PHYSIOLOGICAL AND ENVIRONMENTAL FACTORS INFLUENCING MIGRATION SURVIVAL AND BEHAVIOUR OF HATCHERY SEYMOUR RIVER STEELHEAD SMOLTS (*Oncorhynchus mykiss*) IN COASTAL BRITISH COLUMBIA

by

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Abstract

For anadromous steelhead smolts (*Oncorhynchus mykiss*), physiological condition and spatiotemporal variability in movement patterns, such as routes, have the potential to influence survival, but these aspects of the migration are poorly understood. To investigate route-specific movements and survival during outmigration, I implanted acoustic tags into 243 hatchery steelhead smolts and tracked their migration through coastal British Columbia for up to ~400 km. Two release groups (marine and freshwater) were used to assess survival through the first marine inlet. To better understand how smolt condition influences migration fate, I combined 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 fate. Poorest survival was in the river and marine inlet first encountered by smolts. Survival rates in all other migratory segments did not differ between release groups, suggesting the near-shore marine environment is associated with particularly poor survival for outmigrant steelhead. I present rare evidence of route-specific survival for a migratory species, which was detected though a series of channels ~200 km from release. The westernmost route here was associated with significantly higher survival and was more travelled. A portion of smolts exhibited 'milling patterns' including reversals in migration direction or lateral movements along acoustic subarrays. Redundancy analyses of gene expression, infectious agent loads, and body condition highlighted gene expression profiles indicative of migratory fate. Smolts that were never detected in the river clustered together, far from other groups in ordination space. Smolts that did not make it from the river to the estuary had significantly elevated expression of the immune genes Il-17D and RPL6, and lower expression of the osmoregulatory gene NKA α1b relative to other individuals. Two infectious agents were detected in tagged smolts (*Flavobacterium psychrophilum* and '*Candidatus* Branchiomonas *cysticola*'), neither of which had an influence on survival. My results identify potentially important, yet understudied regions affecting survival of salmonids smolts. I also demonstrate some of the first evidence of gene expression profiles predicting individual migration fate in juvenile salmonids, and highlight potential mechanisms influencing freshwater and early marine survival for steelhead smolts.

Lay Summary

Salmon (*Oncorhynchus* spp.) smolts experience poor survival when migrating from freshwater to the open ocean. To better understand what influences smolt behaviour and survival, I tagged hatchery steelhead (*Oncorhynchus mykiss*) smolts, and tracked them for up to ~400 km in freshwater, and coastal British Columbia. Survival was poorest in the river and first marine inlet encountered by smolts. I identified an important migratory route in the coastal marine system for steelhead smolts. Combining tagging data, and novel gene expression analyses, I identified two immune function genes, and one gene related to saltwater transfer which were associated with individuals that were never detected leaving the river. I also identified two pathogens present in the smolts although neither appeared to influence their survival. My thesis identifies important environmental and physiological factors which influence migration fate during a critical life stage that is tied to salmon population productivity.

Preface

This research was conducted as part of the Salish Sea Marine Survival Project (an international collaborative initiative aimed at determining factors influencing survival for anadromous salmonid populations in the Salish Sea), and as 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 considerable logistical support from my colleagues Dr. Nathan B. Furey and Arthur L. Bass, as well as supervision and guidance from my supervisor, Dr. Scott Hinch. David Welch, Aswea Porter and Erin Rechisky from Kintama Research Services provided support by deploying and recovering acoustic receivers in the marine environment, and considerable involvement with statistical analyses in modelling movements and survival (Chapter 2). Dr Kristi Miller and her staff at the Molecular Genetics Lab (Pacific Biological Station, Nanaimo) were integral in facilitating and guiding me in my genomic laboratory work (Chapter 3). Stephen Vincent, Marc Guimond and the staff at the Seymour River Hatchery assisted with logistical support during tagging and deployment of freshwater receivers. 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: Route-specific movements and survival during early marine migration of hatchery steelhead (*Oncorhynchus mykiss*) smolts in coastal British Columbia

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Chapter 3: Transcriptome profiles relate to migration fate in hatchery steelhead (*Oncorhynchus mykiss*) smolts

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Dedication

This thesis is dedicated to my wife Taryn Lees, my brothers Jonathan and Brian Healy, and my parents, Peter and Janet Healy.

Chapter 1: Introduction

1.1 Salmonids in the Pacific Northwest

Anadromous Pacific salmonids (*Oncorhynchus spp.*) are a group of teleost fish with high cultural, ecological, and economic importance in the Pacific Northwest. They are key components of marine and terrestrial food webs, and are significant species for First Nations, recreational and commercial harvest. Though productivity of salmon in the Pacific Northwest is generally characterized by high interannual variability, declines in abundance and recruitment in some species and stocks has been evident since the early 1990's (Irvine & Akenhead, 2013; Irvine & Fukuwaka, 2011). These trends have focused increased efforts aimed at identifying factors influencing survival across various life stages. Recent responses, such as the Cohen Commission of Inquiry into the Decline of Fraser River Sockeye (*Oncorhynchus nerka;* Cohen, 2012), and the Salish Sea Marine Survival Project (SSMSP, 2017; www.marinesurvivalproject.com) are examples of such efforts. Although the proximate factors driving declining trends in abundance remain poorly understood, numerous hypotheses have been proposed including climatic related changes in oceanic productivity (Atcheson et al. 2012; Hare et al. 1999; Irvine & Fukuwaka, 2011), disease (Arkoosh et al. 2004), predation (Berejikian et al. 2016; Thomas et al. 2016), and competition (Beamish et al. 2004; Irvine & Akenhead, 2013).

1.2 Smolt survival trends

The productivity of salmon populations may strongly be linked to factors that influence the smolt life stage (Irvine & Akenhead, 2013), when juveniles undergo important physiological

changes and migrate from freshwater rearing to ocean rearing. These changes, such as alterations to osmoregulation (Bystriansky, 2006; McCormick & Saunders, 1987; Richards, 2003), body morphology (Nichols et al. 2008) and rapid growth (Beamish & Mahnken, 2001; Beckman et al. 1998; McCormick & Saunders, 1987) allow fish to transition to the marine environment where they will reside as adults prior to returning to freshwater to spawn (Groot & Margolis, 1991). Smolts are exposed to numerous biotic and abiotic factors which can influence their behaviour and migratory success; however, our knowledge of the early marine phase is limited (Drenner et al. 2012). Some studies suggest that the early marine phase is associated with poor survival for juvenile salmonids (Balfry et al. 2011; Clark et al. 2016; Welch et al. 2009, 2011; Goetz et al. 2015; Kendall et al. 2017), but little research has investigated the underlying mechanisms.

1.3 Factors influencing smolt migratory success

Attributes of the environment, such as predator densities, resource availability and barriers to migration are known to influence migratory movements (Alerstam et al. 2003). These factors and their interactions can result in substantial variation in migration routes and behaviour among individuals (Gschweng et al. 2008; Hays et al. 2001). While spatiotemporal variability in movements can be associated with survival (Hewson et al. 2016; Sicurella et al. 2016), examples in the literature remain rare. Survival is expected to be variable across landscapes (Hewson et al. 2016; Sawyer et al. 2009), so identifying regions or routes where survival is particularly poor is an important step in determining where to focus research or conservation efforts (Sawyer et al. 2009). Migration routes have been linked to survival for juvenile salmonids in rivers (Buchanan et al. 2013; Perry et al. 2010, 2013) and through coastal marine systems (Furey et al. 2015), underscoring the importance that trajectories can have on migratory fate. Because survival

during the early marine period is thought to influence population productivity (Moore et al. 2012), critical migration routes could have important links to population-level impacts in salmonids.

The physiological condition of smolts may play an important role in migratory survival (Hostetter et al. 2011; Jeffries et al. 2014). In preparation for leaving natal freshwater rearing areas, smolts must undergo a number of drastic physiological changes, which if comprimised could lead to a reduction in migratory success. Poor condition can influence various aspects of outmigration performance, such as a reduction in predation avoidance capabilities (Hostetter et al. 2012; Mesa, 1994), osmoregulatory failure (Fuss & Hopley, 2003), poor growth (Beamish et al. 2004), and reduced immune functioning (Arkoosh et al. 2006; Hostetter et al. 2011).

Infectious agents (e.g. viruses, bacteria) and immune responses may also play an important role in survival for outmigrating smolts (Jeffries et al. 2014). At present, however, the role of these factors on smolt survival is poorly understood, as it can be particularly difficult to link individual physiology to migration survival (Miller et al. 2014). Infectious agents have the potential to diminish economically important fisheries species (Hoenig et al. 2016; Lafferty et al. 2015), and disrupt important ecological food webs (Buck & Ripple, 2017), thus, further underscoring the need to identify infectious agents of concern in salmonid populations.

The recent incorporation of genomic techniques by fisheries ecologists has made it easier to investigate the role of disease and immune responses on survival in migrating salmonids. By combining physiological biopsies with acoustic telemetry, gene expression profiles associated with immune responses have been correlated with migration fate and spawning success in adult sockeye (Miller et al. 2009, 2011). At present, however, few studies have applied this

methodology to migrating smolts (but see: Jeffries et al. 2014). Disease is thought to be an important factor causing early marine mortality in outmigrating smolts (Van Gaest et al. 2011; Ferguson et al. 2012; Hostetter et al. 2012; Miller et al. 2014), although at present, our knowledge of how infectious agents influence smolts is primarily from lethal sampling. More recent research has utilized non-lethal gill biopsies, which can be taken from smolts without impacting individual survival (Martinelli-Liedtke et al. 1999; Jeffries et al. 2014). These samples can be analyzed for various molecular biomarkers and related to telemetry data to determine how individual physiological condition influences migration survival. Jeffries et al. (2014) used this technique on outmigrating sockeye smolts in Chilko Lake, British Columbia (BC), and found that gene expression profiles related to immune responses and pathogens were predictive of fate during migration (Jeffries et al. 2014). The present work employed similar methodology of Jeffries et al. (2014) to understand how physiology (including the expression of genes, and presence of infectious agents) influences freshwater and early marine survival of steelhead (*Oncorhynchus mykiss*) smolts in coastal British Columbia.

1.4 Steelhead trout studies

Steelhead are an anadromous form of rainbow trout, which are generally considered a Pacific salmon; however, steelhead are known for their complex and plastic life history. Unlike Pacific salmon, steelhead are iteroparous (i.e. can have multiple reproductive events during their life), and have populations which consist of both migratory and non-migratory forms (Kendall et al. 2015). It is estimated that at least 31% of historic populations of steelhead have gone extinct in the last several hundred years in the Pacific Northwest (Gustafson et al. 2007), and in many

southern British Columbia watersheds, average steelhead recruitment has been declining since the 1990s (Ward, 2000).

To investigate several factors related to steelhead smolt migration survival, a four-year acoustic telemetry study was carried out with Seymour River (North Vancouver, British Columbia) hatchery steelhead from 2006-2009 (Balfry et al. 2011). In the last two years of the study, two different release locations were used: (1) a river release site, and (2) a marine release site, located ~18 km to the west of the mouth of the river. The study showed an apparent increase in survival (~7-21% increase) over ~400 km of marine migration for smolts released at the saltwater site, beyond the first marine inlet (i.e. Burrard Inlet; Balfry et al. 2011). These results align with the hypothesis that much of the mortality of migrating smolts can be localized to the early marine portion of the migration; however, with relatively low sample sizes, these estimates of survival were potentially unreliable. One of the years also looked at the effect of vaccinating fish against several known microbes related to disease prior to their release. This enhanced survival slightly, indicating that infectious agents may play a role in migration success for this population. At present, the scope of infectious agents in this system, as well as their contribution to survival is poorly understood.

1.5 Thesis overview and research objectives

To better understand factors influencing survival of outmigrating steelhead smolts, my thesis investigated how landscape-level factors and individual smolt condition relates to migration fate and behaviour. The present work had two primary objectives. First was to use acoustic telemetry to quantify levels of Seymour River hatchery steelhead smolt survival,

including identifying regions and routes of particularly poor survival through the freshwater and early marine portions of migration. Second was to pair telemetry data with non-lethal biopsies and cutting edge genomics techniques to assess the relationship between physiology, infectious agents and migration fate. My hypotheses were: (1) steelhead released past Burrard Inlet, a potential region of poor survival, would experience higher survival than fish that must pass through this migration segment, and (2) individual smolt condition would relate to survival and movement rates, such that: fish that were not osmotically prepared for the marine environment, were positive for infectious agents, and/or showed gene expression profiles indicative of immune responses would exhibit reduced levels of survival through the freshwater and coastal marine environments.

In Chapter 2, I report the findings from a 2015 steelhead smolt acoustic tagging study which quantifies survival during outmigration, and describes other aspects of movements such as travel rates and milling behaviours. The use of two release groups (marine and river), large-scale telemetry arrays, and modified mark-recapture models allowed me to identify regions of particularly poor survival for migrating smolts. Several new telemetry arrays offered the rare opportunity to assess route-specific survival through an important portion of the marine migration in coastal British Columbia. Chapter 3 takes a physiological approach to survival, by investigating the relationship between a suite of physiological biomarkers (i.e. the expression of multiple genes) and infectious agents on survival. This chapter furthers our knowledge of intrinsic factors influencing trends in survival as steelhead migrate through the early freshwater and marine coastal environment on their way to the open ocean. In Chapter 4, my conclusion section, I summarize and synthesize the findings of both studies, highlight how my work has furthered our knowledge of salmonid ecology, suggest avenues of future investigation, and

discuss possible implications to management and conservation of salmonids in the Pacific Northwest.

Chapter 2: Route-specific movements and survival during early marine migration of hatchery steelhead (*Oncorhynchus mykiss*) smolts in coastal British Columbia

2.1 Introduction

Animal migrations are complex and diverse behaviours witnessed across numerous taxa, including insects, birds, mammals, and fish (Chapman et al. 2014). Migrations can confer various benefits to an individual, including access to favourable feeding areas (Igota et al. 2004, Daly et al. 2014), reproductive opportunities (Chapman et al. 2012), and a reduction in predation risk (Skov et al. 2013), but movements can also come at a cost. Spatiotemporal variability in movements may have direct implications for an organism's fitness or survival (Nathan et al. 2008). For example, dynamics of the environment, such as barriers to movement, currents, predator density, and resource availability, have the potential to influence migrations (Alerstam et al. 2003). The interactions of these factors can result in variable migratory behaviours, such as migration routes and timing (Hays et al. 2001, Gschweng et al. 2008, Singh et al. 2012). Such variability can influence an individual's probability of survival (English et al. 2005, Furey et al. 2015, Hewson et al. 2016, Sicurella et al. 2016), but empirical examples remain rare (Holyoak et al. 2008). Linking variation in organismal movements to fitness is at present an understudied aspect of wildlife ecology (Holyoak et al. 2008, Nathan et al. 2008), yet identifying important migratory routes and/or regions may be useful for informing spatial allocation of conservation resources (Hewson et al. 2016) or future industry development along migratory corridors (Sawyer et al. 2009, Cohen 2012). The present study aimed to investigate spatial movements for a migratory species, including how route- and location-specific movements can influence survival for a migratory species. For migratory anadromous salmonids (*Oncorhynchus* spp.) in the northern Pacific, population productivity is generally characterized by high interannual

variability; however, declines in abundance and survival in many species and populations have been evident since the early 1990s (Irvine & Fukuwaka 2011, Irvine & Akenhead 2013). These declines have prompted considerable research to identify factors influencing productivity of these economically, culturally, and ecologically important species (e.g. Cohen 2012). Productivity of salmonid populations can be linked to the marine phase, particularly during the 'smolt' life stage (Irvine & Akenhead 2013), when fish undergo dramatic physiological changes and migrate from freshwater natal areas to the marine environment. At present, however, the specific factors influencing survival during this critical life-history phase are poorly understood.

Acoustic telemetry studies in the Pacific Northwest have shown that the near-shore marine environment is typically associated with low smolt survival. Survival can vary during the first 300−400 km of marine migration, but generally ranges between ~3 and 30% depending on the species or population (Welch et al. 2009, 2011, Balfry et al. 2011, Clark et al. 2016). Even though smolt losses during this initial marine migration can be a relatively small fraction of the losses incurred at sea prior to returning as adults (Welch et al. 2011), poor survival during the early marine period is underscored by the short timeframe over which it occurs (typically ~2−4 weeks as smolts navigate towards offshore feeding grounds (Melnychuk et al. 2010, Welch et al. 2011, Clark et al. 2016). For some species, such as anadromous steelhead trout (*Oncorhynchus mykiss*), this critical period has been linked to declines in both wild and hatchery-based populations in the Pacific Northwest in recent years (Goetz et al. 2015), highlighting the need for a better understanding of the factors influencing survival during this initial coastal marine period.

The Salish Sea is a semi-enclosed marine embayment situated between Vancouver Island and the mainland of British Columbia (Beamish & MacFarlane 2014, Benedict & Gaydos 2015), which forms an important migratory pathway as smolts move from natal rivers to their offshore

feeding grounds. Steelhead and sockeye salmon (*Oncorhynchus nerka*) are typically thought to move through estuaries and the Salish Sea in particularly rapid and highly directed migrations compared with other species of salmonids, which may take up residency for extended periods (Tucker et al. 2009, Melnychuk et al. 2010, Welch et al. 2011). The increased use of acoustic telemetry in recent years has further characterized fine-scale movements of migrating smolts through the Salish Sea. For example, migratory movement patterns and their impacts on survival have been investigated for steelhead smolts at the Northern Strait of Georgia telemetry subarray (spanning between the mainland of British Columbia and Vancouver Island, ~130 km northwest of Vancouver), highlighting route-specific survival trends across this portion of the migration (Furey et al. 2015). Milling patterns have been identified around this subarray, including westward and fully counterclockwise movements (Furey et al. 2015). At present, however, little is known about migratory patterns and survival further along in the migration, where numerous islands and fjords offer the potential for further spatiotemporal variability in smolt migration.

I tracked hatchery-reared steelhead smolts from the Seymour River (North Vancouver, British Columbia) as they migrated nearly 400 km through the freshwater and near-shore marine environment. Previous acoustic telemetry work on this population has suggested that survival is particularly low for smolts migrating through the first marine inlet (~18 km long) encountered on leaving the estuary (Balfry et al. 2011), but sample sizes were low. I used acoustic telemetry with a large sample size of steelhead smolts to quantify survival and movement patterns, and to identify regions and routes associated with poor migratory success. Two release groups were employed to experimentally test the hypothesis that the first marine inlet is a region of particularly low survival for smolts outmigrating from this watershed. New acoustic receiver subarrays were deployed along the migration route at the northern exit of the Salish Sea and in

Johnstone Strait, which provided the ability to assess route-specific survival and migration movements for steelhead smolts at finer spatial and temporal scales than previously possible.

2.2 Methods

2.2.1 Study System

The Seymour River is a regulated system located in North Vancouver, British Columbia (Figure 2.1). Its watershed drains approximately 176km^2 (Balfry et al. 2011) and flows south where its mouth meets Burrard Inlet. The Seymour Hatchery is located just downstream of the Seymour Falls Dam, which blocks historical spawning access to salmonids (*Oncorhynchus spp.*) in the river. The hatchery produces up to 30000 steelhead trout annually, which are typically reared for a year and released as smolts in the spring (Seymour Salmonid Society 2015). If released in the Seymour River below the dam, steelhead smolts migrate downstream to Burrard Inlet (~2.5 km from freshwater release site) and then northwest through the Salish Sea, the Discovery Islands, and Johnstone Strait before reaching Queen Charlotte Sound and ultimately the open Pacific Ocean (Balfry et al. 2011, Welch et al. 2011; Figure 2.1A). Presently, the hatchery loads smolts onto trucks and releases them beyond Burrard Inlet, in response to a study by Balfry et al. (2011), which suggested this region was associated with poor survival

2.2.2 Acoustic tagging

Tagging took place at the Seymour Hatchery (49° 26’ 15.2”N, 122° 58’ 01.1”W) on 14 and 15 May 2015. A total of 243 steelhead smolts (fork length [FL] = 200.2 mm [±0.8 mm SE]; mass [M] = 77.0 g [±1.1 g SE]; Table 1) were randomly removed from hatchery rearing

channels, placed in separated raceways, and restricted from feeding for 24 h prior to surgeries. Surgeries followed Collins et al. (2013) and Furey et al. (2016), and are described in greater detail in Appendix A.1. Surgeries took between 1 and 9 min (mean = 3.5 min [±0.1 min SE]), and surgical instruments were sterilized between each surgery. Following surgeries, fish were placed in separated raceways grouped by release location (i.e. all river-release smolts were grouped together) and allowed to recover for at least 4 d prior to their release. Of the 243 smolts acoustic-tagged (VEMCO V7-2L, 7 mm × 18 mm, ~0.7 g in water; 69 kHz, VEMCO, www.vemco.com), 164 were also non-lethally biopsied using small bone cutting forceps to remove the tips of 2−3 gill filaments for genemoic analyses (i.e. Chapter 2). Previous studies involving much smaller sockeye smolts (~120mm FL) have suggested no impact on the survival of fish receiving this non- lethal gill clip treatment (Martinelli-Liedtke et al. 1999, Jeffries et al. 2014). Tagging procedures followed the University of British Columbia Animal Use Protocol A15-0205.

2.2.3 Acoustic telemetry infrastructure

As steelhead smolts migrated through the Salish Sea, they passed several marine acoustic receiver subarrays (combination of VEMCO VR2W, VR3, and VR4 receivers) originally designed by the Pacific Ocean Salmon Tracking project (Welch et al. 2002) and now maintained by the Ocean Tracking Network Canada (Cooke et al. 2011). These subarrays are located in the Northern Strait of Georgia (NSOG), Queen Charlotte Strait (QCS), and Strait of Juan de Fuca. In 2015, two new marine subarrays were deployed to investigate marine migration north of the Salish Sea in the Discovery Islands (DI) and Johnstone Strait (JS) region using new dual-frequency (69 and 180 kHz) VR4 receivers (Figure 2.1C). In addition, several temporary receivers were deployed in the Seymour River (~2.5 km from release). In sum, this large-scale

acoustic receiver array (comprising over 100 receivers) allowed tagged smolts to be tracked from their point of release to the northern or southern tip of Vancouver Island (Figure 2.1A), an in-water migration distance of up to ~400 km.

