.
Figure A1. Heatmap of host biomarkers in relation to smolt age, *Flavobacterium psychrophilum*, and survival groups for tagged and gill sampled Chilko Lake sockeye smolts (*Oncorhynchus nerka*) in 2016. Red represents elevated expression of host biomarkers. Blue represents decreased expression of host biomarkers. Fl_psy = *Flavobacterium psychrophilum*, abs = absent (i.e. no *F. psychrophilum*), low = low loads of *F. psychrophilum*, high = high loads of *F. psychrophilum*. High loads of *F. psychrophilum* were determined by visually assessing the distribution of relative loads for outliers.