2.2.4 Fish releases

Tagged steelhead were loaded into ~1000-L tanks on trucks and released at 1 of 2 locations: (1) in the lower Seymour River (hereafter, 'river-release') (49°19'18.7" N, 123°00'50.4" W) or (2) directly into saltwater in West Vancouver (hereafter, 'marine-release') (49°20'24.8" N, 123°13'58.2" W; Table 2.1). These two release sites (Figure 2.1B) were chosen to experimentally test the influence of migrating ~18 km through Burrard Inlet on survival to the NSOG subarray. The marine-release group (n = 160) was transported and released on 19 May along with (~20 000) untagged hatchery-reared steelhead smolts. The river-release group (n = 83) was released over the course of 3 days (21−23 May; ~25−30 smolts d^{-1}) to minimize acoustic interference between tags, or 'tag collisions' on the lower river receivers and thus improve detection probability. Each river release included untagged conspecifics (~200−300) to mimic typical hatchery releases

2.2.5 Holding study

To investigate the impacts of gill clipping and tagging on smolts in freshwater and saltwater, 123 steelhead smolts were tagged with 'dummy tags' (same weight and dimensions as the V7 tags used for released smolts) and given at least four days to recover prior to being transported for holding at the University of British Columbia. Eighty of these smolts were also

non-lethally biopsied for gill tissue, for a separate study. Tagged fish were placed in either a saltwater or freshwater 3000-L tank along with a group of untagged conspecifics in each tank (Table 2.1). The duration of the holding study was 18 days, which approximately equals the expected travel time of steelhead smolts between Seymour River and QCS (Balfry et al. 2011, Welch et al. 2011). Tagging procedures were consistent between the holding study and acoustic tagging surgeries. Fish were fed daily (EWOS Canada, www.ewos.com) and tanks were monitored several times per day for mortalities and tag loss. At the end of the study, all fish were anaesthetized briefly (as per the acoustic tagging procedure; see A.1 'Acoustic tagging' in the Appendix) and FL and mass were measured prior to the fish being returned to tanks. The change in mean mass and FL for untagged fish in saltwater and freshwater tanks was calculated. Separate 1-sample t-tests were used to compare the change in mass and length of tagged fish to the mean change in untagged fish for each tank. One fish was removed from these analyses due to measurement error.

2.2.6 Survival analyses

To estimate segment-specific and cumulative survival of acoustic-tagged smolts during migration, I used a spatial mark−recapture model approach (e.g. Welch et al. 2009, Clark et al. 2016). Estimates of survival (ϕ), subarray detection probability (p), and their associated variances were calculated using variants of the Cormack-Jolly-Seber (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. See the Appendix for comprehensive details of analyses described below.

Survival analyses followed several steps. First, I screened the data for false detections, forming detection histories for each tagged individual, and then assessed goodness of fit of the data to the model. Separate Mann-Whitney U-tests were used to assess whether mean arrival dates at marine subarrays (NSOG, DI, JS, and QCS) differed by release groups or between the routes themselves along subarrays (e.g. if mean arrival date differed between Discovery Passage vs. Sutil Channel). Next, I tested whether release location had an impact on ϕ and p to assess whether it was reasonable to estimate only one survival parameter for the two release groups in each area where migration routes were shared (i.e. NSOG to DI, DI to JS, JS to QCS). There was no evidence of an effect, so for subsequent analyses, all tagged smolts were pooled in the common migration corridor (NSOG to QCS). Finally, I used Akaike's information criterion (AIC) to assess whether FL, tag burden (the ratio of mass of acoustic tag in air to fish mass), and non-lethal gill tissue sampling affected survival. To test these effects, I compared the performance of the base model with 3 other models; each of these models was the same as the base model, but also included an additive effect for one of the three covariates of interest (Table 2.2). To account for model selection uncertainty (i.e. similar candidate model weights; Table 2.2), I model averaged across the four models used to test these effects to generate final estimates of ϕ and p for each migration segment. I then used the segment-specific survival estimates to calculate survival rates per unit time and distance, and cumulative survival estimates from release.

2.2.7 Route-specific use and survival

Along the marine acoustic subarrays (NSOG, DI, JS, and QCS), initial detection counts of smolts were compiled into histograms to assess the distribution of smolts across each

subarray. Few fish were detected on the Juan De Fuca line, so no distribution was created for this subarray. I further assessed route-based movements and survival in the Discovery Islands region (Figure 2.1C) using a spatial multi-state mark−recapture model. Similar to the CJS model, multi-state models estimate survival (defined as S as opposed to ϕ for CJS models) and detection probability (p), but they also estimate the probability of movement between states (i.e. route use; ψ). I used this approach to test whether steelhead were more likely to migrate through the DI using (1) Discovery Passage (between Vancouver Island and Quadra Island) or (2) Sutil Channel (between Quadra Island and Cortes Island) (Figure 2.1C), and whether the 2 routes resulted in different survival. Only one fish was detected migrating through Desolation Sound (between Cortes Island and the BC mainland) and thus was excluded from analyses. Route choice was assigned based on the location of last detection on the DI subarray. Six fish detected at the DI were removed from the analysis because they were last detected on NSOG (i.e. they probably did not migrate north). As multi-state models do not perform well near the boundaries of 0 and 1, I also used bootstrapping to gain additional estimates of route-specific survival through the DI.

A similar multi-state model selection approach was used to assess route-specific survival to the DI based on route along the NSOG subarray (Malaspina Strait, to the east of Texada Island versus the Strait of Georgia to the west). For these analyses, route use was assigned based on the location of first detection on the NSOG subarray. To further investigate disproportional route use around Texada Island, I used a proportional test of the initial receiver detection position for all smolts, while taking into account channel width of the Malaspina Strait relative to the subarray as a whole.

For these analyses, I used R with the package 'RMark' (Laake 2013) to construct models using the program 'MARK' (White & Burnham 1999). Model assumptions include equal

survival probability, equal probability of detection, and instantaneous sampling. Typically, detection probability and survival at the final subarray (QCS in the present study) cannot be independently estimated as there are no further subarrays along the migration route. One solution is to select a value for detection probability based on knowledge of the area, or performance of subarrays in similar environments (i.e. Welch et al. 2011, Clark et al. 2016). A pilot study involving double-tagged (VEMCO V9-1H and V4-1H acoustic tags) Seymour River steelhead in 2015 allowed me to more accurately estimate detection probability at QCS than has previously been employed (E.L. Rechisky *unpublished data*).

2.2.8 Travel and survival rates

Travel times were calculated from release or departure from one subarray to arrival at the next subarray. Departure was defined as the last detection along a subarray, and arrival as the first detection along a subsequent subarray. For river-release fish, the travel time from release to the estuary could not be accurately estimated because fish were randomly released over the course of three days. Next, travel rate in all segments was calculated as distance divided by travel time, where distances were measured for each fish as the shortest in-water distance between the central point of each subarray. For the subarrays spanning multiple channels at NSOG and DI, I measured the distance to the central point of each channel and then calculated an average across all detected fish. To assess the influence of individual smolt FL on marine migration rates (in km d^{-1}), separate generalized linear models were run for each segment migrated in common between the two release groups (i.e. NSOG to DI, DI to JS, and JS to QCS). To assess the relationship between estuary residence (duration between first and last detections) and survival to NSOG, a binomial generalized linear model was generated with survival from the estuary to NSOG as the

binary response and residence time in the river as the explanatory. I tested the significance of the model by comparing the difference in residual deviances between the model and a null model.

To scale survival by distance and time, I converted survival estimates to survival rates. Model-averaged survival estimates were converted to survival rates per day and per km as: $S^{1/d}$, where S = estimated survival and d = the mean travel time (d) or mean distance travelled (km). Survival rates based on route between DI and JS were also assessed, to take into account the differences in migration distance (and thus expected differences in migration time) between Desolation Sound and Sutil Channel. A detailed description of survival rate analysis can be found in the Appendix (see A.9 Survival rates)

2.2.9 Milling patterns during migration

I assessed individual smolt behaviour to identify unusual or unexpected migratory behaviours across the marine portion of migration. Smolt migratory sequences were assessed for two aspects of milling: (1) 'lateral movements' along a subarray, defined when smolts were first detected in one channel along a subarray, and next detected on another channel along that same subarray (i.e. first detected in Sutil Channel, then detected in Discovery Passage without first being detected at another subarray), and (2) 'reverse migrations,' classified as making a reversal in direction from one subarray to a previous subarray along the migration corridor (i.e. movement going against the generally expected migration direction).

2.3 Results

2.3.1 Survival

Estimated segment-specific survival in freshwater (release to river mouth ~2.5 km downstream) was 79% (95% CI: 67−90%). When accounting for the distance of this migratory segment, survival rates here were particularly low: 0% per 100 km (0− 0.1% per 100 km). For river-release smolts, subsequent segment-specific survival from the Seymour River mouth to NSOG was 27% (15−44%), while marine-release smolts experienced 65% (54−74%) survival from release to NSOG. For marine-release fish, the survival rate from release to NSOG was 96% d^{-1} (94−97% d^{-1}) compared with 87% d^{-1} (81−91% d^{-1}) for river-release fish travelling between the river estuary and NSOG. When considering survival rates per 100 km to the NSOG array, these differences were more apparent (marine-release: 70% per 100 km [63−77% per 100 km]; river-release: 40% per 100 km [26−51% per 100 km]; Figure 2.2).

Although sample size was limited for river-release smolts in the marine environment (Table A2 in the Appendix), there was no evidence that segment-specific survival beyond NSOG differed between release groups (Table 2.3). I therefore pooled release groups from NSOG to QCS to produce one estimate in each of the remaining migration segments. Segment-specific survival increased between NSOG and the DI (83% [70−91%]) and DI to JS subarrays (84% [56−96%]), but decreased slightly in the segment from JS to QCS to 61% (38−80%; Figure 2.2). Segment-specific survival estimates are summarized in Table 2.4. Cumulative survival from NSOG to QCS was 42% (27−57%), and mean survival rates from NSOG to QCS were 70% per 100 km (61−78% per 100 km) and 91% d^{-1} (88−93% d^{-1}). Total survival from release to QCS (~400 km) was 9% (3−15%) for river-release fish and 27% (17−38%) for marine-release fish (Figure 2.3).

2.3.2 Route selection

At NSOG, more steelhead were initially detected in the Strait of Georgia to the west of Texada Island (n = 63) than to the east, in Malaspina Strait (n = 44; Table A2 in the Appendix). Malaspina Strait is much narrower than the Strait of Georgia, and when accounting for channel width, significantly more fish used the eastern route (width of each channel relative to the width of the subarray as a whole; proportions test, $\chi^2 = 16.504$, df = 1, $p < 0.0001$). Use of Malaspina Strait was mostly by marine-release fish; only 3 of 15 river-release smolts detected along NSOG were detected in the Malaspina Strait.

Along the DI subarray, fish predominantly were first detected in Discovery Passage (n = 72) compared with Sutil Channel (n = 37) and Desolation Sound (n = 2; Figure 2.4). Multi-state model results indicated a transition probability (i.e. probability of route use; ψ) of 77% (64−86%) for Discovery Passage and 23% (13−36%) for Sutil Channel. Smolts showed no indication of skewed distributions of arrival positions along the JS subarray, and a slight tendency for migrating toward the southern shore along the QCS subarray (Figure 2.4). Multi-state model selection results suggested that migration route at the NSOG subarray (i.e. east vs. west of Texada Island) did not influence survival to the DI (Table A1 in the Appendix); however, there is strong evidence that route selection at the DI impacted survival of smolts to the subsequent subarray at JS. The top-ranked model for the segment between the DI and JS considered the two routes (Discovery Passage to the west, and Sutil Channel to the east) separately and was strongly supported (97.3% of corrected AIC [AICc] weight; Table 2.5). Survival estimates through Discovery Passage and Sutil Channel to JS were 100% (0−100%) and 47% (19− 77%), respectively (bootstrapped estimates: Discovery Passage: 98% [87−100%];

Sutil Channel: 46% [23− 72%]). When factoring in the difference in migration route distances and time spent migrating between the 2 routes, survival was estimated to be 97% per 100 km (82−100% per 100 km) for Discovery Passage, compared with 48% per 100 km (24−73% per 100 km) for Sutil Channel. When considering migration time, migration rates were 99% d^{-1} (92−100% d^{-1}) and 84% d^{-1} (71−93% d^{-1}) for Discovery Passage and Sutil Channel, respectively. Raw detections can be found in Table A2 in the Appendix.

2.3.3 Effect of fork length, gill clipping, and tag burden

When assessing the effects of FL, gill clipping, and tag burden on survival, model selection results revealed similar model weights across all candidate models, including the base model (base model: ϕ[release × $segment_{Release\ to\ NSOG}$ + $segment_{NSOG\ to\ QCS}$] p(site); Table 2.2). This base model had the largest weight for any model, with 30.9% of the AICc weight. Addition of covariates (FL, gill clipping, tag burden), however, did not greatly improve the amount of deviance explained relative to the base model (Table 2.2). The model that included FL as a covariate had 30.7% of the AICc weight. In this model, the coefficient estimate for FL was slightly positive with a 95% CI spanning zero (0.014 [−0.001 to 0.029]), indicating limited evidence that larger fish survived better than smaller fish. Mean tag burden for acoustic-tagged smolts was low at only 2.2% (±0.6% SD), and the AICc weight for the model that included tag burden was 24.5%. The model containing gill clipping had the lowest model weight at 14.0%. The coefficients for both tag burden and gill clipping in their respective models were negative, with 95% CIs slightly overlapping zero (mean tag burden: −31.7 [−69.99 to 6.63]; mean gill clip: −0.19 [−0.61 to 0.23]), indicating limited evidence for weak negative effects on survival by both gill clipping and tag burden.

2.3.4 Timing and travel rates

Fish released in the river (between 21 and 23 May) were detected in the estuary between 21 May and 16 June (mean: 27 May [±5 d SD]), highlighting that some individuals remained in freshwater for several weeks (~20% spent more than 1 week). Of the detections in the estuary, 52 (of 66 total) smolts were last detected on the farthest downstream receiver. Mean estuary residence time (duration between first and last detections) was 1.1 d (±0.2 d SE). Smolts that remained in the river for extended periods were slightly less likely to survive between the estuary and NSOG than those that moved out of the river much more rapidly (binomial generalized linear model [GLM], $\chi^2 = 10.492$, df = 1, $p < 0.01$). Initial and final detections of smolts on the river estuary receivers were predominantly observed between sundown and sunrise (Figure A1 in the Appendix).

Mean travel time from release to QCS was 19.2 d (±1.2 d SE) for marine-release smolts. River-release smolts took an average of 12.8 d (±0.9 d SE) to migrate from the Seymour River estuary to QCS. In the first marine segment (to NSOG), migration rate was similar between release groups, with river-release fish travelling 17.3 km d^{-1} (±1.9 km d^{-1} SE) and marine-release fish travelling 17.2 km d^{-1} (±1.0 km d^{-1} SE). River-release fish mean arrival date was slightly later at NSOG (Mann-Whitney U-test, W = 1071, $p < 0.0001$), DI (Mann-Whitney U-test, W = 900, $p < 0.0001$), and JS (Mann-Whitney U-test, W= 389, $p = 0.001$) subarrays than for marine-release fish, which was expected given the similar migration rates between release groups, slightly later dates of river releases, and extra time river-release fish took to migrate in freshwater. Along the NSOG subarray, mean arrival dates were slightly later at the Malaspina Strait (Mann-Whitney U-test, W = 392, $p < 0.0001$), and no differences in mean arrival date was

detected among the three subarrays spanning the DI (Mann-Whitney U-test, W = 1521, p = 0.082).

Travel rates were fastest in the segment between DI and JS (Figure 2.5). Between NSOG and DI, travel rates were 21.4 km d^{-1} (±5.3 km d^{-1} SE) and 22.6 km d^{-1} (±1.5 km d^{-1} SE) for river- and marine-release fish, respectively. Between the DI and JS subarrays (~80 km distance), travel rates became more variable and mean rates more than doubled, with marine-release fish travelling 41.7 km d^{-1} (±2.3 km d^{-1} SE) and river-release fish slightly faster at 54.6 km d^{-1} (±8.6 km d^{-1} SE; Figure 2.5). For the final segment of migration between JS and QCS, travel rate slowed slightly to 34.5 km d^{-1} (±1.6 km d^{-1} SE) for marine-release smolts, and 30.2 km d^{-1} (±4.6 km d^{-1} SE) for river-release smolts. FL did not influence migration rate in any migration segment (NSOG to DI: GLM, $F_{1,75} = 0.927$, p = 0.339; DI to JS: GLM, $F_{1,54} = 1.283$, p = 0.2624; JS to QCS: GLM, $F_{1,25} = 0.017$, p = 0.896).

2.3.5 Milling patterns during migration

Of all released fish, 11% (n = 26) exhibited milling patterns, such as lateral movements along a subarray or reverse migrations in the marine environment. After release, five marine-release smolts were subsequently detected on the Seymour River estuary receivers ~18 km east of the release site (i.e. opposite to the expected migration direction). Only one of these fish was later detected on marine receivers as far north as the JS subarray. At NSOG, two marine-release fish made lateral movements along the subarray. Only one of these fish was a successful migrant to the DI, where it was then classified as a 'reverse migrant' as it was re-detected on the NSOG subarray to the south. All reverse migrations detected within the marine environment (n = 9)

began somewhere along the DI subarray, with eight being from the marine-release group. Fifty-five percent of these reversals in migration at the DI began in Sutil Channel, while 45% began in Discovery Passage. Only three of these reverse migrants eventually made it back to the DI after re-detection on the NSOG subarray, with none successfully migrating to JS. Fourteen fish made lateral movements along the DI subarrays (marine- release: n = 12; river-release: n = 2). For these fish 78% were first detected in Sutil Channel (n = 11) and then migrated to Discovery Passage. Eight fish that showed this change in channel migration behaviour made it at least to the JS subarray, and 5 successfully migrated through the system to QCS.

2.3.6 Holding study

At the end of the 18 day holding study, no mortality or tag loss was observed for steelhead in either freshwater or saltwater tanks. Tagging did not affect either final aggregated mean mass in either tank (saltwater: 1-sample t-test, $t_{39} = -0.211$, $p = 0.834$; freshwater: 1-sample t-test, $t_{36} = -0.982$, $p = 0.333$) or final aggregated mean FL in freshwater (1-sample t-test, $t_{36} = -1.320$, $p = 0.195$) of fish. Tagged smolts in saltwater grew slightly larger (~2 mm on average) relative to untagged smolts (1-sample t-test, $t_{39} = 5.003$, $p < 0.001$). Fish were returned to their tanks after the experiment and monitored daily, with no tag expulsion being noted for several months after the study.

2.4 Discussion

This study highlights the poor overall survival of hatchery steelhead (*Oncorhynchus mykiss*) smolts migrating through freshwater and the early marine environment. I tracked

steelhead smolts for up to ~400 km and provide further evidence that the early marine period of migration through Burrard Inlet is associated with low survival for juvenile salmonids. Cumulative survival for steelhead trout to QCS (~15−30 d) was only ~9% and ~27% for river- and marine-release groups, respectively. These survival estimates are similar to those estimated in previous years using the same population (Balfry et al. 2011) and are consistent with survival estimates for other stocks and species migrating through the Salish Sea to QCS, including Cultus Lake sockeye smolts (~3−30% survival) (Welch et al. 2009) and Cheakamus River steelhead (27% survival) (Melnychuk et al. 2007).

Within the lower Seymour River, segment-specific survival was estimated to be ~79% from release to the estuary receivers, which is notably low considering this migratory segment represents less than 1% (~2.5 km) of the total distance through the study system. Longer freshwater residency periods were associated with slightly poorer overall survival to NSOG; however, this finding may be a result of survival being associated with time, rather than any specific characteristic of this landscape. Potential residualization by steelhead (a process where smolts remain in freshwater and do not migrate to the ocean) may have influenced our estimates of freshwater survival, although residualization in hatchery fish is typically ~5% and is least likely when fish are released in small groups close to the estuary (Hausch & Melnychuk 2012), as occurred in our study. In addition, a recent rock slide 2 km up stream of the release site posed a migratory barrier to smolts (S. J. Healy, *unpublished data*), and ~80% of final estuary detections were on the downstream estuary receiver, indicating directed movements into the marine environment. Thus, the likelihood of residualization for this population in 2015 was low.

Once beyond the Seymour River, smolts entered Burrard Inlet, which was a region of pronounced poor survival for migrating smolts. This marine inlet accounted for nearly half of the

total loss of river-release smolts to the NSOG subarray (~130 km), even though only encompassing ~13% (18 km) of the total distance. In contrast, marine-release smolts, which did not have to migrate though the inlet, experienced ~2.3-times higher survival to NSOG and ~3-times higher survival to QCS (Figures 2.2, 2.3), indicating that the effect of the high initial losses incurred within Burrard Inlet persisted in the subsequent phases of the marine migration. Survival rates (both per day and per 100 km) to NSOG were also significantly higher for marine-release smolts (Figure 2.2), and thus survival differences cannot be simply attributed to a slightly longer migration for river-release smolts alone. Our results therefore support Balfry et al.'s (2011) suggestion that Burrard Inlet is a region of particularly poor survival for Seymour hatchery steelhead.

A lack of receiver infrastructure in Burrard Inlet meant I was not able to assess which direction river-release smolts migrated initially on leaving the estuary. This may have influenced my survival estimates from the estuary to NSOG, particularly if any smolts migrated east (i.e. into a fjord, the Indian Arm) after leaving the estuary. Balfry et al. (2011), who did position receivers farther east of the Seymour River mouth in Burrard Inlet, found that very few smolts (~2−5%) migrated east after leaving the Seymour River estuary, and those that did generally were later detected heading west into the Salish Sea. Even if a similar proportion of my tagged smolts migrated east into Indian Arm and were never detected again, this would still not explain the difference in survival between release groups to the NSOG subarray. Therefore, I consider that my experimental release groups and receiver subarray setup were effective in estimating survival for smolts travelling through this marine inlet.

Predation may contribute to the poor survival observed through freshwater and the first marine inlet (i.e. Burrard Inlet). Though I did not directly assess or observe predation, numerous

species of birds that prey on juvenile salmonids are prevalent in the area at the time of outmigration, including common mergansers (*Mergus merganser*), double-crested cormorants (*Phalacrocorax auritus auritus*), and great blue herons (*Ardea herodias fannini*) (Butler et al. 2015). In the lower Columbia River system, juvenile steelhead are one of the most vulnerable salmonids to predation by waterbirds (Collis et al. 2001), which can account for up to 28% of mortality for outmigrating smolts (Evans et al. 2016). Harbour seals (*Phoca vitulina*), which are at carrying capacity in the Strait of Georgia (Olesiuk 1999), target salmon (Yurk & Trites 2000, Thomas et al. 2017) and may be a major source of early marine mortality for outmigrating steelhead smolts in nearby Puget Sound (Berejikian et al. 2016). Other predators, such as Pacific spiny dogfish (*Squalus suckleyi*), are common in the broader region and have the potential to prey on smolts and influence survival (Beamish et al. 1992, Beamish & Sweeting 2009). Having never been exposed to predators in a wild setting, hatchery steelhead may be particularly susceptible to predation (Osterback et al. 2014). I detected predominantly nocturnal movements into the estuary by smolts, which is generally considered a predator avoidance behaviour and has been observed in clear freshwater systems (Chapman et al. 2013, Chase et al. 2013, Clark et al. 2016, Furey et al. 2016). Predation has been hypothesized as a major driver of mortality for other populations of steelhead and sockeye migrating short distances to the sea (Welch et al. 2004, Melny chuk et al. 2007). In nearby Howe Sound (immediately northwest of Burrard Inlet), near-shore mortality of migrant coho salmon (*Oncorhynchus kisutch*) and steelhead smolts is thought to be influenced by predation 'bottlenecks' (Melnychuk et al. 2013), and such spatial constrictions are also characteristic of Burrard Inlet. Releasing smolts with higher densities of co-migrants may have contributed to an increase in survival to NSOG for marine-release fish, due to a reduction in per-capita predation risk (e.g. 'predator swamping'; Furey et al. 2016);

however, we consider any reduction in predation risk associated with co-migrant densities to be small and ephemeral considering the large densities of salmonid smolts migrating through the Salish Sea during this time of year (Peter man et al. 1994, Tucker et al. 2009). Future research could examine movements of smolts at finer scales and/or investigate predator behaviour and feeding on smolts to help determine why this landscape appears to be a high risk to smolt survival.

My results provide another example of route-specific survival in migratory species (see Skalski et al. 2002, Perry et al. 2010, 2013, Furey et al. 2015, Hewson et al. 2016). In the DI region, the majority of smolts (~77%) used the westernmost route (Discovery Passage), and those that did benefited by experiencing over twice as high survival to JS (~80 km) as those migrating through Sutil Channel to the east. The Discovery Passage subarray was deployed slightly farther to the north (~18 km) than the other DI subarrays, which may have influenced my estimates of survival, as the distance for this western route to the JS subarray was ~20−25% shorter than those to the east. However, the ~two-fold higher survival advantage for smolts taking Discovery Passage cannot be simply attributed to this difference in migration distance alone. Even when factoring in migratory distance, survival rates per 100 km were still estimated to be approximately twice as high for smolts migrating through Discovery Passage compared with Sutil Channel. I am therefore confident that these differences in route-specific survival are ecologically relevant.

Several factors may be contributing to variable use of migratory routes through the early portion of marine migration. Outmigrating salmonid smolts in the marine environment are thought to orient using Earth's magnetic field (Putman et al. 2014a,b) and make directed migrations toward feeding grounds irrespective of currents (Thorstad et al. 2004, Hedger et al.

2008, Melnychuk et al. 2010). Strong surface currents, however, still influence smolt movements (Booker et al. 2008, Mork et al. 2012); therefore, the migratory trajectories of steelhead smolts are likely a combination of active swimming and the surface currents they experience. Furey et al. (2015) hypothesized that tidal currents in the Salish Sea contributed to westward and even counterclockwise movements observed in ~30−50% of steelhead around the NSOG subarray. Similarly, these same tidal currents (with mean surface currents towards the northwest; Foreman et al. 2012) may be pushing or guiding steelhead smolts to the most western route through Discovery Passage. In tidally driven river delta systems, smolts have been found to select routes with increased flow (Perry et al. 2010, Steel et al. 2013), and if this holds true in the early marine environment, it could explain why Seymour steelhead smolts displayed higher use of Discovery Passage, as this route contains some of the strongest tidal currents in the region (Foreman et al. 2012).

It is currently unclear what is causing differential survival between routes travelled through the DI. Higher water velocities may contribute to faster migration times and subsequent increase in survival (Steel et al. 2013). Navigation through Sutil Channel or Desolation Sound is more complex due to the presence of numerous islands and fjords (Figure 2.1C). Discovery Passage, in contrast, provides a more direct route to the JS and QCS subarrays. When factoring in differences in time spent travelling each route, survival rates per day were still higher for Discovery Passage (~70 km) than for Sutil Channel (~110 km), although with slightly overlapping confidence intervals between the two routes, suggesting that the differences in survival between these routes are likely not a result of just migration time alone. Smolts may encounter varying levels of predation pressure (Newman & Brandes 2010, Perry et al. 2010) due to higher densities of seal haul-outs (Berejikian et al. 2016, Thomas et al. 2017) or other

predators depending on which route they select. Concentrations of nutrients, phytoplankton, and zooplankton are known to vary within the Salish Sea (Peña et al. 2016), thus influencing availability of food and potentially affecting survival for outmigrating smolts. We did not detect an effect of survival based on route selection at the NSOG subarray, as was found for steelhead smolts by Furey et al. (2015), possibly due to interannual differences in predator abundance or food availability. The spatiotemporal extent of predators and/or food distributions and their influence on smolt survival within the Salish Sea itself are poorly understood and warrant further research.

The holding study and model selection results suggest that survival was not biased by the acoustic tagging procedure. At the end of the 18 day holding period, I found no evidence that tagging affected smolt survival in either saltwater or freshwater. Studies have generally found minimal or no impacts from acoustic tagging on factors such as swimming performance, feeding, or survival in juvenile salmonids, particularly when tag burdens are kept lower than ~4−6% (Welch et al. 2007, Collins et al. 2013, Neville et al. 2015) as was done in our study (mean tag burden = 2.21%). I detected no tag loss over the duration of the holding study, suggesting that my survival analyses were not biased by smolts shedding acoustic tags while swimming. There was little support for models including the effect of tag burden and gill clipping on survival, consistent with studies suggesting the non-lethal gill clipping procedure has minimal or no impact on growth or survival of juvenile salmonids (Martinelli-Liedtke et al. 1999, Jeffries et al. 2014). Additionally, there was limited evidence to suggest that FL significantly influenced survival. Regardless, I model averaged across the four candidate models including these covariates (gill clipping, tag burden, FL; Table 2.2), which captures their potential impacts on the estimates of survival.

Mean migration rates for steelhead in the Salish Sea ranged between ~15 and 30 km d^{-1} (~0.9−1.8 body lengths s^{-1}) depending on migration segment, a rate similar to those previously estimated in this region for steelhead (Melnychuk et al. 2010, Balfry et al. 2011) and sockeye smolts (Welch et al. 2009). Between the DI and JS arrays, steelhead smolt migration rates increased and were more variable (~25− 60 km d^{-1}; Figure 2.5), and in some cases approached rates (~70−100 km d^{-1}; Figure 2.5) comparable to those observed for smolts migrating downstream in large freshwater rivers (Melnychuk et al. 2010, Clark et al. 2016). The increased migration rates observed between the DI and JS is likely a result of the narrow channels strongly directing the migration, but may also be influenced by selective tidal-stream transport (e.g. Metcalfe & Arnold 1997). Although the mean flow due to tides is close to zero in this region, tidal currents peak at up to ~4.5 m s^{-1} in some areas within the DI (Foreman et al. 2012), dwarfing the maximum sustained swimming speeds of the smolts. These rapid currents would allow smolts to travel much faster than is typically estimated in marine waters when they flow in the direction of migration, assuming the smolts were able to at least partially shelter from the effect of the tides when the current runs opposite to their migration direction.

A small subset (~11%) of tagged smolts exhibited apparent milling patterns between NSOG and JS, including reversals in migratory direction and lateral movements along arrays. Steelhead are thought to take relatively rapid and directed migrations towards the open ocean as smolts (Hartt & Dell 1986, Welch et al. 2011), but recently, more complex milling behaviours have been identified for a substantial number of steelhead smolts (30−50%) tracked at the NSOG subarray (Furey et al. 2015). This previous research considered milling at a finer geographic scale (i.e. between individual receivers along a single subarray), which likely accounts for the larger percentage of milling behaviours observed compared with the present study. With the

addition of new subarrays in 2015, I found that these milling patterns are still present further along in the migration, particularly at the DI subarray. This could be a result of strong tidal surface currents influencing migration patterns in this portion of the Salish Sea (Foreman et al. 2012) or other factors such as the distribution of food or predators. For smolts making lateral movements along the DI subarray, 64% of those last detected migrating through Discovery Passage survived to JS. In fact, the only milling patterns at the DI that resulted in a successful migration to QCS were by those fish that eventually migrated through Discovery Passage, further underscoring Discovery Passage as an important migratory corridor. It seems likely that at least some of the observed milling behaviour may have been caused by predation. If a tagged smolt is consumed by a predator, the predator's movements could potentially be tracked while the tag remains in the predator's gut; however, I was unable to assess this directly.

The present study identifies critical regions and important corridors for outmigrating steelhead smolts, and could help inform conservation and management of salmonids migrating through the Salish Sea. Determining important migratory regions and routes for smolts may be crucial for informing future development decisions and allocating conservation resources in the region. I have shown that the freshwater environment and the first estuarine inlet are two regions of particularly low survival for smolts, and the brief residence in these regions suggests that piscivorous predators may play a large role in impacting smolt survival here. My results also provide empirical evidence that route selection can influence survival during migration, a concept for which empirical evidence remains rare (Holyoak et al. 2008). Large differences in survival between channels through the Discovery Islands were identified, and most smolts were detected migrating through the more favourable route. My study signifies that this understudied region is a potentially important corridor for juvenile salmonids in the Salish Sea. As mortality

rates in the early marine period are thought to directly impact adult returns and population productivity in steelhead (Moore et al. 2012), these results suggest that route-selection during outmigration could be associated with population-level impacts to survival and productivity. This information underscores the importance of identifying critical regions and predominant migratory pathways for these culturally, ecologically, and economically important species.

2.5 Chapter 2 tables

Table 2.1: Summary table of Seymour River hatchery steelhead (*Oncorhynchus mykiss*) smolts used for release and for holding study. N/A = not applicable

Group	Tag Type; frequency	Release Dates	Mean fork length (mm; SD)	Mean weight (g; SD)	Number	Number gill clipped
Lower Seymour (river-release)	V7-*2L*; 69 kHz	May 21-23, 2015	201.7 (15.2)	78.6 (21.0)	83	57
West Vancouver (marine-release)	V7-*2L*: 69 kHz	19-May-15	199.4 (13.2)	75.5 (17.0)	160	107
Holding (saltwater)	Dummy V7; N/A	N/A	200.2 (14.6)	76.9 (18.1)	63	40
Holding (saltwater)	Untagged	N/A	197.6 (12.3)	70.7 (15.6)	34	0
Holding (freshwater)	Dummy V7; N/A	N/A	196.4 (12.6)	71.0 (13.6)	60	40
Holding (freshwater)	Untagged	N/A	200.2 (11.8)	74.8 (14.7)	33	0

Table 2.2: Ranking of Cormack-Jolly-Seber models based on Akaike's information criterion adjusted for low sample size and for overdispersion (QAICc) to test the effect of fork length, gill sampling, or tag burden on survival. ΔQAICc = QAICc − QAICcmin, where min indicates the QAICc for the best model; ϕ = survival; p = detection probability; NSOG = Northern Strait of Georgia; QCS = Queen Charlotte Strait. The base model is indicated in **bold**

Model	No. of parameters	QAICc	ΔQAICc	Weight
φ(release*segment$_{\text{Release to NSOG}}$[a] + segment$_{\text{NSOG to QCS}}$) *p*(site[b])	10	542.281	0	0.309
φ(release*segment$_{\text{Release to NSOG}}$[a] + segment$_{\text{NSOG to QCS}}$ + Fork Length) *p*(site[b])	11	542.3	0.019	0.307
φ(release*segment$_{\text{Release to NSOG}}$[a] + segment$_{\text{NSOG to QCS}}$ + Tag Burden) *p*(site[b])	11	542.76	0.478	0.245
φ(release*segment$_{\text{Release to NSOG}}$[a] + segment$_{\text{NSOG to QCS}}$ + Gill Clip) *p*(site[b])	11	543.867	1.585	0.14

a - Segment length to NSOG differed by release group

b - Only river-released smolts were used to estimate *p* for the estuary receivers

Table 2.3: Cormack-Jolly-Seber model selection results of test of whether release groups (release) could be pooled for survival analyses north of the Northern Strait of Georgia (NSOG) subarray. QAICc = corrected Akaike's information criteria with low sample size and modified for overdispersion; ΔQAICc = QAICc − QAICcmin; QDeviance = model deviance adjusted for overdispersion; QCS = Queen Charlotte Strait.

Model	No. of parameters	QAICc	ΔQAICc	Weight	QDeviance
φ(release * segment$_{\text{Release to NSOG}}$[a] + segment$_{\text{NSOG to QCS}}$) p(site[b])	10	543.585	0	0.928	24.793
φ(release * segment$_{\text{Release to QCS}}$[a]) p(site[b])	13	549.354	5.77	0.052	24.299
φ(release * segment$_{\text{Release to NSOG}}$[a] + segment$_{\text{NSOG to QCS}}$) p(release * segment)	14	551.55	7.965	0.017	24.39
φ(release * segment$_{\text{Release to QCS}}$[a]) p (release * segment)	16	555.282	11.697	0.003	23.892

a - Segment length to NSOG differed by release group

b - Only river-released smolts were used to estimate p for the estuary receivers

Table 2.4: Estimates of segment-specific survival, survival rates (per day and per 100 km) and detection probability for each subarray across the study system. N/A = not applicable; NSOG = Northern Strait of Georgia; DI = Discovery Islands; JS = Johnstone Strait; QCS = Queen Charlotte Strait

Parameter	Release group	Segment	Estimate (%)	SE	Lower 95% CI	Upper 95% CI
Survival (ϕ)	River release	River	78.8	5.9	65.1	88.1
	River release	River mouth to NSOG	27.3	7.3	15.3	43.6
	Marine Release	Release to NSOG	64.6	5.3	53.8	74.1
	Combined	NSOG to DI	83.1	5.2	70.4	91
	Combined	DI to JS	83.7	9.8	55.8	95.5
	Combined	JS to QCS	60.8	11.3	38	79.7
Detection Probability (*p*)	River Release	River	100	0	0	100
	Combined	NSOG	81.8	5	70	89.6
	Combined	DI	94.4	3.5	82.2	98.4
	Combined	JS	71	9.4	50	85.7
	Combined	QCS	Fixed at 73.0			
Survival per day	River release	River	N/A	N/A	N/A	N/A
	River release	River mouth to NSOG	87.1	6.2	75.1	99.2
	Marine Release	Release to NSOG	95.7	3.1	89.5	100
	Combined	NSOG to DI	95.4	3.5	88.6	100
	Combined	DI to JS	91.3	7.5	76.6	100
	Combined	JS to QCS	85.1	6.2	73	97.3
Survival per 100 km	River release	River	0	0	0	0.01
	River release	River mouth to NSOG	40.4	7.6	27.1	56.1
	Marine Release	Release to NSOG	70.3	4.6	60.6	78.5
	Combined	NSOG to DI	75.7	7.1	59.1	86.8
	Combined	DI to JS	78.8	12.3	45.8	93.9
	Combined	JS to QCS	60.6	11	38.7	80

Table 2.5: Multi-state model selection results to test if survival to the Johnstone Strait subarray was influenced by route selection in the Discovery Islands. QAICc= Akaike's Information Criteria with low sample size and modified for overdispersion; ΔQAICc= QAICc-QAICcmin. NSOG = Northern Strait of Georiga, QCS = Queen Charlotte Strait

	Model	No. of parameters	QAICc	ΔQAICc	Weight	QDeviance
Survival separate between routes	S(release * segment$_{\text{Release to NSOG}}$[a] + route[c] * segment$_{\text{NSOG to QCS}}$) p (site[b]) ψ (segment)	12	573.011	0	0.973	15.851
Survival the same between routes	S(release * segment$_{\text{Release to NSOG}}$[a] + segment$_{\text{NSOG to QCS}}$) p (site[b]) ψ (segment)	11	580.209	7.198	0.0266	25.143

a - Segment length to NSOG differed by release group

b - Only river-released smolts were used to estimate *p* for the estuary receivers

c - The route parameter was used to provide independent estimates for the channels in the Discovery Islands. All smolts were assumed to have a common migration route (except for differences in release locations) from release to the Discovery Islands. Routes split as fish migrated east or west around Quadra Island (i.e. Discovery Passage and Sutil Channel), then rejoined for the final segment between Johnstone Strait and Queen Charlotte Strait.

2.6 Chapter 2 figures

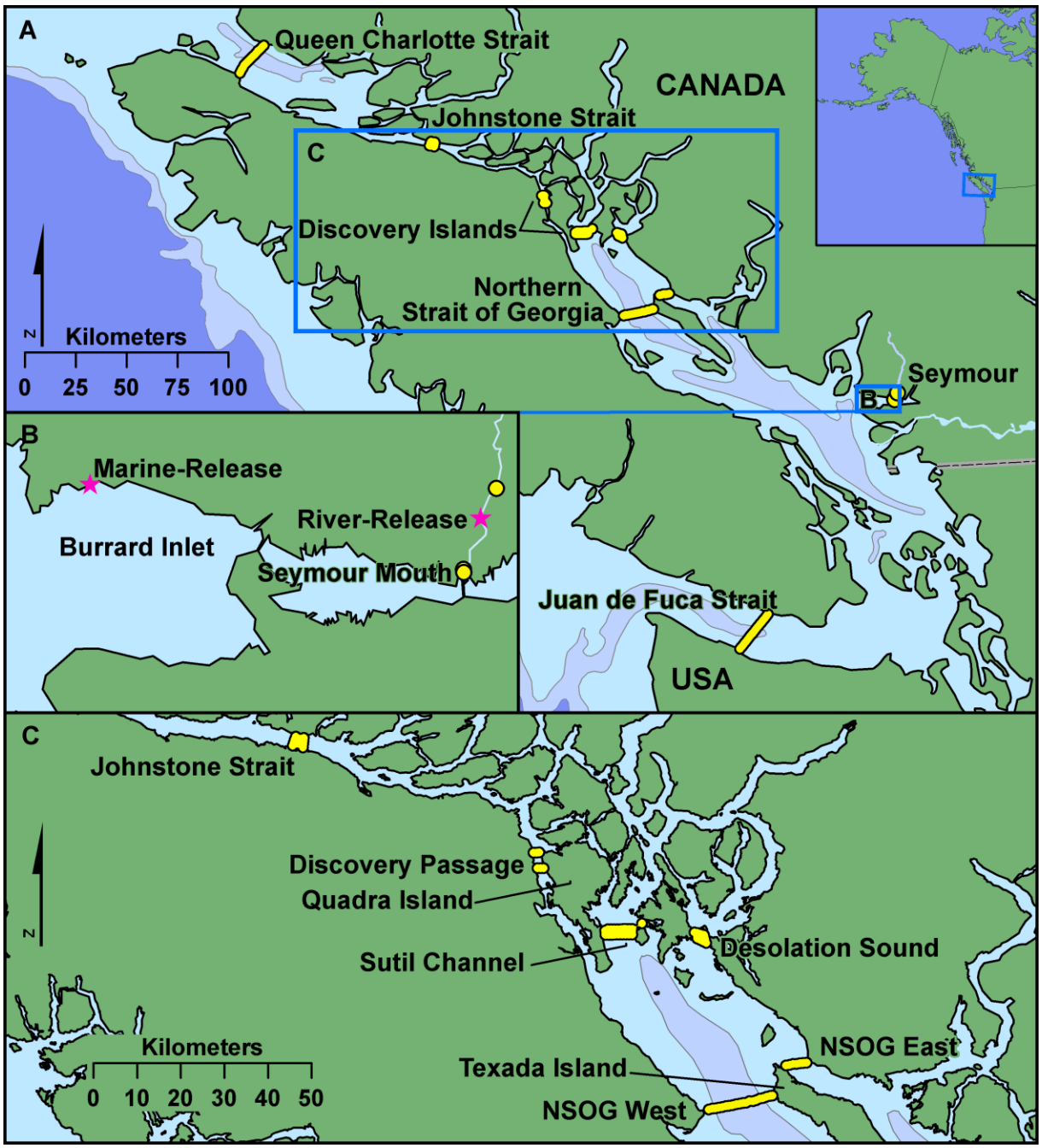

Figure 2.1: (A) Study area for Seymour steelhead (*Oncorhynchus mykiss*) in 2015. Yellow circles and lines represent either individual receivers or a receiver subarray. (B) Close up of Burrard Inlet and Seymour River. (C) Close-up of the Northern Strait of Georgia, Discovery Islands, and Johnstone Strait region. Fish were tagged at the Seymour River hatchery in May, then transported and released at either West Vancouver ('marine-release'; n = 160) or the lower Seymour River ('river-release'; n = 83), indicated by the stars. The depth contours show the 200 and 500 m isobaths

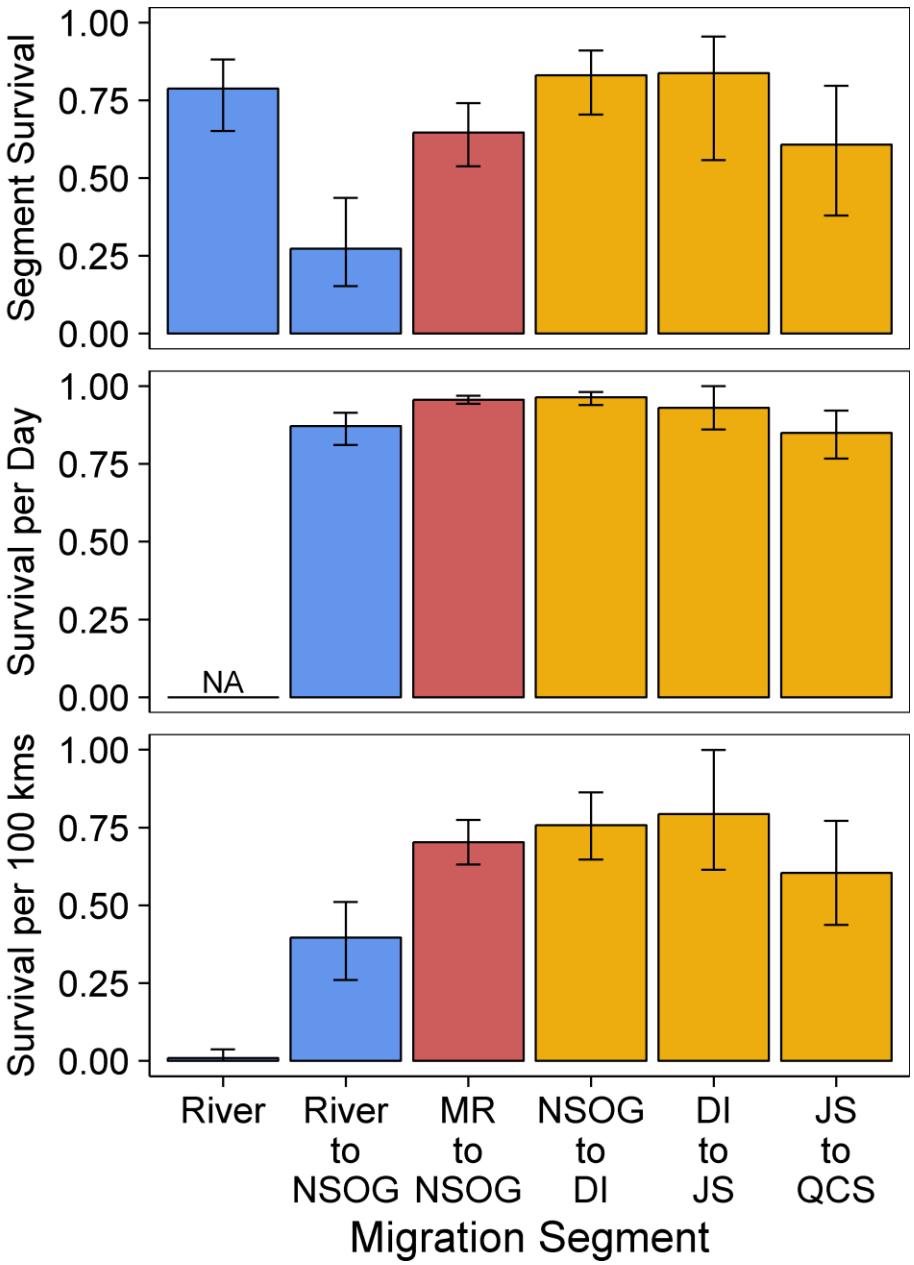

Figure 2.2: Model-averaged survival (±95% CI) for acoustic-tagged steelhead (*Oncorhynchus mykiss*) smolts (A) per migration route segment, (B) per day, and (C) per 100 km. River- and marine-release (MR) smolts are shown in blue and red, respectively. Estimates to the Northern Strait of Georgia subarray were kept separate for each release group, and were combined for all subsequent detection sites (shown in orange). Survival per day could not be estimated in the river because smolts were released randomly over three days, or for marine-release smolts as they were released ~18 km west of the Seymour estuary. na = not applicable; NSOG = Northern Strait of Georgia; QCS = Queen Charlotte Strait; DI = Discovery Islands; JS = Johnstone Strait

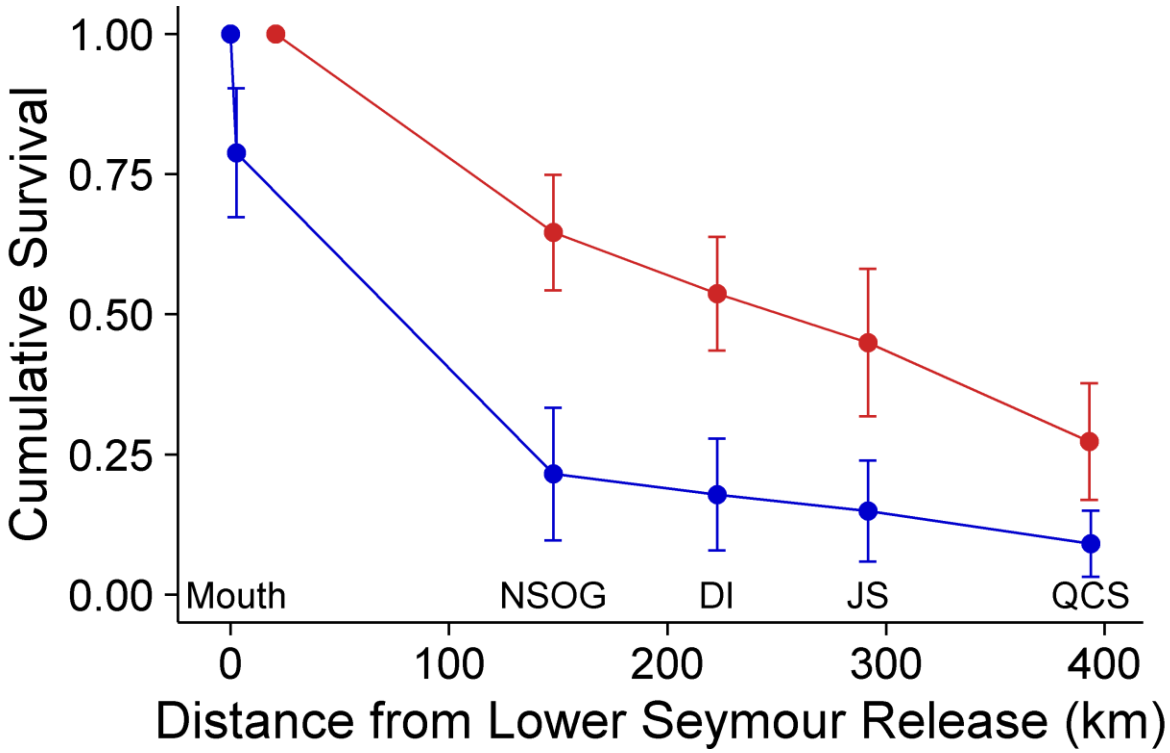

Figure 2.3: Cumulative survival (±95% CI) of steelhead *(Oncorhyncus mykiss)* smolts from release to the Queen Charlotte Strait (QCS) subarray. River- and marine-released smolts are shown in blue and red, respectively. Mouth=mouth of the Seymour River, NSOG=Northern Strait of Georgia, DI=Discovery Islands, and JS=Johnstone Strait arrays.

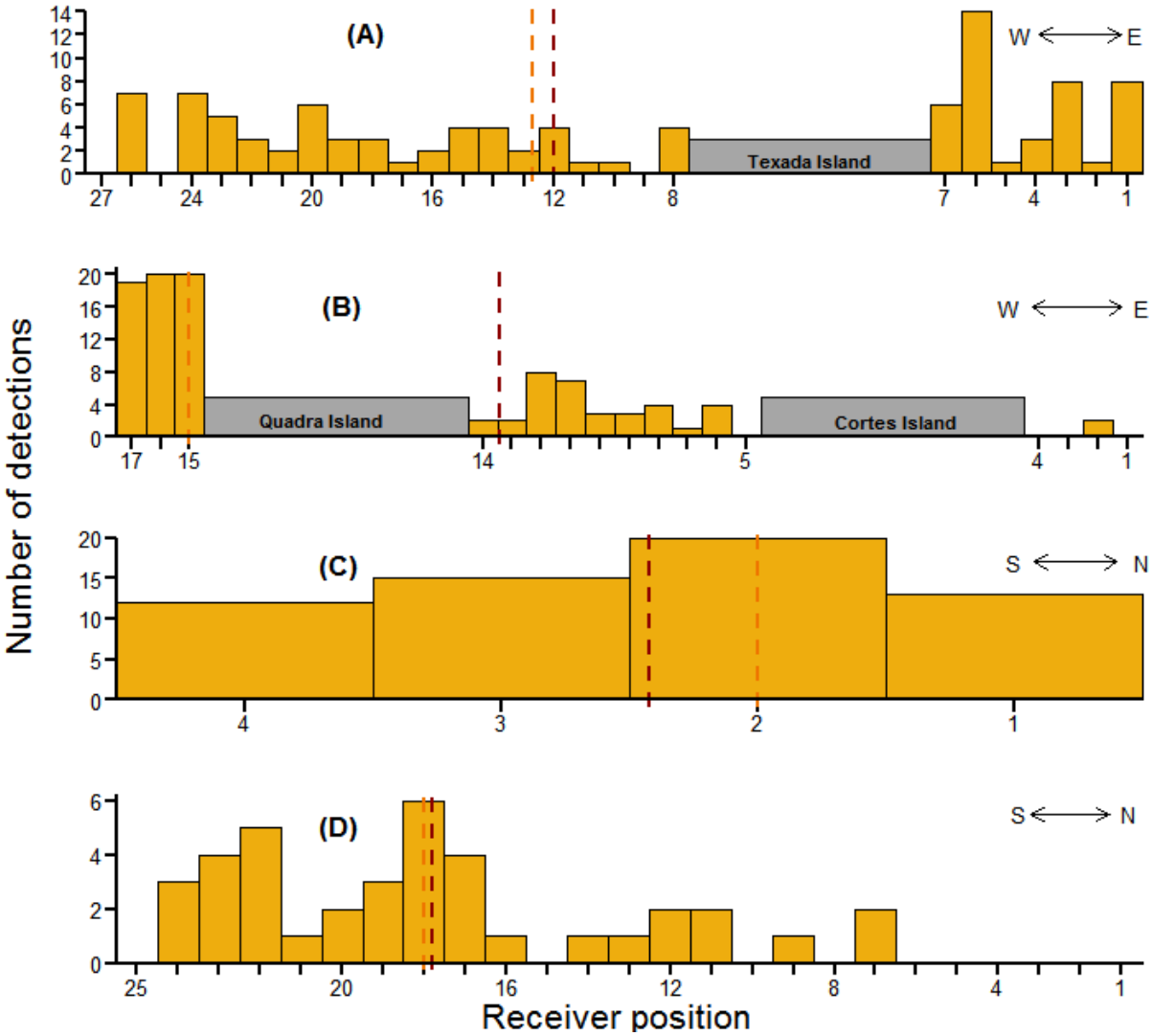

Figure 2.4: Distribution of first detections for tagged steelhead (*Oncorhynchus mykiss*) smolts across the marine subarrays at (A) Northern Strait of Georgia, (B) Discovery Islands, (C) Johnstone Strait, and (D) Queen Charlotte Strait. Values along the x-axis represent individual receiver locations along the subarrays (oriented ~west−east for A and B and ~south−north for C and D), while grey boxes indicate islands interrupting some of the subarrays. As the distributions are of first detections along arrays, the figure does not necessarily reflect the final routes fish used along subarrays (i.e. through the Discovery Islands). Brown and red dashed vertical lines are mean and median of distribution along arrays, respectively.

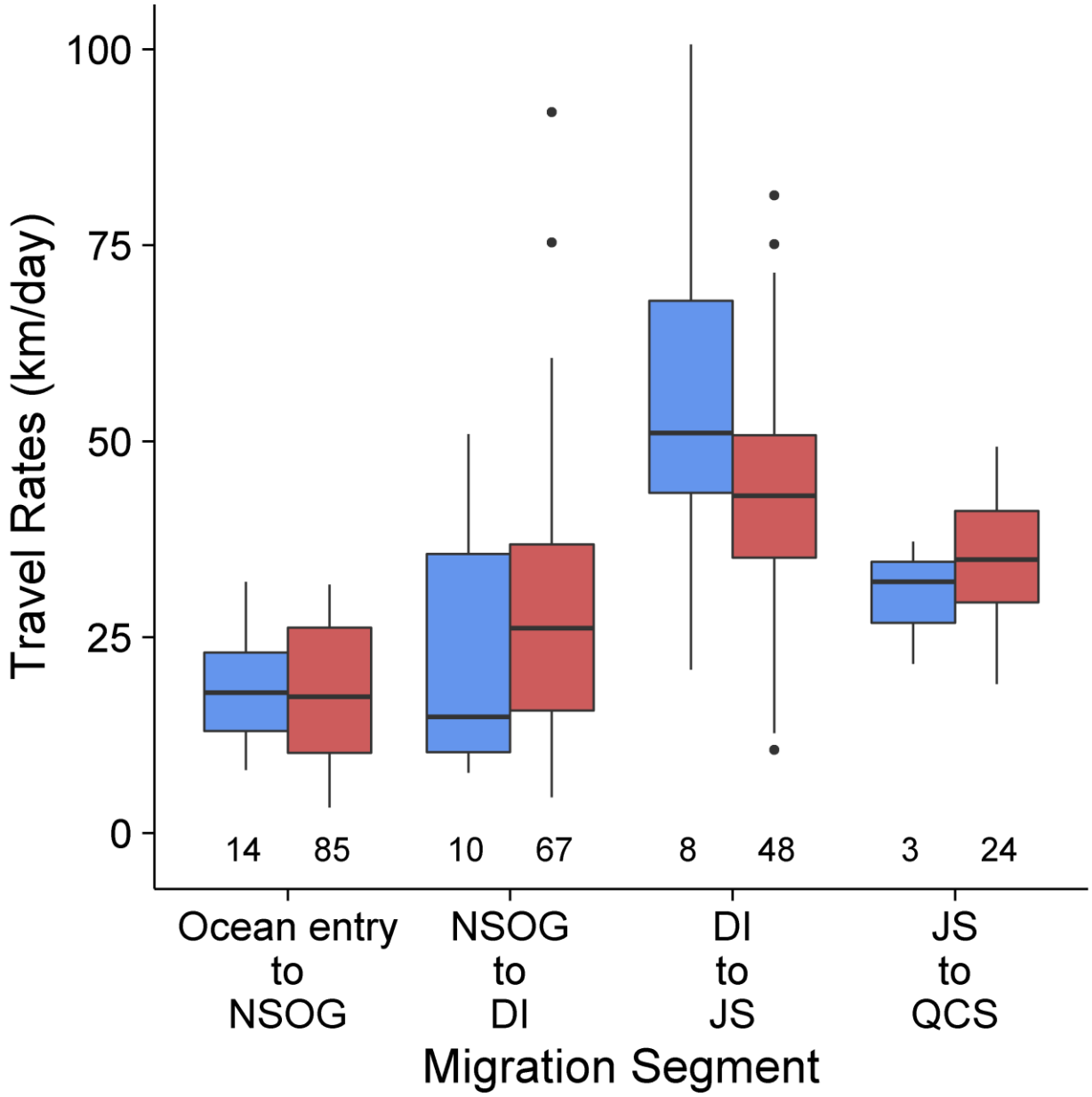

Figure 2.5: Segment-specific migration travel rates (km day^{-1}) for river-release (blue) and marine-release (red) steelhead *(Oncorhyncus mykiss)* smolts. Boxes represent the 1st (bottom) and 3rd (top) quartiles, horizontal lines indicate the median, and vertical whiskers depict maximum and minimum values. Outliers are shown as black dots, and sample sizes are shown underneath each box. Ocean entry represents when smolts left the estuary (for river-release individuals), or were released in the ocean (marine-release individuals). NSOG = Northern Strait of Georgia; QCS = Queen Charlotte Strait; DI = Discovery Islands; JS = Johnstone Strait.

Chapter 3: Transcriptome profiles relate to migration fate in hatchery steelhead (*Oncorhynchus mykiss*) smolts

3.1 Introduction

Large-scale migrations are an important life history component for Pacific anadromous salmonids (*Oncorhynchus* spp.). Towards the end of their freshwater residence, individuals undergo dramatic physiological changes prior to migrating to the marine environment as smolts (Groot and Margolis 1991). For smolts, the period of outmigration through freshwater and marine coastal regions can be associated with particularly poor survival (Balfry et al. 2011; Clark et al. 2016; Friedland et al. 2014; Welch et al. 2011). Declining productivity in some species and stocks (Irvine and Akenhead 2013) has been linked to the smolt life stage (Goetz et al. 2015; Kendall et al. 2017), underscoring the need to identify factors influencing survival during this critical period. An increased understanding of processes linked to outmigration survival could be used by managers to enhance the predictive capabilities of population productivity models (Beamish and Mahnken 2001; Evans et al. 2014; Irvine and Fukuwaka 2011), and to improve conservation measures for species or stocks in decline. Studies focusing on the smolt life stage have suggested various factors which can influence survival, including predation (Berejikian et al. 2016; Hostetter et al. 2012), environmental conditions (Beamish et al. 2000; Friedland et al. 2014), and food availability (Beamish and Mahnken 2001; Hertz et al. 2016).

Physiological condition can also play an important role in survival for smolts during outmigration (Hostetter et al. 2011; Jeffries et al. 2014). The smoltification process is energetically-intensive and consists of various key physiological changes enabling fish to transition from freshwater to the marine environment (Groot and Margolis 1991; Hanson et al. 2011). Most importantly, smolts must undergo shifts in ion regulation at the gills (Stefansson et

al. 2007), which if compromised could contribute to reduced estuary or early marine survival (McCormick et al. 2009). Size- or growth-related factors may also influence fate for outmigrating smolts because alterations in body size and morphology are important for entering marine systems (Beamish et al. 2004; Nichols et al. 2008). However, our current understanding of how smolt condition influences fate is lacking, as few studies have directly linked individual physiology with migration fate (but see: Evans et al. 2014; Hostetter et al. 2011; Jeffries et al. 2014).

An understudied aspect of the smolt life stage is the role that disease and immune responses play on migration performance (Miller et al. 2014; but see Jeffries et al. 2014). Smolts can be exposed to infectious agents during freshwater rearing and upon entering the marine environment (Bakke and Harris, 1998). Infected individuals may be less capable of successfully migrating (Jeffries et al. 2014) or at greater risk to predation along migratory pathways (Hostetter et al. 2012; Miller et al. 2014). Recent studies with outmigrating steelhead smolts (*Oncorhynchus mykiss*) in the Columbia River system have linked poor external body condition to increased levels of infectious agents and reduced survival (Evans et al. 2014; Hostetter et al. 2011). Few studies on disease in migrating populations have linked infectious agents to fate directly, because it can be particularly challenging to observe mortality in wild systems (La and Cooke 2011; Miller et al. 2014). At present, population-level monitoring for diseases in the Pacific Northwest is limited (Miller et al. 2014), particularly for species in decline, such as steelhead (Scheuerell et al. 2009; Smith et al. 2000). Thus, a necessary step in determining the role disease plays in migrating populations will be identifying infectious agents and intrinsic factors (e.g. stress and immune responses) which are associated with individual smolt outmigration fate.

Recent advancements in transcriptomics technology (quantifying the expression levels of mRNA in a tissue sample) have vastly improved our ability to study an organism's physiology. High-throughput real-time quantitative polymerase chain reaction (HT-qRT-PCR) is a powerful and sensitive tool that allows researchers to simultaneously assess tissue samples from many individual fish against multiple assays. Assays can be chosen to target the expression of genes in the tissue, and/or assess the presence and loads of infectious agents within the sample itself (Miller et al. 2016). This technology uses microfluidics and is used in medical fields (Diercks et al. 2009; Michelet et al. 2014; Spurgeon et al. 2008), but more recently has been adopted by fisheries ecologists (Evans et al. 2011; Jeffries et al. 2014; Miller et al. 2011), and demonstrated as a reliable methodology for use in salmonid infectious agent studies (Miller et al. 2016). The resulting data can be combined with biotelemetry to identify associations between gene expression, infectious agents, and survival for individual migrating salmonids (Miller et al. 2009, 2011; Evans et al. 2011). Jeffries et al. (2014) demonstrated this approach by combining non-lethal gill biopsies with acoustic telemetry and found that infectious agents and immune gene expression profiles of Sockeye salmon smolts (*Oncorhynchus nerka*) were predictive of migration fate in fresh water.

Acoustic telemetry is an effective tool for studying multiple aspects of smolt outmigration ecology. Individual movements, as well as survival and migration rates can be estimated across large distances of both freshwater and marine migration (e.g. Clark et al. 2016; Melnychuk et al. 2007; Moore et al. 2010; Welch et al. 2009, 2011). In 2015, an acoustic tagging study took place with hatchery steelhead (*O. mykiss*) smolts from the Seymour River (North Vancouver, British Columbia) (Healy et al. 2017). This study used both river and marine release locations to test the hypothesis (put forth by Balfry et al. 2011) that the initial segment of the

marine pathway (Burrard Inlet) was associated with poor survival for migrating smolts. The study concluded that the river and Burrard Inlet were regions of particularly poor survival (Healy et al. 2017), and thus identified regions where external and physiological factors may be particularly important determinants of migration fate.

The primary objective of the present study was to investigate the relationship between the physiological condition of Seymour River steelhead smolts and outmigration fate. I collected non-lethal gill biopsies from acoustically-tagged steelhead (same fish from Chapter 2) at the Seymour River Hatchery, and used HT-qRT-PCR to screen for multiple infectious agents. Additionally, I assessed the expression of a suite of host genes, and related gene expression profiles and infectious agents to migration fate. A previous study with this population found that vaccination of smolts against several microbes (*Aeromonas salmonicida*, *Listonella anguillarum* and *Vibrio salmonicida*) appeared to enhance survival, indicating that infectious agents may play a role in migratory success (Balfry et al. 2011). My hypothesis was that smolts in poor condition (i.e. positive for infectious agents, showed aberrant expression of immune genes consistent with an immune response, and/or were not osmotically prepared for entering the marine environment) would have poor survival through the freshwater and the early marine portions of migration.

3.2 Methods

3.2.1 Study system

The Seymour River is a regulated river located in North Vancouver, British Columbia (Figure 3.1). Its watershed flows south into the marine system, Burrard Inlet, which separates the city of Vancouver from North Vancouver. The Seymour River hatchery, located downstream of

the Seymour Falls dam, produces up to 30,000 steelhead trout annually (Seymour Salmonid Society, www.seymoursalmon.com). If steelhead smolts are released in the lower Seymour River, they migrate south to Burrard Inlet, then typically to the northwest through the Salish Sea (Balfry et al. 2011, Welch et al. 2011), which is a semi-enclosed marine system situated between Vancouver Island and the mainland of British Columbia. Smolts then must navigate through the Discovery Islands, Johnstone Strait, and Queen Charlotte Sound, before ultimately making their way to the open ocean (Figure 3.1). At present, the hatchery releases most of its steelhead in saltwater, beyond Burrard Inlet, after an earlier study (Balfry et al. 2011) suggested this inlet may be associated with poor survival.

3.2.2 Acoustic Tagging and Gill Sampling

Tagging took place at the Seymour River Hatchery (49°26'15.2"N 122°58'01.1"W) between May 14th and 15th, 2015 (University of British Columbia Animal Use Protocol: A15-0205). Steelhead smolts (fork length [FL] = 200.2 mm [±0.8 SE]; mass [M] = 77.0 g [±1.1 SE]; n=243) were removed from hatchery rearing channels, placed in separated raceways and starved for 24 hours prior to surgeries. Following Collins et al. (2013) and Furey et al. (2016), fish were randomly (i.e. haphazardly) selected, acoustically tagged (Vemco V7 acoustic transmitters; 7 mm x 18 mm, ~0.7 g in water; 69 kHz, Vemco Ltd., Bedford, NS; www.vemco.com), and measured for mass and FL (total air exposure < 1 min). Acoustic tagging procedures are described in more detail in Appendix A.1. During acoustic tagging, 164 smolts were non-lethally biopsied for gill tissue using small bone cutting forceps to remove 2-3 gill filaments (Jeffries et al. 2014), which were placed in RNA*later* (Life Technologies, Grand Island, NY). Gill samples were stored at 4^{o}C for 24-48 hours before being frozen at -80^{o}C prior to laboratory work.

Following surgery, fish were placed in separate pens within a flow-through raceway (grouped by release location) and allowed to recover for at least four days prior to release.

3.2.3 Releases and telemetry infrastructure

Tagged steelhead were transported in ~1000-L tanks on trucks, and released at one of two locations: 1) directly into saltwater ('marine-release') ~18 km west of the Seymour River estuary (49°20'24.8"N, 123°13'58.2"W; FL = 199.4 mm [±1.0 SE], M = 75.5g [±1.3 SE], n = 160) or 2) in the lower Seymour River ('river-release') (49°19'18.7"N 123°00'50.4"W; FL = 201.7 mm [±1.7 SE], M = 78.6g [±2.3 SE], n=83]) (Figure 3.1). These release locations (same used by Balfry et al. 2011) were selected for a parallel study using acoustic telemetry to test the hypothesis that Burrard Inlet was a region of poor survival for migrating steelhead (Healy et al. 2017; i.e. Chapter 2 of this thesis). Marine-released fish were transported and released on May 19th, and river-released fish were released over the course of three days (May 21st-23rd) to maximize detections (i.e. minimize acoustic interference between tags, or 'tag collisions') in the estuary. Marine-released fish were released with ~20 000 untagged conspecifics produced by the hatchery which were transported by separate trucks. River-released fish were released with several hundred untagged hatchery conspecifics.

Steelhead were tracked by a suite of acoustic receiver subarrays (combination of Vemco VR2W, VR3 and VR4), originally set up by the Pacific Ocean Salmon Tracking (POST) project (Welch et al. 2003), and now maintained by the Ocean Tracking Network (OTN) Canada (Cooke et al. 2011) and the Pacific Salmon Foundation. Three additional receivers (Vemco VR2W) were placed in freshwater: one ~1.5-km upstream of the release site, and two at the mouth of the Seymour River to monitor estuary residence time (duration between first and last detection).

These fixed freshwater and marine arrays allowed us to track smolts from their point of release to the northern or southern tip of Vancouver Island (~400 km; Figure 3.1).

3.2.4 Laboratory work

Steelhead smolt gill samples were analysed for 57 host genes and 18 infectious agents (run in duplicate) using high-throughput real-time quantitative polymerase chain reaction (HT-qRT-PCR) on the Fluidigm Biomark™ platform (Fluidigm, San Francisco, CA, USA). This technology uses microfluidics, and allows for 96 different samples against 96 different assays to be run on a single dynamic array (Miller et al. 2016). Host gene assays were selected based on important processes related to smoltification (Beamish & Mahnken 2001, Havird et al. 2013, Nilsen et al. 2007, Stefansson et al. 2007) and immune/stress responses to potential infectious agents (e.g. Henriksen et al. 2015b, Raida & Buchmann 2008) (Table 3.1). Infectious agent assays were selected based on pre-analyses screening of pooled gill samples (i.e. Miller et al. 2016), as well as prior knowledge of agents known to infect salmonids in the Pacific Northwest (Jeffries et al. 2014; Miller et al. 2014, 2016).

Genomic assessments took place at the Fisheries and Oceans Canada Pacific Biological Station (Nanaimo, British Columbia). Gill RNA extraction methods followed those previously described (Bass et al. 2017. Jeffries et al. 2014, Miller et al. 2011). Gill filaments were removed from RNA*later* (Life Technologies, Grand Island, NY) vials and homogenized using Magmax™-96 for Microarrays Kits (Ambion Inc, Austin, TX, USA). Gill filaments were homogenized using TRI-reagent™ (Ambion Inc, Austin, TX, USA), then 1-bromo-3-chloropropane was added to the homogenate. 100 µL aliquots of the aqueous phase were placed in 96-well plates prior to RNA extraction. RNA was eluted with RNAse, and purity was assessed

and normalized to 15 ng μL^{-1} using the A_{260}/A_{280} method using a Biomek FXP liquid handling instrument (Beckman-Coulter, Mississauga, ON, Canada). Normalized RNA (0.25 μg) was used to make cDNA using VILO (SuperScript VILO MasterMix; Life Technologies) and PCR cycling at 25°C for 5 min, 24°C for 60 min and 85°C for 5 min according to the Biomark protocol.

To account for the small assay volumes used by the Biomark platform, suspended cDNA (1.25 μL) was pre-amplified with a 5 μL mix of primers corresponding to all 75 PCR assays, by cycling 15 times in a PCR machine at 95 °C for 10 min, 95 °C for 10 s, and 60 °C for 4 min. All microbe assays (n = 18) were run in duplicate, while host biomarkers (n = 54) were run singularly. Three reference genes (run in duplicate) were included on each array. Two of these reference genes are commonly used with salmonid samples on the Biomark platform (COIL and 78d16.1; Miller et al. 2016, Teffer et al. 2017), and EF1a was also included based on prior transcriptome studies with *O. mykiss* (Gunnarsson et al. 2017, Stefansson et al. 2007). After specific target amplification (STA), ExoSAP-IT™ (Affymetrix, Santa Clara, California) was used to remove unincorporated primers by PCR cycling at 37 °C for 15 min and 80 °C for 15 min, then samples were diluted 1:5 in DNA Suspension Buffer (TEKnova, Hollister, California). Artificial positive constructs (cloned DNA sequence standards corresponding to all infectious agent assays, as outlined in Miller et al. 2016) were run in a panel of six serial dilutions on each dynamic array. For host gene assays, 5 3-X serial dilutions of host DNA were run on each dynamic array using 1 μL from each pooled sample. These dilutions allowed for the calculation of host gene assay efficiencies.

Two 96.96 Biomark Fluidigm dynamic arrays were loaded in preparation for qPCR (each with identical assays, but different smolt samples). Sample mix (5 μL) was prepared using 1x TaqMan Universal Master Mix (Life Technologies), 20x GE Sample Loading Reagent

(Fluidigm), and amplified cDNA. Assay mix (5 μL) was prepared with 10 μM primers and 3 μM probes for each assay. Sample mix and assay mix were then added into the inlets on the Fluidigm 96.96 dynamic array. PCR was performed on the Biomark with the following conditions: 50 ^{o}C for 2 min, 95 ^{o}C for 10 min, followed by 40 cycles of 95 ^{o}C for 15 s and 60 ^{o}C for 1 min.

3.2.5 Statistical analyses

Biomark Real-Time analysis software was used to determine cycle threshold (Ct) for each assay. Amplification curves of all assays were visually evaluated for unusual curve shapes. Using R statistical software (R Core Team 2015), efficiencies were calculated for each assay using the slope of a regression between Ct values and serial dilutions. Points falling significantly outside of the linear relationship between Ct and known RNA concentration (typically found on the extreme ends; i.e. lowest RNA concentration), were removed to improve the accuracy of assay efficiency estimates. Only assays with efficiencies between 1.80 and 2.20, and with proper amplifications on both dynamic arrays, were considered for subsequent analyses. In total, 24 assays did not meet these criteria and were removed, leaving a total of 33 host gene assays, and 15 infectious agents. One housekeeping gene (EF1a) was removed due to poor efficiency (<1.80). Host gene expression was normalized with the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen 2001) with the first delta as the average of the two reference genes, and the second delta the pooled sample made at the cDNA step. Individual samples were assessed for indication of poor quality (low expression of reference genes), and five samples were removed that were higher than 2*SD from the mean Ct of either reference gene. Thus, all subsequent analyses were completed with 114 samples (Table 3.2).

To assess survival of gill biopsied smolts, acoustic data were compiled into detection histories for each individual with available paired genomic data (n = 114). Survival in the river was calculated by dividing the number of river-released individuals detected in the estuary by the number released. Survival to NSOG was calculated in a similar manner; however, separate estimates were calculated for each release group (i.e. for marine-release smolts from release to NSOG, and for river-released individuals travelling from the estuary to NSOG). To identify migration fate, individual smolts were then categorized into one of three groups based on regions where survival was known to be poorest (Healy et al. 2017; Chapter 2 of this thesis): 1) smolts that were released in the river, and never detected (i.e. assumed river mortalities; RM), 2) smolts from either release group that did not survive the initial portion of the marine migration to the NSOG subarray (UN), and 3) individuals that were successful migrants to at least the NSOG subarray (SU).

One of the primary interests of the present study was to detect infectious agents and determine their impacts on migration fate. I first converted infectious agent assay Ct's to copy number (amount of RNA copies in sample; i.e. load), and then log transformed to improve normality. For each infectious agent detected, an ANOVA was run comparing loads among migration fate groups. I also compared the presence of each detected infectious agent among fate groups using a separate Pearson's χ^2 goodness-of-fit test.

To describe any interrelationships between infectious agents, smolt body condition, and migration fate on gene expression, I used constrained ordination in the form of redundancy analyses (RDA). In preparation for RDA, a new variable, 'relative infectious agent burden' (RIB; Bass, *In Prep*) was first calculated by:

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

where L_i is the RNA load of the *i*th infectious agent, *L*max$_i$ is the maximum load of the *i*th agent, summed across all infectious agents (*m*) infecting the individual. Thus, this metric considers infectious agent(s) present, as well as their relative load. I modelled smolt mass (g) as a function of fork length (mm), and used the residuals of this relationship as a metric of body condition (i.e. larger length-weight residuals = larger mass for a given fork length). An overall RDA model was run using the package *vegan* (Borcard et al. 2011, Oksanen et al. 2008) in R.

RDA combines regression with PCA (Zuur et al. 2007) to test the relationship of multiple explanatory factors on a response matrix of data (in the present case, the gene expression matrix). Separate Monte Carlo permutations tests can be used to assess the significance of the entire model (i.e. whether the response matrix is associated with any explanatory variables), investigate which individual RDA axes represent variation that is more structured than random (i.e. test if gene clustering on individual canonical axes is not just randomly distributed), as well as test which individual explanatory factors are significant predictors of the response matrix (Legendre et al. 2011). Monte Carlo permutation tests calculate a p-value based on the proportion of permuted test statistic values larger than the true unpermuted value of the statistic for a one-tailed ANOVA test (Borcard et al. 2011). For my analyses, the gene expression matrix of all individuals was the response variable, and scaled explanatory variables included both infectious agents, RIB, length-weight residuals, and migration fate (model: Gene expression matrix ~ '*Ca.* B. *cysticola'* + *F. psychrophilum* + RIB + length-weight residuals + migration fate). The model fit, as well as axes and terms, were assessed using Monte Carlo permutation tests. Any genes that were tightly linked in RDA ordination space to migratory fate groups were

further assessed by one-way ANOVAs with post-hoc Tukey's honest significance tests. Five genes in closest ordination space to the RM group (Il-17D, RPL6, MMP13, IFNa and C5aR), as well as five closest to SU smolts (NKA α1b, NKA b1, hep, SAA and C7) were chosen for these analyses (see Results).

To test if length-weight residuals (i.e. body condition) varied by migration fate groups, I used a one-way ANOVA with post-hoc Tukey's honest significance tests. Next, to test if length-weight residuals predicted residence time (as in Hanson et al. 2011), a generalized linear model (GLM) was run with log transformed residence time as the response, and length-weight residuals as the explanatory variable. Additionally, because most residence times were less than one day (Healy et al. 2017), I categorized smolts by either 'long' residency (>24 hrs), or 'short' residency (<24 hrs), and used a one-way ANOVA to see if length-weight residuals differed between these two groups.

Because any potential differences between the two release groups (river- and marine-release) could bias my results, I carried out several tests assessing the physiological condition of these groups. First, I ran a separate RDA model with a similar structure to the previously described RDA model, with the exception of replacing migration fate with release group (model: Gene expression matrix ~ '*Ca.* B. cysticola' + *F. psychrophilum* + RIB + length-weight residuals + release group). Release group was appropriate to assess in a separate model, because migration fate and release group are confounding variables (e.g. only river-release fish could be classified 'river mortalities'). Thus, the inclusion of both of these factors in one RDA may have resulted in overfitting the model. The model fit, and the significance of axes and terms were tested using Monte Carlo permutations tests. I also compared the presence of each infectious agent by using Pearson's χ^2 goodness-of-fit tests, with the null hypothesis that presence did not differ between

release groups. To assess if loads of infectious agents differed between release groups, I ran separate ANOVAs comparing release groups for each infectious agent detected. All statistical analyses were completed with R (RStudio, v1.0.136, www.rstudio.com), and assumptions for statistical tests (including normality, variances, etc) when appropriate were visually assessed.

3.3 Results

Smolt survival was poorest in two regions of the migration. In particular, for river-released smolts with accompanying gene expression data, 37 of 46 were detected in the estuary (80% survival) just ~2.5 km downstream of the release site (Table 3.2). In the marine environment, survival varied between groups to the NSOG array. For river-released smolts that had to travel through Burrard Inlet, only 13 were detected at NSOG (35% survival), compared to 46 of 68 marine-released smolts (68% survival; Table 3.2), which were released just beyond Burrard Inlet. These survival calculations agree with survival estimates from a parallel study using both biopsied and non-biopsied steelhead smolts which also found the river and Burrard Inlet to be regions of poor survival (Healy et al. 2017).

Two of 18 infectious agents monitored (Table 3.1) were detected, both of which were bacteria. *Flavobacterium psychrophilum* was present in 71 samples, and '*Candidatus* Branchiomonas cysticola' in 15 samples. There was no indication that loads (ANOVAs: *F. psychrophilum,* $F_{2,68} = 1.069$, $p = 0.35$; '*Ca.* B. cysticola*',* $F_{1,13} = 0.013$, $p = 0.91$) or presence (*F. psychrophilum,* $\chi^2 = 0.382$, $df = 2$, $p = 0.826$; '*Ca.* B. cysticola*',* $\chi^2 = 1.543$, $df = 2$, $p = 0.462$) of these infectious agents were associated with migration fate. No difference in loads were detected between release groups (ANOVAs: '*Ca.* B. cysticola', $F_{1,13} = 0.220$, $p = 0.647$; *F.*

psychrophilum, $F_{1,69} = 0.704$, $p = 0.404$). '*Ca.* B. cysticola' presence did not vary between groups (marine-released: ~15%; river-released: ~11%; $\chi^2 = 0.097$, df = 1, $p = 0.755$); however, presence of *F. psychrophilum* was determined to be higher in marine-released (~72%) relative to river-released smolts (~48%; $\chi^2 = 5.866$, df = 1, $p = 0.015$).

The RDA model including migration fate (model: Gene expression matrix ~ '*Ca.* B. cysticola' + *F. psychrophilum* + RIB + length-weight residuals + migration fate) was significant (Monte Carlo permutations test, $F_{6,107} = 1.495$, $p = 0.005$; Figure 3.2). All five factors ('*Ca.* B. cysticola', *F. psychrophilum*, RIB, length-weight residuals, migration fate) combined accounted for ~8% of the variance in the gene expression data. The first two RDA axes were determined to be significant (Monte Carlo permutations tests, RDA1, $F_{1,107} = 4.229$, $p = 0.001$; RDA2, $F_{1,107} = 2.024$, $p = 0.039$) and explained 3.5%, and 1.6% of the variance in the gene expression data, respectively (i.e. cumulatively: ~5.2%). Two explanatory factors were found to be significantly related to the gene expression matrix ($p < 0.05$): migration survival (Monte Carlo permutations test, $F_{2,107} = 1.5013$, $p = 0.045$), and length-weight residuals (Monte Carlo permutations test, $F_{1,107} = 2.494$, $p = 0.003$) (Table 3.3). RDA1 was most associated with several genes loading positively (i.e. Il-17D, C5, Il-15, CD8a, C4B, SHOP21 and MHCI) and negatively (i.e. NKA α1b, Il-1B, ATP5G3-C, SAA, NKA b1, IgMs, hep, and NKA a3) along this axis (Figure 3.2). RDA2 was positively associated with C7, HSC70, MHCI, CD8a, NKA α1c and hep, and most negatively with NKA a3, Il-1B , Il-17D, RPL6, ATP5G3-C, and MX.

The overall RDA ordination revealed survival fate groups clustered separately in ordination space along the first two axes (Figure 3.2). Successful smolts (SU) clustered negatively on RDA1 and positively on RDA2, while RM individuals (river-released smolts that failed to reach the estuary) clustered far away from all other fate groups (positively on RDA1,

negatively on RDA2). RM smolts clustered closest with five immune genes (Il-17D, RPL6, MMP13, IFNa and C5aR). In contrast, successful migrants through the system were associated with the osmoregulatory genes NKA α1b, and NKA b1, but were also in close ordination space with the immune genes C7, SAA and hep. These genes closest to SU smolts were also furthest in ordination space from RM smolts (Figure 3.2). Closer analyses of these ten candidate genes revealed that Il-17D (ANOVA, $F_{2,111} = 11.065$, $p<0.0001$), NKA α1b (ANOVA, $F_{2,111} = 3.607$, $p = 0.03$), and RPL6 (ANOVA, $F_{2,111} = 5.687$, $p = 0.004$) were the best predictors of migration fate (i.e. with p values < 0.05) (Figure 3.3). On the RDA ordination, UN smolts (i.e. assumed to have not survived in the marine environment pre-NSOG) clustered closest to the center of the RDA ordination.

Measures of river residency were tested as continuous (time between first and last estuary detection) and categorical (duration <24 hrs, or >24 hrs) variables. Smolt length-weight residuals on their own did not influence estuary residency by time (GLM, $F_{1,35} = 1.852$, $p = 0.182$), or duration (ANOVA, $F_{1,35} = 2.361$, $p = 0.133$) (Figure 3.4). However, length-weight residuals were higher for RM smolts compared to the other fate groups (ANOVA, $F_{2,111} = 5.589$, $p = 0.005$) (Figure 3.4).

The RDA investigating the relationship between release group on gene expression indicated the model (Gene expression matrix ~ '*Ca.* B. cysticola' + *F. psychrophilum* + RIB + length-weight residuals + release group) was significant (Monte Carlo permutations test, $F_{5,108} = 1.911$, $p = 0.001$). The five explanatory factors accounted for ~8% of the variance in gene expression data. The first two axes were significant (RDA1: $F_{1,108} = 4.464$, $p = 0.001$; RDA2: $F_{1,108} = 2.834$, $p = 0.003$), and three terms were significant predictors of gene expression (release group: $F_{1,108} = 3.191$, $p = 0.002$; length-weight residuals: $F_{1,108} = 2.45$, $p = 0.004$; and RIB: $F_{1,108}$

= 2.137, p = 0.033) (Table 3.4). River-released smolts were most associated with CD83, but also several other immune (e.g. Il-1B, C5aR, RPL6, MX) and osmoregulatory genes (e.g. NKA a3, NKA b1), while marine-released smolts were primarily clustered with C7 and HSC70 (Figure 3.5).

3.4 Discussion

The present study shows that the Seymour River and the first marine embayment (Burrard Inlet) were associated with poor survival for biopsied smolts, particularly given their short distances (~2.5 km and ~18 km, respectively). As expected, my survival calculations are consistent with a parallel telemetry study reporting survival estimates of biopsied and non-biopsied steelhead (Healy et al. 2017; i.e. Chapter 2 of this thesis), as well as prior work using the same marine and release locations (Balfry et al. 2011). The present study enhances our knowledge of how the physiological condition of hatchery steelhead smolts can influence migration fate through these high-risk landscapes. By combining acoustic telemetry with HT-qRT-PCR of non-lethal gill biopsies, I identified several important genes which were related to fate, as well as identified several infectious agents present in the population of hatchery steelhead.

Two infectious agents, '*Ca.* B.cysticola' and *F. psychrophilum*, were detected in gill samples from acoustically tagged smolts, but neither had any apparent influence on smolt gene expression profiles or migration fate. '*Ca.* B. cysticola' is a recently discovered bacterium that may be associated with proliferative gill disease (Toenshoff et al. 2012), however, '*Ca.* B. cysticola' is not necessarily always associated with mortality (Bass et al. 2017, Gunnarsson et al.

2017, Teffer et al. 2017). Presence of this infectious agent in a fish may be the result of a secondary infection (Tengs & Rimstad 2017), however, some studies have hypothesized that '*Ca.* B. cysticola' could be part of the normal microflora present on the gills (Steinum et al. 2009, Toenshoff et al. 2012). Thus, the association between the presence of this infectious agent and disease in salmonids warrants further investigation. *F. psychrophilum* is a common bacteria associated with mortality of salmon in aquaculture facilities worldwide (Nematollahi et al. 2003), but its presence does not always equate to disease (Decostere et al. 2000, Nematollahi et al. 2003). Susceptibility to *F. psychrophilum* infection for juvenile rainbow trout (non-anadromous *Oncorhynchus mykiss*) may be age-dependent, as older individuals (>5 months) are most successful at avoiding disease states (Decostere et al. 2001), which may help explain why this infectious agent did not influence smolt survival in the present study. An important limitation is that PCR can detect RNA of an infectious agent in fish, but cannot distinguish between individuals in a carrier or disease state. Recent work has paired histopathology with HT-qRT-PCR techniques, and identified a suite of host genes which can distinguish between disease states for viral infections (Miller et al. 2017). Applying this methodology to other types of infectious agents (e.g. bacteria) will vastly improve our ability to identify important genes indicative of fish in disease states, and identify more clear links between infectious agents and migration fate.

The use of gill biopsies in the present study likely limited my ability to detect infectious agents. Many bacteria and viruses are thought to enter fish via the gills, gut or skin (Khimmakthong et al. 2013, Schönherz et al. 2012, Tobback et al. 2010), but can then move to infect other internal tissues in later stages of infection (Bradford et al. 2010). Therefore, because only steelhead gills were biopsied, I may have missed infectious agents present in other tissues. Furthermore, because of the small sizes of gill tissue taken from smolts, I had to normalize RNA

to a concentration ~25% of levels typically used with larger samples taken from adult fish (Miller et al. 2016). These low concentrations likely contributed to an increase in false negatives for infectious agents. Therefore, I consider it likely that there was a higher presence of the two detected agents in the population than I estimated here (62% for *F. psychrophilum* and ~13% for '*Ca.* B. cysticola'), as well as other infectious agents I may not have detected (or did not assay for) which could have influenced migration fate.

Positioning of smolts across the top two RDA ordination axes provided insight into the variances in physiological condition relating to survivors to at least NSOG (SU) and river-released smolts that failed to reach the estuary (RM). Genes related to the smoltification process were in close ordination space to survivors, including important osmoregulatory isoforms associated with the saltwater transition (e.g. NKA α1b and b1; (Richards 2003; Stefansson et al. 2007). Additionally, hepcidin (hep), which has been linked to inflammation and iron metabolism (Ganz 2012; Raida and Buchmann 2009) as well as C7, which is hypothesized to link the acute and adaptive immune systems (Gonzalez et al. 2007) were positively associated with successful smolts. In contrast, RM smolts showed association primarily with genes indicative of an inflammatory response, such as Il-17D (Zou and Secombes 2016), Il-15 (Komatsu et al. 2009; Wang et al. 2007), RPL6 (Kumar et al. 2014; Miller et al. 2014), and MMP13, which may signify chronic inflammation at the gills (Castro et al. 2013; Krasnov et al, 2012; Tadiso et al. 2011). Of the immune genes we investigated, Il-17D and RPL6 were the most predictive of fate for migrating smolts, with RM individuals showing significantly higher relative expression of these genes than other individuals. Transcriptome signals related to inflammatory genes at the gills have previously been linked to survival in salmonids in multiple studies, regardless of the cause (Drenner 2006; Jeffries et al. 2012, 2014; Miller et al. 2011, 2014; Teffer et al. 2017).

Multiple mechanisms can induce inflammatory responses, including aquatic contaminants (Eder et al. 2009; Schmidt-Posthaus et al. 2001), stress (Castro et al. 2011; Verleih et al. 2015), and infectious agents (Kvellestad et al. 2005; Raida et al. 2011; Raida and Buchmann 2009; Tadiso et al. 2011). Thus, an indication of an inflammatory response in RM fish suggests these individuals were in poor condition relative to other smolts, which could have reduced swimming performance (Castro et al. 2013), and/or increase susceptibility to predation (Hostetter et al. 2012; Tucker et al. 2016) in freshwater. Alternately, gill inflammation along with reduced indicators of smoltification may result in failure of fish to migrate out of the river system (Sutherland et al. 2014).

The ability to adapt to changes in salinity is integral for smolts migrating from freshwater natal streams to the marine environment (Robertson and Mccormick 2012; Schreck et al. 2006; Stich et al. 2015). In the present study, the expression of N^+, K^+-ATPase isoform α1b (NKA α1b) was associated with migration fate, with RM smolts showing lower relative expression of NKA α1b compared to other migration fate groups. NKA α1b is thought to be particularly important for saltwater entry (Bystriansky 2006), and higher expression of this isoform at the gills can be associated with the parr-smolt transition in the spring (Robertson and Mccormick 2012; Stefansson et al. 2007), suggesting that steelhead which were never detected in the river estuary may not have been fully developed as smolts to enter the marine environment. Similarly, a positive association with the stress gene heat shock protein 70 (HSC70; Boone and Vijayan 2002; Lewis et al. 2010) for successful smolts could indicate an increased tolerance for transfer to seawater (Niu et al. 2008). The expression patterns of other osmoregulatory genes (NKA α1c, b1, and a3) were not particularly indicative of survival; however, NKA α1c levels tend to not be associated with transfer to saltwater (Richards et al. 2003).

The freshwater survival results should be interpreted cautiously because I cannot directly conclude that all RM smolts represented mortalities. Juvenile steelhead are known to exhibit migratory plasticity, with some individuals in the population showing anadromy, while others remain in freshwater as residents (i.e. 'residualize'; reviewed in Kendall et al. 2015). As parr transform into smolts, they develop a more fusiform body morphology (i.e. smaller length-weight residuals in this study) that prepares them for marine migration (Nichols et al. 2008, Stefansson et al. 2007). In species with both migratory and non-migratory forms (such as steelhead), larger length-weight residuals (sometimes referred to by a similar metric: 'condition factor') can be an indication of freshwater residualization (Hausch & Melnychuk 2012, Tipping et al. 2003). My results indicate that RM smolts had larger length-weight residuals than other groups. Similarly, lower levels of NKA α1b (such as was seen for RM individuals) could be an indication of a smolt residualizing in freshwater (Hanson et al. 2011). Even though the river estuary receivers had a detection probability of 100% (Healy et al. 2017), there were no detections here for the last 11 days prior to recovery; therefore, some of the RM smolts may have remained in the river as residents, or delayed for longer in the river prior to emigration. If smolts remained in the river, this likely resulted in an underestimation of river survival from the acoustic telemetry data alone. While stream home ranges can be small for juvenile resident trout (<1-2 km; Hartman et al. 2012, Harvey et al. 2005), residency rates are typically only ~5 % in hatchery steelhead (Hausch & Melnychuk 2012), and smolts released close to the river estuary (such as in the present study) are significantly less likely to residualize (Hausch & Melnychuk 2012). Therefore, it is unlikely that all the RM smolts represent residualized fish. Regardless, combining acoustic telemetry with HT-qRT-PCR allowed me to detect physiological differences at the molecular level which demarcates RM smolts from other fate groups.

An important consideration is that my RDA results are on ordinations that explain ~8% of the variance in the gene expression data. While this is low, previous work with adult sockeye salmon using principal component analyses related migration survival to axes which explained ~12% of the variance in the data, but with substantially more genes (>12,000 genes; Miller et al. 2011). Other external factors can influence smolt gene expression (Evans et al. 2011), including temperature (Beckman et al. 1998; Verleih et al. 2015), light levels (Stefansson et al. 2007), and other infectious agents that were not included in my panel of assays. Additionally, survival can be influenced by many factors such as currents/flow (Perry et al. 2013), food availability (Beamish and Mahnken 2001) and predators (Berejikian et al. 2016; Hostetter et al. 2012). Therefore, for the present study, it is not surprising that my methodology explained <10% of the variance in the data, given the limited explanatory variables in the RDA models, as well as other genes for which assays were not run (or that did not meet my quality standards for efficiency) on the qPCR dynamic arrays.

Infectious agent and gene expression profiles were found to be slightly different between the two release groups, which could confound interpretation of my results. Marine-released smolts had higher detected presence of *F. psychrophilum*, and clustered with different genes in RDA ordination space than river-released smolts (Figure 3.5). These variable immune gene expression profiles could be a result of the disparate presence of *F. psychrophilum* among groups. My acoustic tagging procedure was kept consistent across all surgeries, with smolts being haphazardly selected from tanks, and tagging alternating between release groups (i.e. both release groups were tagged simultaneously, and alternated between taggers throughout). Thus, the gene expression differences I detected between groups was most likely due to chance. Regardless, this slight difference in gene expression by group may have biased my results.

Although these differences in physiological state may have contributed to the poor survival observed to NSOG for river-released smolts relative to marine-released, survival was determined to be similar to previous estimates (Balfry et al. 2011). Other external factors, such as predation, likely play a more prominent role in survival through these early portions of marine migration (Berejikian et al. 2016; Healy et al. 2017) and may explain why differences in smolt condition didn't appear to significantly influence survival here.

Linking telemetry with transcriptome profiles and infectious agents, such as in the present study, allows the rare opportunity to identify factors operating at the molecular level which influence migratory fate for smolts. Disease likely plays an important role in migration survival, but can be particularly challenging to study, as mortality is seldom observed in migrating fish (Miller et al. 2014). While the present study found no indication that infectious agents influenced migration fate, I found immune gene profiles which were predictive of fate for migrating steelhead. I highlighted the early riverine portion of outmigration to be a region where the expression of several genes were particularly important determinants of fate, consistent with similar work with sockeye smolts in British Columbia (Jeffries et al. 2014). Because factors operating during this critical life stage can be linked to population productivity (Irvine & Akenhead 2013, Kendall et al. 2017, Moore et al. 2012), identifying relationships between smolt physiology and migration fate will be crucial for future conservation and increased population predictive capabilities.

3.5 Chapter 3 tables

Table 3.1: Primer and probe sequences corresponding to assays used in HT-qRT-PCR analyses on hatchery steelhead (Oncorhynchus mykiss) smolts.

Gene Abbreviation	Infectious agent/ host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Efficiency
re.sal	*Renibacterium salmoninarum*	Microbe	Bacteria	F: CAACAGGGTGGTTATTCTGCTTTC R: CTATAAGAGCCACCAGCTGCAA P: CTCCAGCGCCGCAGGAGGAC	1.89
vi_sal	Vibrio salmonicida	Microbe	Bacteria	F: GTGTGATGACCGTTCCATATTT R: GCTATTGTCATCACTCTGTTTCTT P: TCGCTTCATGTTGTGTAATTAGGAGCGA	1.87
fl_psy	*Flavobacterium psychrophilum*	Microbe	Bacteria	F: GATCCTTATTCTCACAGTACCGTCAA R: TGTAAACTGCTTTTGCACAGGAA P: AAACACTCGGTCGTGACC	1.84
c_b_cys	*'Candidatus* Branchiomonas cysticola*'*	Microbe	Bacteria	F: AATACATCGGAACGTGTCTAGTG R: GCCATCAGCCGCTCATGTG P: CTCGGTCCCAGGCTTTCCTCTCCCA	1.80
ae_sal	*Aeromonas salmonicida*	Microbe	Bacteria	F: TAAAGCACTGTCTGTTACC R: GCTACTTCACCCTGATTGG P: ACATCAGCAGGCTTCAGAGTCACTG	2.03
vi_ang	*Vibrio anguillarum*	Microbe	Bacteria	F: CCGTCATGCTATCTAGAGATGTATTTGA R: CCATACGCAGCCAAAAATCA P: TCATTTCGACGAGCGTCTTGTTCAGC	1.82
ic_hof	*Ichthyophonus hoferi Sphaerothecum*	Microbe	Mesomycetozoean	F: GTCTGTACTGGTACGGCAGTTTC R: TCCCGAACTCAGTAGACACTCAA P: TAAGAGCACCCACTGCCTTCGAGAAGA	1.86
lo_sal	*Loma salmonae*	Microbe	Microsporidian	F: GGAGTCGCAGCGAAGATAGC R: CTTTTCCTCCCTTTACTCATATGCTT P: TGCCTGAAATCACGAGAGTGAGACTACCC	1.81
pa_the	*Paranucleospora theridion*	Microbe	Microsporidian	F: CGGACAGGGAGCATGGTATAG R: GGTCCAGGTTGGGTCTTGAG P: TTGGCGAAGAATGAAA	1.60
ce_sha	*Ceratonova shasta*	Microbe	Myxozoan	F: CCAGCTTGAGATTAGCTCGGTAA	1.81

Gene Abbreviation	Infectious agent/ host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Efficiency
				R: CCCCGGAACCCGAAAG P: CGAGCCAAGTTGGTCTCTCCGTGAAAAC	
pa_min	*Parvicapsula minibicornis*	Microbe	Myxozoan	F: AATAGTTGTTTGTCGTGCACTCTGT R: CCGATAGGCTATCCAGTACCTAGTAAG P: TGTCCACCTAGTAAGGC	1.78
p_pse	*parvicapsula pseudobranchia*	Microbe	Myxozoan	F: CAGCTCCAGTAGTGTATTTCA R: TTGAGCACTCTGCTTTATTCAA P: CGTATTGCTGTCTTTGACATGCAGT	2.13
cr_sal	*Cryptobia salmocidica*	Microbe	Protozoan	F: TCAGTGCCTTTCAGGACATC R: GAGGCATCCACTCCAATAGAC P: AGGAGGACATGGCAGCCTTTGTAT	1.84
ic_mul	*Ichthyophthirius multifiliis*	Microbe	Protozoan	F: AAATGGGCATACGTTTGCAAA R: AACCTGCCTGAAACACTCTAATTTTT P: ACTCGGCCTTCACTGGTTCGACTTGG	1.84
sch	Salmon (gill) chlamydia	Microbe	Virus	F: GGGTAGCCCGATATCTTCAAAGT R: CCCATGAGCCGCTCTCTCT P: TCCTTCGGGACCTTAC	1.82
vhsv	Viral hemorrhagic septicemia virus	Microbe	Virus	F: ATGAGGCAGGTGTCGGAGG R: TGTAGTAGGACTCTCCCAGCATCC P: TACGCCATCATGATGAGT	1.60
prv	Piscine reovirus	Microbe	Virus	F: TGCTAACACTCCAGGAGTCATTG R: TGAATCCGCTGCAGATGAGTA P: CGCCGGTAGCTCT	1.90
ihnv	Infectious hematopoietic necrosis virus	Microbe	Virus	F: AGAGCCAAGGCACTGTGCG R: TTCTTTGCGGCTTGGTTGA P: TGAGACTGAGCGGGACA	1.81
ATP5G3-C	ATP synthase	Host Gene	Ion transport / Metabolism	F: GGAACGCCACCATGAGACA R: CGCCATCCTGGGCTTTG P: AGCCCCATTGCCTC	1.81
C4B	Complement component 4B	Host Gene	Immune	F: TCCAACCACATCGCATTATCC R: ATCTCTGACACCACTGACCACAA	1.83

Gene Abbreviation	Infectious agent/ host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Efficiency
				P: ATAGACAGGCTTCCC	
C5	Component factor 5	Host Gene	Immune	F: TGGCAAGGACTTTTTCTGCT R: AGCACAGGTATCCAGGGTTG P: CTGGCAGGGATTGCATCAAATC	1.93
C5aR	Complement component 5a receptor 1	Host Gene	Immune	F: ACGCACCTTGAGGGTCATT R: CAGTGGAAACCAGCACAGG P: TTGCCGTGTCGCTGAGCTTCTT	1.92
C7	Complement component C7 precursor	Host Gene	Immune	F: ACCTCTGTCCAGCTCTGTGTC R: GATGCTGACCACATCAAACTGC P: AACTACCAGACAGTGCTG	1.80
CCT5	T-complex protein 1 subunit epsilon	Host Gene	Immune	F: CCTCAGTGGGAGGTCCCTAAT R: CCCCAGGTAGTCAAAATGATCCT P: CTTCTGAAGTCATCTATCT	1.74
CD4	Cell receptor	Host Gene	Immune	F: CATTAGCCTGGGTGGTCAAT R: CCCTTTCTTTGACAGGGAGA P: CAGAAGAGAGAGCTGGATGTCTCCG	1.91
CD83	Cluster of differentiation 83	Host Gene	Immune	F: GATGCACCCCTTGAGAAGAA R: GAACCCTGTCTCGACCAGTT P: AATGTTGATTTACACTCTGGGGCCA	1.82
CD8a	Cluster of differentiation 8a	Host Gene	Immune	F: ACACCAATGACCACAACCATAGAG R: GGGTCCACCTTTCCCACTTT P: ACCAGCTCTACAACTGCCAAGTCGTGC	1.81
CIRP	Cold inducible RNA binding protein	Host Gene	Stress/ Osmoregulation	F: AAGCTGTGATTGTGCTCTAAAGAC R: TCCCACTTAGCATTCCATCCTTG P: CTCCTTCAGTTCTGTAATGC	N/A
COMMD7	COMM domain containing protein 7	Host Gene	Immune	F: CAAAGCCAGTATGGACTGTTTCAG R: TTGTTTTCTGCTGCCCCTCTA P: ACCTGATCGCCAGTAGCATGAGCATGTAC	1.80
CXCR4	C-X-C chemokine receptor type 4	Host Gene	Immune/ osmoregulatory	F: GGAGATCACATTGAGCAACATCA R: GCTGCTGGCTGCCATACTG	1.82

Gene Abbreviation	Infectious agent/ host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Efficiency
				P: TCCACGAAGATCCCCA	
FYB	FYN-T-binding protein	Host Gene	Immune	F: TGCAGATGAGCTTGTTGTCTACAG R: GCAGTAAAGATCTGCCGTTGAGA P: CTCAACGATGACATCCACAGTCTCCC	1.88
GHR	Growth hormone receptor	Host Gene	Growth	F: TGGGAAGTTGAGTGCCAGACT R: CACAAGACTACTGTCCTCCGTTGA P: TGGGAGAGCCAGCCAGCCTGC	N/A
GR-2	Glucocorticoid receptor 2	Host Gene	Growth	F: TCCAGCAGCTATGCCAGTTCT R: TTGCCCTGGGTTGTACATGA P: AAGCTTGGTGGTGGCGCTG	N/A
hep	Hepcidin	Host Gene	Immune	F: GAGGAGGTTGGAAGCATTGA R: TGACGCTTGAACCTGAAATG P: AGTCCAGTTGGGGAACATCAACAG	1.93
HSC70	Heat shock cognate 70 protein	Host Gene	Stress	F: GGGTCACACAGAAGCCAAAAG R: GCGCTCTATAGCGTTGATTGGT P: AGACCAAGCCTAAACTA	1.86
HSP90	Heat shock protein 90	Host Gene	Stress	F: TGGGCTACATGGCTGCCAAG R: TCCAAGGTGAACCCAGAGGAC P: AGCACCTGGAGATCAA	1.63
HTA	HIV-1 Tat interactive protein	Host Gene	Immune	F: CTTGTAACAGTTCGACATGGCTTATT R: TGGTGAAGCATTTCTGTATGTCAA P: TCTGTACTGAGCATCCCCGCACATTACA	1.83
IFNa	Interferon alpha	Host Gene	Immune	F: CGTCATCTGCAAAGATTGGA R: GGGCGTAGCTTCTGAAATGA P: TGCAGCACAGATGTACTGATCATCCA	1.87
IGF-1R	Insulin-like growth factor 1	Host Gene	Growth	F: TGAAGAGCCACCTGAGGTCACT R: TCAGAGGTGGGAGGTTGAGACT P: CGGGCTAAAGACCCGTCCCAGTCC	1.99
IgMs	Immunoglobulin	Host Gene	Immune	F: CTTGGCTTGTTGACGATGAG R: GGCTAGTGGTGTTGAATTGG P: TGGAGAGAACGAGCAGTTCAGCA	1.86
IgT	Immunoglobulin tau	Host Gene	Immune	F: AGCACCAGGGTGAAACCA R: GCGGTGGGTTCAGAGTCA	2.10

Gene Abbreviation	Infectious agent/ host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Efficiency
				P: AGCAAGACGACCTCCAAAACAGAAC	
Il-10	Interleukin 10	Host Gene	Immune	F: CGACTTTAAATCTCCCATCGAC R: GCATTGGACGATCTCTTTCTTC P: CATCGGAAACATCTTCCACGAGCT	N/A
Il-11	Interleukin 11	Host Gene	Immune	F: GCAATCTCTTGCCTCCACTC R: TTGTCACGTGCTCCAGTTTC P: TCGCGGAGTGTGAAAGGCAGA	1.94
IL-15	Interleukin 15	Host Gene	Immune	F: TTGGATTTTGCCCTAACTGC R: CTGCGCTCCAATAAACGAAT P: CGAACAACGCTGATGACAGGTTTTT	1.85
Il-17D	Interleukin 17D	Host Gene	Immune	F: CAACAGAAGTGCGAACGATG R: GATGCCACATCGCATAACAG P: TGGTCGAGTATCTTTCGTGTGTTTGC	1.91
IL-1B	Interleukin 1 beta	Host Gene	Immune	F: AGGACAAGGACCTGCTCAACT R: CCGACTCCAACTCCAACACTA P: TTGCTGGAGAGTGCTGTGGAAGAA	1.83
IL-1R	Interleukin-1 receptor complex	Host Gene	Immune	F: ATCATCCTGTCAGCCCAGAG R: TCTGGTGCAGTGGTAACTGG P: TGCATCCCCTCTACACCCCAAA	1.80
IL-8	Interleukin 8	Host Gene	Immune	F: GAGCGGTCAGGAGATTTGTC R: TTGGCCAGCATCTTCTCAAT P: ATGTCAGCGCTCCGTGGGT	1.97
JUN	Transcription factor	Host Gene	Immune	F: TTGTTGCTGGTGAGAAAACTCAGT R: CCTGTTGCCCTATGAATTGTCTAGT P: AGACTTGGGCTATTTAC	N/A
MARCH2	Salmo salar E3 ubiquitin-protein ligase MARCH2	Host Gene	Immune/ stress	F: GCACCTGCGATAGAAGAGCAT R: GAGATGGAATCCGCAGAAGCT P: ACTTGTTTAACCATGCTGTGCGACTCTTCCT	1.85
MHC1	Major histocompatibility complex class II	Host Gene	Immune	F: GCGACAGGTTTCTACCCCAGT R: TGTCAGGTGGGAGCTTTTCTG P: TGGTGTCCTGGCAGAAAGACGG	1.99
MHCII	Major	Host	Immune	F: TGCCATGCTGATGTGCAG	1.64

Gene Abbreviation	Infectious agent/ host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Efficiency
	histocompatibility complex class II	Gene		R: GTCCCTCAGCCAGGTCACT P: CGCCTATGACTTCTACCCCAAACAAAT	
MMP13	Matrix metalloproteinase-13	Host Gene	Immune	F: GCCAGCGGAGCAGGAA R: AGTCACCTGGAGGCCAAAGA P: TCAGCGAGATGCAAAG	1.81
MMP25	Matrix metalloproteinase-25 precursor	Host Gene	Immune	F: TGCAGTCTTTTCCCCTTGGAT R: TCCACATGTACCCACACCTACAC P: AGGATTGGCTGGAAGGT	1.75
MX	Antiviral protein	Host Gene	Immune	F: AGATGATGCTGCACCTCAAGTC R: CTGCAGCTGGGAAGCAAAC P: ATTCCCATGGTGATCCGCTACCTGG	1.82
NKA a1a	Na+/K+ ATPase a1a subunit	Host Gene	Osmoregulatory	F: CCAGGATCACTCAATGTCACTCT R: GCTATCAAAGGCAAATGAGTTTAATATCATTGTAAAA P: ACGATTACATTATAAGGCAATACT	N/A
Nka a1a	Na+/K+ ATPase a1a subunit	Host Gene	Osmoregulatory	F: AGGAAGCCTTCCAGAACGCT R: CAATCAAACTGGAAGCCCTCA P: AATCCCCAGGCAAAGTGGCCCA	N/A
NKA a1b	Na+/K+ ATPase a1b subunit	Host Gene	Osmoregulatory	F: GCTACATCTCAACCAACAACATTACAC R: TGCAGCTGAGTGCACCAT P: ACCATTACATCCAATGAACACT	1.87
NKA a1c	Na+/K+ ATPase a1c subunit	Host Gene	Osmoregulatory	F: AGGGAGACGTACTACTAGAAAGCAT R: CAGAACTTAAAATTCCGAGCAGCAA P: ACAACCATGCAAGAACT	1.81
NKA a3	Na+/K+ ATPase a3 subunit	Host Gene	Osmoregulatory	F: GGAGACCAGCAGAGGAACAG R: CCCTACCAGCCCTCTGAGT P: AAGACCCAGCCTGAAATG	1.80
NKA b1	Na+/K+ ATPase subunit beta 1	Host Gene	Osmoregulatory	F: CGTCAAGCTGAACAGGATCGT R: CCTCAGGGATGCTTTCATTGGA P: CCTTGGCCTGAAGTTG	1.80
NKCC	Na+/K+,2Cl-contransporter	Host Gene	Osmoregulatory	F: GATGATCTGCGGCCATGTTC	N/A

Gene Abbreviation	Infectious agent/ host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Efficiency
				R: AGACCAGTAACCTGTCGAGAAAC P: CTCCAGAAGGCCCAACTT	
RPL6	Ribosomal protein L6	Host Gene	Immune	F: CGCCACCACAACCAAGGT R: TCCTCAGCCTCTTCTTCTTGAAG P: AGATCCCCAAGACTCTGTCAGACGCCT	1.81
SAA	Serum amyloid protein A	Host Gene	Immune	F: GGGAGATGATTCAGGGTTCCA R: TTACGTCCCCAGTGGTTAGC P: TCGAGGACACGAGGACTCAGCA	1.87
SAP	Serum amyloid P	Host Gene	Immune	F: CAACGTCTCAAAGCCCATTT R: GCCTCGTTCTTGCTCAGAGT P: CTGCTATGACCATGTGTCAGAGGTTC	1.59
SHOP21	Salmon hyperosmotic protein 21	Host Gene	Stress/Immune	F: GCGGTAGTGGAGTCAGTTGGA R: GCTGCTGACGTCTCACATCAC P: CCTGTTGATGCTCAAGG	2.00
STAT1	Activator of transcription 1-alpha/beta	Host Gene	Immune	F: TGTCACCGTCTCAGACAGATCTG R: TGTTGGTCTCTGTAAGGCAACGT P: AGTTGCTGAAAACCGG	1.75
TCRb	T-cell receptor beta	Host Gene	Immune	F: TCACCAGCAGACTGAGAGTCC R: AAGCTGACAATGCAGGTGAATC P: CCAATGAATGGCACAAACCAGAGAA	1.75
TF	Transferrin	Host Gene	Immune	F: TTCACTGCTGGAAAATGTGG R: GCTGCACTGAACTGCATCAT P: TGGTCCCTGTCATGGTGGAGCA	1.72
TNF-a	Tumor necrosis factor alpha	Host Gene	Immune	F: GGGGACAAACTGTGGACTGA R: GAAGTTCTTGCCCTGCTCTG P: GACCAATCGACTGACCGACGTGGA	2.10
Coil	Reference Gene	Reference Gene	Reference	F: GCTCATTTGAGGAGAAGGAGGATG R: CTGGCGATGCTGTTCCTGAG P: TTATCAAGCAGCAAGCC	1.82
EF1a	Elongation factor 1 alpha	Reference Gene	Reference	F: CGGAACGACGGTCGATCT R: GCTCACATCGCCTGCAAGT P: CTCCTTGAGCTCGCTG	1.74
786d16.1	Si:dkey-78d16.1	Referenc	Referenc	F: GTCAAGACTGGAGGCTCAGAG	1.81

Gene Abbreviation	Infectious agent/ host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Efficiency
P	protein [Danio rerio]	e Gene	e	R: GATCAAGCCCCAGAAGTGTTTG P: AAGGTGATTCCCTCGCCGTCCGA	

Table 3.2: Summary table of Seymour River hatchery steelhead (*Oncorhynchus mykiss*) smolt tagging and survival data by release group.

Tagging data				Assumed river mortalities (RM)	Survived River		Unsuccessful to NSOG (UN)	Successful past NSOG (SU)	
Release location	**Sample size**	**length (mm; SD)**	**weight (g; SD)**	**Count**	**Count**	**Segment Survival (%)**	**Count**	**Count**	**Cumulative Survival (%)**
River-release	46	203.9 (15.9)	81.3 (23.6)	9	37	80	24	13	28
Marine-release	68	202.7 (12.6)	80.0 (17.8)	N/A	N/A	N/A	22	46	68

Table 3.3: Summary statistics for the Redundancy analysis (RDA) of the migration fate model (Model: Gene expression matrix ~ cbcys + flavo + RIB + length-weight residuals + migration fate) of Seymour River steelhead. Significant p values are shown in **bold**.

Variable	DF	Variance	F	P
Relative infectious agent burden	1	0.4496	1.580	0.099
'*Candidatus* Branchiomonas cysticola'	1	0.2594	0.9115	0.515
Flavobacterium psychrophilum	1	0.2794	0.9820	0.451
Length-weight residuals	1	0.7097	2.4940	**0.003**
Migration Fate	2	0.8544	1.5013	**0.045**
Residual	107	30.4475		

Table 3.4: Summary statistics for the Redundancy analysis (RDA) of the model testing for an effect of release group on gene expression (Model: Gene expression matrix ~ '*Ca*. B. cysticola' + *F. psychrophilum* + RIB + residuals + release group). Significant p values are shown in **bold**.

Variable	DF	Variance	F	P
Release group	1	0.8958	3.1911	**0.002**
Relative infectious agent burden	1	0.5999	2.1370	**0.033**
Length-weight residuals	1	0.7013	2.4984	**0.004**
'*Candidatus* Branchiomonas cysticola'	1	0.2629	0.9366	0.463
Flavobacterium psychrophilum	1	0.2227	0.7933	0.637
Residual	108	31.2913		

3.6 Chapter 3 figures

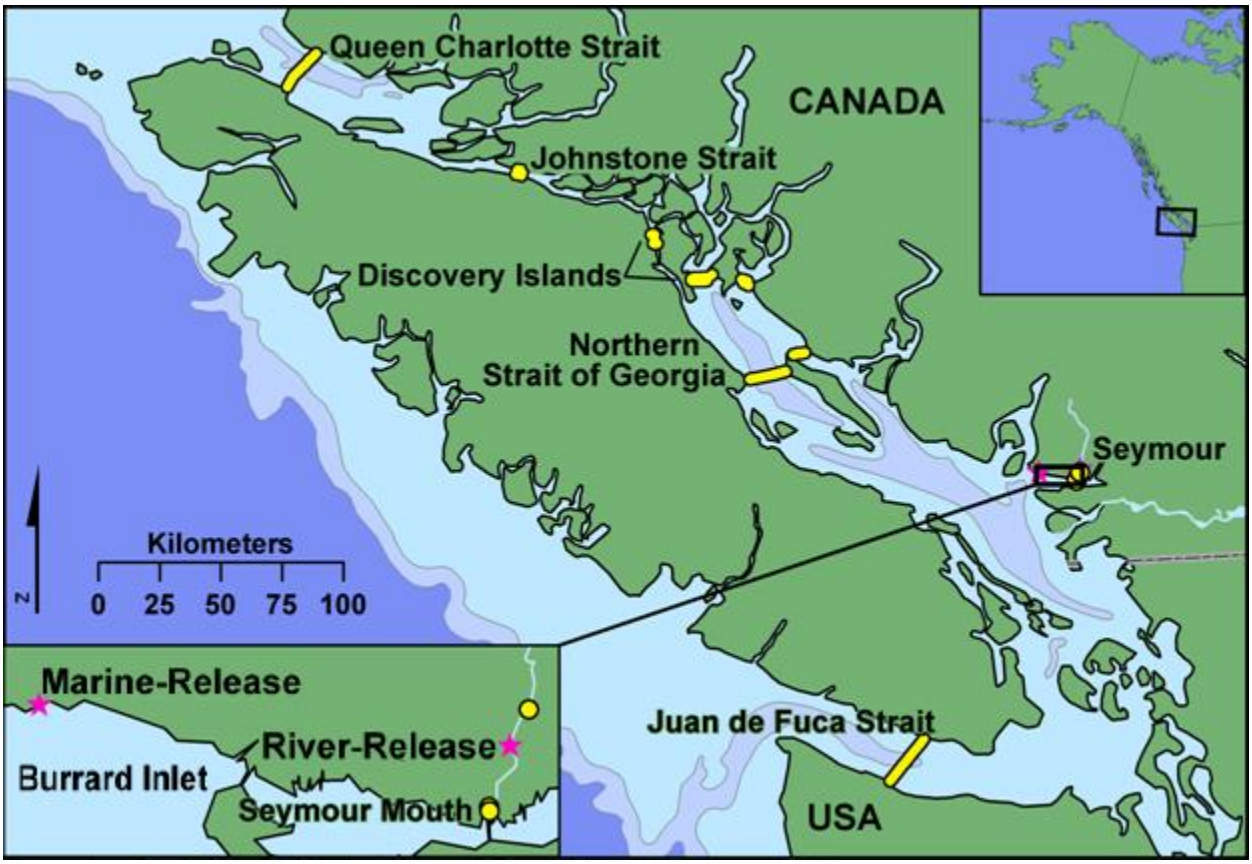

Figure 3.1: Map of the 2015 study area for Seymour steelhead (*Oncorhynchus mykiss*). Yellow circles and lines represent either individual receivers, or a receiver subarray. The inset (bottom left) shows a close up of Burrard Inlet and the Seymour River, with release locations (stars). Fish were tagged at the Seymour River hatchery in May, then transported and released at either West Vancouver ('Marine-release'; n = 160) or the lower Seymour River ('River-release'; n = 83). The depth contours show the 200 and 500 meter isobaths.

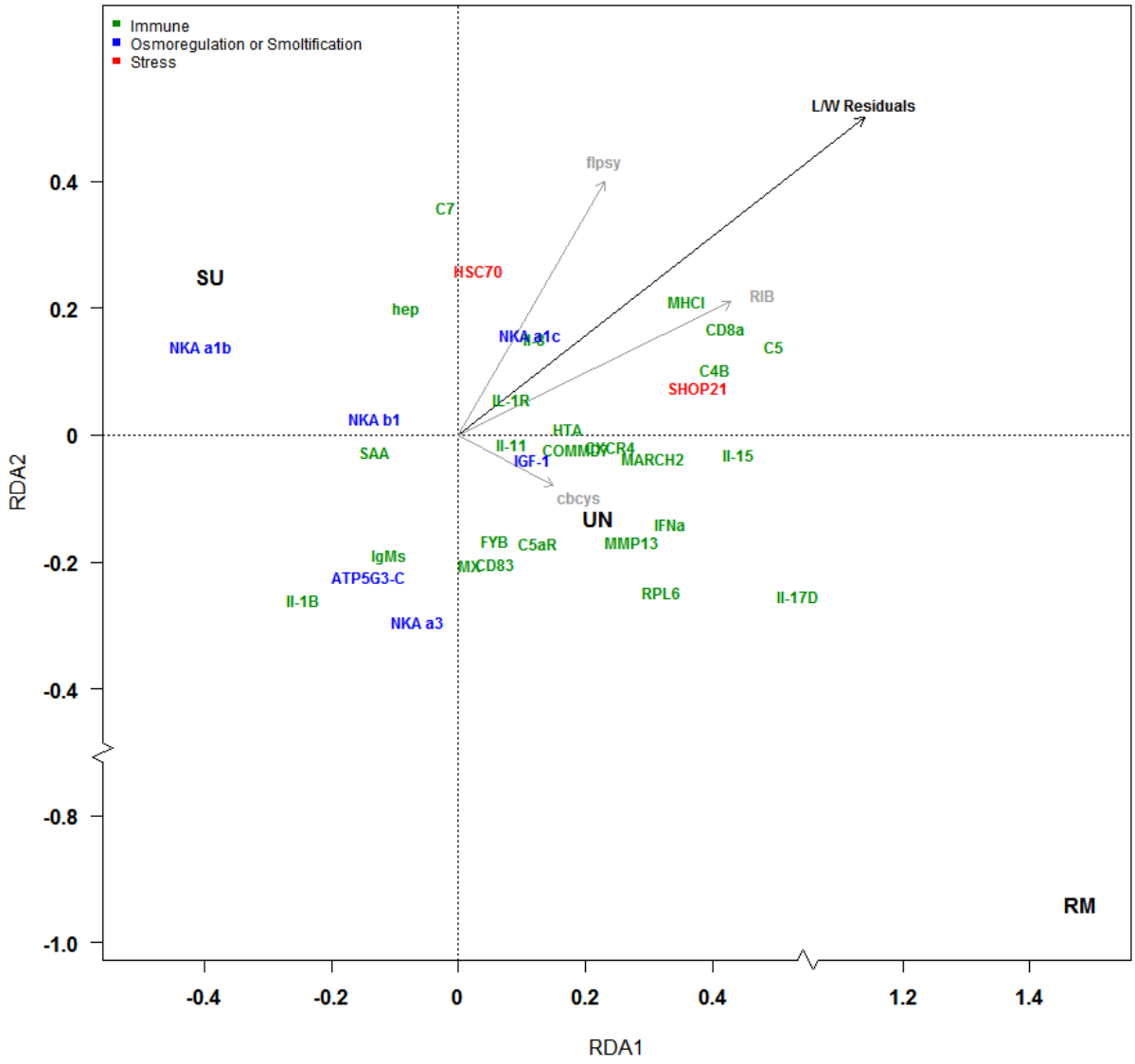

Figure 3.2: Redundancy analyses (RDA) ordination plot of Seymour River hatchery steelhead (*Oncorhynchus mykiss*) gene expression data from non-lethal gill biopsies. RDA1 and RDA2 were determined to be significant, and all significant covariates are in black, while non-significant variables ($p>0.05$) are in grey. Migration fate centroids are shown by: RM = "River mortalities" (river-released smolts never detected on the estuary receivers), SU = "Successful migrants" to at least the NSOG subarray (both release groups), UN = "Unsuccessful migrants" (both release groups not detected at, or beyond NSOG). Genes are coloured according to their primary known function from the available literature, however, many genes are known to have multiple physiological associations. Note: Both RDA1 and RDA2 have axes breaks.

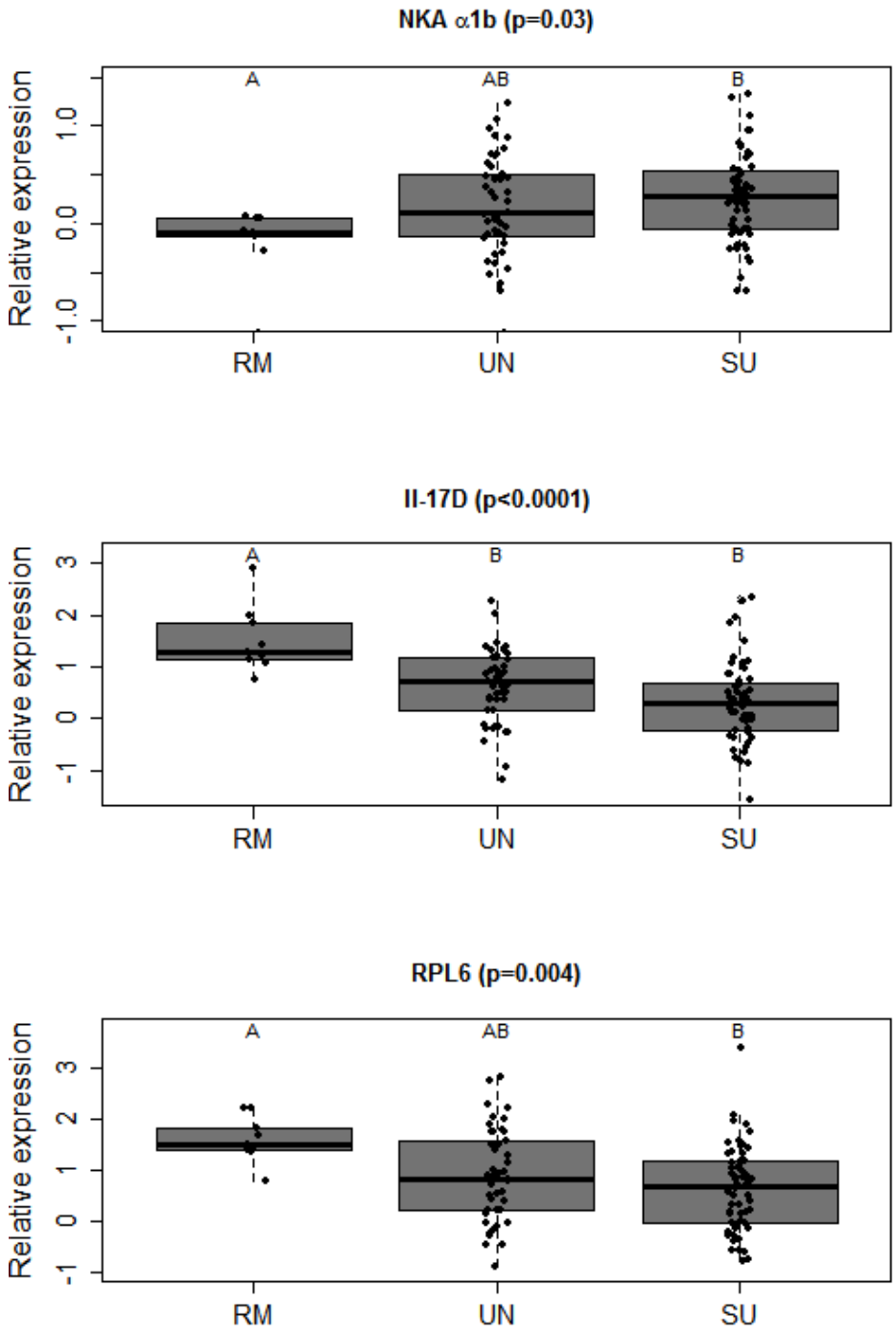

Figure 3.3: Boxplots of relative gene expression of three genes significant ($p < 0.05$) between migration fate groups. Individual hatchery steelhead (*Oncorhynchus mykiss*) smolts are shown by the black dots, while migration fate groupings are shown by the individual boxes. RM = "River mortalities" (or smolts never detected on the estuary receivers), SU = "Successful migrants" to at least the NSOG subarray, UN = "Unsuccessful migrants" (did not make it to NSOG). Different letters denote statistical significance between fate groups for each gene.

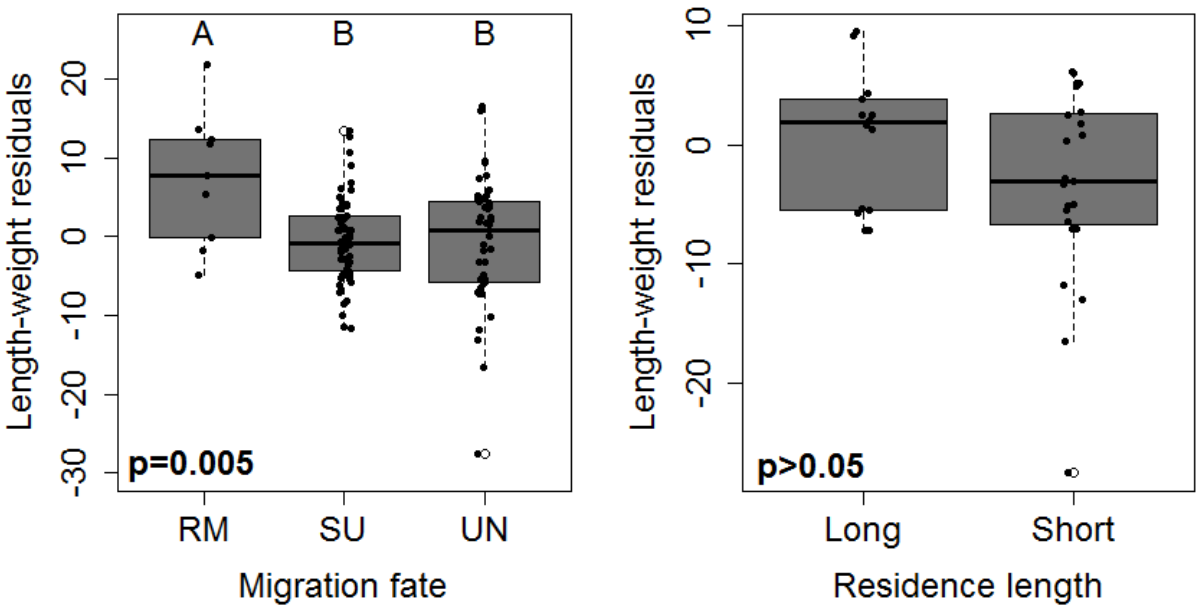

Figure 3.4: Relationship between length-weight residuals of hatchery steelhead (*Oncorhynchus mykiss*) smolts and migration fate (left). Estuary residence period (for just river-released smolts) is shown on the right by duration ('long' = >24 hrs; 'short'= <24 hrs; right). Letters above each migration fate group (left) shows statistical significance between groups. Each black point represents an individual smolt.

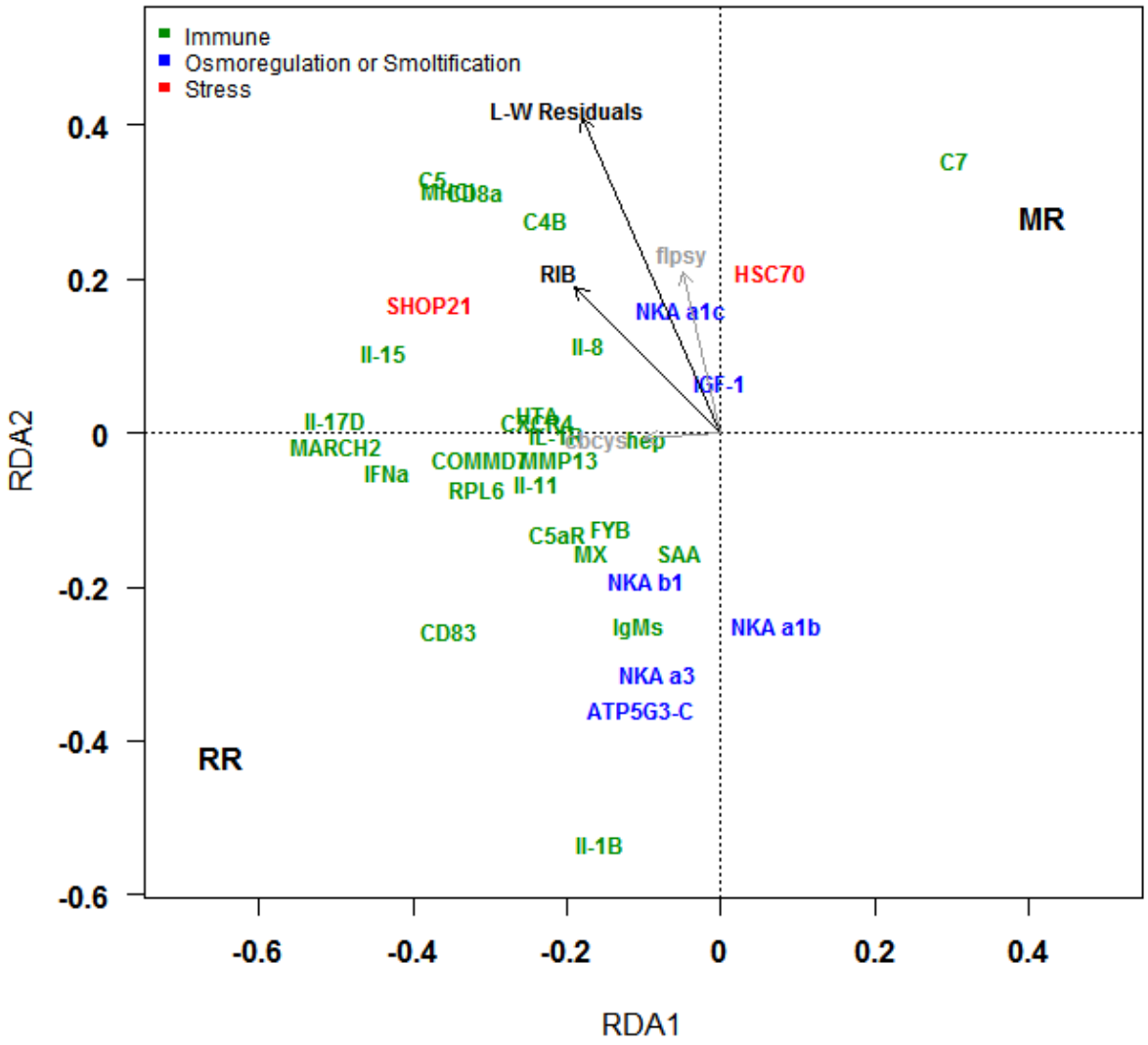

Figure 3.5: Redundancy analyses (RDA) ordination plot of Seymour River hatchery steelhead (*Oncorhynchus mykiss*) showing differences between release groups. RDA1 and RDA2 were determined to be significant, and all significant ($p<0.05$) covariates are in black, while non-significant variables are in grey. Migration release group centroids are shown by: MR = Marine-release and RR = River-release. Genes are coloured according to their primary known function from the available literature, however, many genes are known to have multiple physiological associations.

Chapter 4: Summary and conclusions

The migration from freshwater to the marine environment is a challenging and risky endeavour for Pacific salmonid (*Oncorhynchus spp.*) smolts. Individuals must undergo important physiological alterations to make the transition into marine systems, where they will reside for several years before returning to natal freshwater rearing areas as adults (Groot & Margolis 1991). During outmigration, numerous external/environmental factors have the potential to influence smolt movements and survival (Lawson et al. 2004, Thompson & Beauchamp 2014) including individual physiological condition (Evans et al. 2014) and disease (Jeffries et al. 2014, Miller et al. 2014). The influence of such factors on survival during this life stage is an understudied aspect of salmonid ecology. The migration from freshwater to the marine environments is typically associated with poor survival (Welch et al. 2009, Clark et al. 2016), and this important stage can be linked to recent declines in productivity in some species (Goetz et al. 2015, Kendall et al. 2017). Therefore, the need to identify and understand factors influencing outmigration survival will be critical for future conservation measures of stocks or species in decline.

My thesis identified multiple factors which can influence outmigration survival for hatchery steelhead smolts in coastal British Columbia. This work enhances our understanding of the ecology of outmigrant hatchery smolts in the Salish Sea, and may have broader implications for future management and conservation of salmonids in the Pacific Northwest. In Chapter 2, I used acoustic telemetry to assess and describe landscape-level survival and behaviour for migrating steelhead smolts. I used a marine and a freshwater release location, as well as modified Cormack-Jolly-Seber mark-recapture models to estimate survival across broad segments of migration (~400 km distance). The addition of new receiver arrays in 2015 allowed me to

identify route-specific movements and survival through the Discovery Islands. In Chapter 3, I investigated how smolt physiological condition relates to outmigration fate. To do this, I took non-lethal gill biopsies from tagged smolts and used high-throughput real-time quantitative polymerase chain reaction (HT-qRT-PCR) to assess samples for a suite of host genes related to stress, immune function, and osmoregulation. I also screened these samples for the presence of multiple infectious agents known or suspected to cause disease in salmonids, and successfully detected two in the population. Using redundancy analyses, I assessed how these gene expression profiles and infectious agents are linked to migration fate. Below, I summarize my findings, discuss how these results have increased our knowledge of factors influencing survival, and suggest how this work may help inform future studies and/or management of salmonids in the Pacific Northwest.

4.1 Burrard Inlet and the Seymour River

Survival for migratory species can vary spatially across landscapes (Sawyer et al. 2009, Furey et al. 2015, Hewson et al. 2016). Thus, understanding where mortality occurs is a necessary step in determining important regions to focus conservation and management efforts (Sawyer et al. 2009). In Chapter 2, I identified several landscapes of particularly poor survival for hatchery steelhead smolts from the Seymour River, North Vancouver. Specifically, the Seymour River and first estuarine inlet (Burrard Inlet) smolts encountered upon leaving freshwater were regions where survival was poor. I hypothesized that predation likely contributes to survival here due to the large densities of piscivorous predators in the region (Olesiuk 1999, Butler et al. 2015). Additionally, these high-risk landscapes identified by my work show similar spatial characteristics of those known to be exploited by predators in nearby

systems (e.g. Hostetter et al. 2012, Melnychuk et al. 2013, Evans et al. 2016). Further work is needed to identify important predators, as well as to understand the impacts that predation may have on population-level productivity (Berejikian et al. 2016, Thomas et al. 2016, Kendall et al. 2017). While it seems likely that predation contributes to these region-specific trends in survival, other factors likely also play a role. These include food availability, and the influence of anthropogenic activities including pollution or contaminants from the surrounding developed area. Future studies should focus on these high-risk landscapes, and could benefit from pairing acoustic telemetry with other spatiotemporal data sources such as primary productivity, temperature, contaminant concentrations, and predator densities and/or movements.

4.2 Hatchery practices and management

After a study published by Balfry et al. (2011), which hypothesized that Burrard Inlet was a region of poor survival, the Seymour Salmonid Society altered their hatchery release strategy. In an effort to increase adult recruitment to the Seymour River, managers altered their smolt release location from the lower Seymour River, to a saltwater site. At present, the hatchery loads one-year-old steelhead smolts into tanks on trucks, and releases them in West Vancouver at the same marine release site as the present study (Seymour Salmonid Society, *pers. comm.*). Using much larger sample sizes than Balfry et al. (2011), my work confirms that this strategy may be effective in enhancing the number of steelhead smolts making it to the open ocean by as much as three-fold relative to the historic release strategy. Caution should be taken with this strategy, however, as releasing smolts past Burrard Inlet could be associated with other negative ramifications. Increasing the number of smolts surviving to the open ocean may not necessarily equate to more returning adults. If predation is a large contributor to poor survival through the

river and Burrard Inlet, migration through these regions could act as a natural mechanism to remove poor quality individuals from the gene pool (Genovart et al. 2010, Hostetter et al. 2012). These individuals may otherwise die later on in the migration, as other factors such as food availability (Beamish & Mahnken 1999), competition (Daly et al. 2012), and productivity (Irvine & Fukuwaka 2011) can still act to limit survival during the offshore life stage.

Juveniles are thought to imprint on natal water cues (Ueda 2012), and sequential imprinting likely continues during active smolt outmigration (Keefer and Caudill 2014). Therefore, bypassing a potentially important first stage of migration (e.g. the river and Burrard Inlet), could result in decreased homing abilities (Gunnerod et al. 1988, Heggberget et al. 1991), and an increase in straying behaviour for returning adults (i.e. return migration to non-natal sites; Quinn & Dittman 1990). Since the hatchery began releasing smolts beyond Burrard Inlet, managers have noted an apparent increase in straying behaviour for returning adult Seymour steelhead (Seymour Salmonid Society, *pers. comm.*). These strays could have potential negative implications to the ecology and/or genetic diversity to nearby populations and freshwater systems (reviewed in Keefer & Caudill 2014). Therefore, the assumed benefit of releasing steelhead smolts beyond Burrard Inlet may not be worth the potential costs, and should be considered carefully by managers when co-ordinating hatchery releases. To determine if this release strategy is effective, larger acoustic tags that are programmed to transmit during the outgoing smolt and return adult migrations (e.g. Welch et al. 2009) could be used to study smolt to adult survival, and river return fidelity based on release location.

4.3 Route selection

Determining important migratory routes for salmonid smolts will be crucial for informing the spatial extent of industry development in coastal British Columbia. My work identified Discovery Passage as a particularly important route for Seymour hatchery steelhead, in terms of use and survival. Industries known to have the highest impacts on marine systems in Canada's Pacific waters include commercial fishing, land-based activities, and marine shipping traffic (Ban et al. 2010). These industries also have the potential to act as stressors for migrating smolts in the region. The identification of important migratory routes could help better inform future operations of these types of activities across migratory corridors. For example, there has been increasing concern in recent years regarding the possibility of disease transfer between salmon in open net-pen farms and migrating salmon (Johansen et al. 2011, Miller et al. 2014). Such interactions have been suggested to be one of the leading causes of wild salmonid declines in many regions of the world (Ford & Myers 2008), yet these impacts are largely unknown (Kent 2011, Miller et al. 2014). The Discovery Islands contain one of the largest open net-pen farming industries in the north Pacific (Price et al. 2011); however, farms are not spatially distributed evenly throughout the channels and fjords in the area (Foreman et al. 2015). While my work does not directly implicate fish farms in influencing survival for migrating steelhead smolts, it does imply that smolts may be at varying levels at risk to disease exposure from farms depending on migration routes taken through the region.

Little is known about smolt outmigration movements and behaviour in the coastal marine environment. My results suggest that the Discovery Islands are an important region of migration for which future studies should focus. Considering that factors operating during the smolt life stage are thought to be important for population productivity (Irvine and Akenhead 2013,

Kendall et al. 2017), migratory routes selected here could be linked to population-level impacts for salmonids. At present, what influences route selection and survival for smolts navigating through this portion of the migration is poorly understood. Understudied potential factors of interest include tides, currents, and the spatial distribution of predators and/or food. Repeating similar studies as the present will be necessary to determine if Discovery Passage remains an important migratory corridor across different years, species or stocks of salmonid smolts.

4.4 Smolt condition

Individual smolt condition can play an important role in migratory fate. Disease and immune responses can influence the success of smolts migrating through high-risk landscapes (Jeffries et al. 2014), however, these aspects of migrations are understudied. By combining acoustic telemetry with HT-qRT-PCR in Chapter 3, I have shown that gene expression profiles can be predictive of migration fate for hatchery steelhead smolts. Three genes (one osmoregulatory, and two immune function genes) were differentially expressed by river-released smolts never detected in the estuary. Redundancy analyses revealed that these smolts never detected in the estuary clustered furthest away from other fate groups in ordination space, and highlighted potentially important genes for future investigation. The present work did not find evidence that infectious agents had an influence on migration fate; however, the expression profiles of several immune genes could suggest infectious agents and/or disease play a role in smolt outmigration survival. At present, there are few empirical examples investigating the influence that disease and immune responses play in migrating juvenile salmonids (but see: Hostetter et al. 2012, Jeffries et al. 2014). Additionally, population-level monitoring for diseases in the Pacific Northwest is limited (Miller et al. 2014), particularly for species such as steelhead

(Smith & Ward 2000; Scheuerell et al. 2009). In order to more clearly determine any links between infectious agents and migration fate, future acoustic telemetry studies may benefit from screening for a wider array of infectious agents, and/or focusing on specific transcriptomal biomarkers indicative of disease states (Miller et al. 2017).

Identification of important physiological factors influencing smolt survival may help inform conservation, and improve managers' predictive capabilities for assessing stocks and/or adult returns. Smolt survival can vary substantially among years (Irvine and Akenhead 2013, Kendall et al. 2017), however, the proximate factors influencing survival during this period are poorly understood. Important physiological indicators (such as specific genes or infectious agents) which influence smolt migration fate could be incorporated into population models (e.g. Johnston et al. 2000), therefore enhancing our ability to estimate adult recruitment in subsequent years (Burke et al. 2013).

4.5 Summary

This thesis identified several important factors influencing migration survival for outmigrating hatchery steelhead smolts. My work highlights how landscape-level factors influence migratory fate for juvenile salmonids, and determined important migratory regions and routes for steelhead smolts in coastal British Columbia. Combining acoustic telemetry with novel genomic techniques, my work confirms that intrinsic factors can influence migratory fate, particularly during early freshwater portions of migration. Collectively, the results of this thesis enhance our understanding of factors which may influence population productivity, and adds to our knowledge of the migration ecology of salmonid smolts in the Pacific Northwest.

References

Alerstam, T., Hedenstro, A. and Susanne, A. 2003. Long-distance migration: evolution and determinants. Oikos, 103:247–260

Arkoosh, M.R., Kagley, A.N., Anulacion, B.F., Boylen, D.A., Sandford, B.P., Loge, F.J., and Collier, T.K. 2006. Disease susceptibility of hatchery Snake River spring-summer Chinook salmon with different juvenile migration histories in the Columbia River. Journal of Aquatic Animal Health 18: 223–231

Bakke, T.A., and Harris, P.D. 1998. Diseases and parasites in wild Atlantic salmon (*Salmo salar*) populations. Canadian Journal of Fisheries and Aquatic Sciences, 55:247–266

Balfry, S., Welch, D.W., Atkinson, J., Lill, A.,Vincent, S. 2011. The effect of hatchery release strategy on marine migratory behaviour and apparent survival of Seymour river steelhead smolts (*Oncorhynchus mykiss*). PLoS ONE 6:e14779

Ban, N. C., Alidina, H.M., and Ardron, J.A. 2010. Cumulative impact mapping: Advances, relevance and limitations to marine management and conservation, using Canada's Pacific waters as a case study. Marine Policy 34:876–886

Bass, A.L., Hinch, S.G., Teffer, A.K., Patterson, D.A., and Miller, K.M. 2017. Survey of microparasites present in adult migrating Chinook salmon (*Oncorhynchus tshawytscha*) in southwestern British Columbia determined by high-throughput quantitative polymerase chain reaction. Journal of Fish Diseases 40:453:477

Beamish, R.J., and Mahnken, C. 2001. A critical size and period hypothesis to explain natural regulation of salmon abundance and the linkage to climate and climate change. Progress in Oceanography, 49:423–437

Beamish, R.J., Mahnken, C., and Neville, C.M. 2004. Evidence that reduced early marine growth is associated with lower marine survival of coho salmon. Transactions of the American Fisheries Society, 133: 26–33

Beamish, R.J., and MacFarlane, G. 2014. The Sea Among Us: The Strait of Georgia. Harbour Publishing, Madeira Park, B.C.

Beamish, R.J., and Sweeting, R.M. 2009. Spiny Dogfish in the Pelagic Waters of the Strait of Georgia and Puget Sound. In: Biology and Management of Dogfish Sharks. American Fisheries Society, Bethesda, Maryland, p 110–118.

Beamish, R.J., Noakes, D.J., McFarlane, G.A., Sweeting, R., King, J., and Pinnix, W. 2000. Trends in coho marine survial in relation to the regime concept. Fisheries Oceanography 9:114–119

Beamish, R.J., Thomson, B.L., and McFarlane, G.A. 1992. Spiny dogfish predation on chinook and coho salmon and the potential effects on hatchery-produced salmon. Transactions of the American Fisheries Society 121:444–455

Beckman, B.R., Larsen, D.A., Moriyama, S., Lee-Pawlak, B., and Dickhoff, W.W. 1998. Insulin-like growth factor-I and environmental modulation of growth during smoltification of spring chinook salmon (*Oncorhynchus tshawystscha*). General and Comparative Endocrinology, 109:325–335

Beeman, J.W., and Maule, A.G. 2006. Migration depths of juvenile chinook salmon and steelhead relative to total dissolved gas supersaturation in a Columbia River reservoir. Transactions of the American Fisheries Society 135:584–594

Benedict, A.D., and Gaydos, J.K. 2015. The Salish Sea. Jewel of the Pacific Northwest. Sasquatch Books, Seattle, WA.

Berejikian, B.A., Moore, M.E., and Jeffries, S.J. 2016. Predator-prey interactions between harbor seals and migrating steelhead trout smolts revealed by acoustic telemetry. Marine Ecology Progress Series 543:21–35

Borcard, D., Gillet, F., and Legendre, P. 2011. Numerical Ecology with R. Springer. NY

Booker, D.J., Wells, N.C., and Smith, I.P. 2008. Modelling the trajectories of migrating Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 65:352–361

Boone, A.N., and Vijayan, M.M. 2002. Constitutive heat shock protein 70 (HSC70) expression in rainbow trout hepatocytes: Effect of heat shock and heavy metal exposure. Comparative Biochemistry and Physiology Part C 132:223–233.

Bradford, M.J., Lovy, J., Patterson, D.A., Speare, D.J., Bennett, W.R., Stobbart, A.R., and Tovey, C.P. 2010. Parvicapsula minibicornis infections in gill and kidney and the premature mortality of adult sockeye salmon (*Oncorhynchus nerka*) from Cultus Lake, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 67:673–683

Buchanan, R.A., Skalski, J.R., Brandes, P.L., and Fuller, A. 2013. Route use and survival of juvenile Chinook salmon through the San Joaquin River Delta. North American Journal of Fisheries Management 33:216–229

Buck, J.C., and Ripple, W.J. 2017. Infectious agents trigger trophic cascades. Trends in Ecology & Evolution 32:681-694

Burke, B.J., Peterson, W.T., Beckman, B.R., Morgan, C., Daly, E.A. and Litz, M. 2013. Multivariate models of adult Pacific Salmon returns. PLoS ONE 8: e54134

Butler, R.W., Couturier, A.R., and Dickson, E. 2015. Status and distribution of marine birds and mammals in Burrard Inlet and Indian Arm, British Columbia 2011-13. Pacific Wildlife Foundation & Bird Studies Canada. Port Moody, BC and Port Rowan, Ontario.

Bystriansky, J.S. 2006. Reciprocal expression of gill Na+/K+-ATPase -subunit isoforms 1a and 1b during seawater acclimation of three salmonid fishes that vary in their salinity tolerance. Journal of Experimental Biology 209:1848–1858.

Castro, R., Zou, J., Secombes, C.J., and Martin, S.A.M. 2011. Cortisol modulates the induction of inflammatory gene expression in a rainbow trout macrophage cell line. Fish and Shellfish Immunology 30(1):215–223.

Chapman, J.W., Bell, J.R., Burgin, L.E., Reynolds, D.R., Pettersson, L.B., Hill, J.K., Bonsall, M.B., and Thomas, J.A. 2012. Seasonal migration to high latitudes results in major reproductive benefits in an insect. Proceedings of the National Academy of Sciences of the United States of America 109:14924–14929

Chapman, E.D., Hearn, A.R., Michel, C.J., Ammann, A.J., Lindley, S.T., Thomas, M.J., Sandstrom, P.T., Singer, G.P., Peterson, M.L., MacFarlane, R.B., and Klimley, A.P. 2013. Diel movements of out-migrating Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) smolts in the Sacramento/San Joaquin watershed. Environmental Biology of Fishes 96:273–286

Chapman, B.B., Hulthen, K., Wellenreuther, M., Hansson, L.A., Nilsson, J.A., and Bronmark, C. 2014. Patterns of animal migration. In: Hansson LA, Akesson S (eds) Animal movement across scales. Oxford University Press, Oxford, UK, p 11-35

Chase, R., Hemphill, N., Beeman, J., Juhnke, S., Hannon, J., and Jenkins, A.M. 2013. Assessment of juvenile coho salmon movement and behavior in relation to rehabilitation efforts in the Trinity River, California, using PIT tags and radiotelemetry. Environmental Biology of Fishes 96:303–314

Clark, T.D., Furey, N.B., Rechisky, E.L., Gale, M.K., Jeffries, K.M., Porter, A.D., Casselman, M.T., Lotto, A.G., Patterson, D.A., Cooke, S.J., Farrell, A.P., Welch, D.W., and Hinch, S.G. 2016. Tracking wild sockeye salmon smolts to the ocean reveals distinct regions of nocturnal movement and high mortality. Ecological Applications 26:959-978

Cohen, B.I. 2012). Inquiry into the decline of sockeye salmon in the Fraer River - final report. Public Works and Government Services Canada, Ottawa. http://publications.gc.ca/site/eng/432516/publication.html

Collins, A.L., Hinch, S.G., Welch, D.W., Cooke, S.J., and Clark, T.D. 2013. Intracoelomic acoustic tagging of juvenile sockeye salmon: swimming performance, survival, and postsurgical wound healing in freshwater and during a transition to seawater. Transactions of the American Fisheries Society 142:515–523

Collis, K., Roby, D.D., Craig, D.P., Ryan, B.A., and Ledgerwood, R.D. 2001. Colonial waterbird predation on juvenile salmonids tagged with passive integrated transponders in the Columbia river estuary: vulnerability of different salmonid species, stocks, and rearing types. Transactions of the American Fisheries Society 130: 385–396.

Cooke, S.J., Iverson, S.J., Stokesbury, M.J.W., Hinch, S.G., Fisk, A.T., VanderZwaag, D.L., Apostle, R., and Whoriskey, F. 2011. Ocean tracking network Canada: a network approach to addressing critical issues in fisheries and resource management with implications for ocean governance. Fisheries 36:583–592

Cormack, R.M. 1964. Estimates of survival from the sighting of marked animals. Biometrika 51:429–438

Daly, E.A., Scheurer, J.A., Brodeur, R.D., Weitkamp, L.A., Beckman, B.R., and Miller, J.A. 2014. Juvenile steelhead distribution, migration, feeding, and growth in the Columbia River estuary, plume, and coastal waters. Marine and Coastal Fisheries 6:62–80

Decostere, A., Lammens, M., & Haesebrouck, F. 2000. Difficulties in experimental infection studies with *Flavobacterium psychrophilum* in rainbow trout (*Oncorhynchus mykiss)* using immersion, oral and anal challenges. Research in Veterinary Science 69:165–169

Decostere, A., Haese, E.D., Lammens, M., Nelis, H., and Haesebrouck, F. 2001. In vivo study of phagocytosis, intracellular survival and multiplication of *Flavobacterium psychrophilum* in rainbow trout, *Oncorhynchus mykiss* (Walbaum), spleen phagocytes. Journal of Fish Diseases 24:481–487.

Diercks, A., Kostner, H., and Ozinsky, A. 2009. Resolving cell population heterogeneity: real-time PCR for simultaneous multiplexed gene detection in multiple single-cell samples. PLoS ONE, 4: e6326

Drenner, S.M., Clark, T.D., Whitney, C.K., Martins, E.G., Cooke, S.J., and Hinch, S.G. 2012. A synthesis of tagging studies examining the behaviour and survival of anadromous salmonids in marine environments. PLoS ONE 7:1–13.

Du, L., Qin, L., Wang, X., Zhang, A., Wei, H., and Zhou, H. 2014. Characterization of grass carp (*Ctenopharyngodon idella*) IL-17D: molecular cloning, functional implication and signal transduction. Developmental and Comparative Immunology, 42:220–228.

Eder, K.J., Leutenegger, C.M., Köhler, H.R., and Werner, I. 2009. Ecotoxicology and environmental safety effects of neurotoxic insecticides on heat-shock proteins and cytokine transcription in Chinook salmon (*Oncorhynchus tshawytscha*). Ecotoxicology and Environmental Safety 72:182–190

Engish, K.K., Koski, W.R., Sliwinski, C., Blakley, A., Cass, A.,Woodey, J.C. 2005. Migration timing and river survival of late-run Fraser River sockeye salmon estimated using

radiotelemetry techniques. Transactions of the American Fisheries Society 134:1342–1365

Evans, T.G., Hammil, E., Kaukinen, K., Schulze, A.D., Patterson, D.A., English, K.K., and Miller, K.M. 2011. Transcriptomics of environmental acclimatization and survival in wild adult Pacific sockeye salmon (*Oncorhynchus nerka*) during spawning migration. Molecular Ecology 20: 4472–4489

Evans, A.F., Hostetter, N.J., Collis, K., Roby, D.D., and Loge, F.J. 2014. Relationship between juvenile fish condition and survival to adulthood in steelhead. Transactions of the American Fisheries Society 143:899–909

Evs, A.F., Payton, Q., Turecek, A., Cramer, B., Collis, K., Roby, D.D., Loschl, P.J., Sullivan, L., Skalski, J., Weiland, M., and Dotson, C. 2016. Avian predation on juvenile salmonids: spatial and temporal analysis based on acoustic and passive integrated transponder tags. Transactions of the American Fisheries Society 145:860–877

Ford, J.S. and Myers, R. A. 2008. A global assessment of salmon aquaculture impacts on wild salmonids. PLoS Biology 6:411–417

Forman, M.G.G., Stucchi, D., Garver, K.A., Tuele, D., Isaac, J., Grime, T., Guo, M., and Morrison, J. 2012. A circulation model for the Discovery Islands, British Columbia. Atmosphere-Ocean 50:301–316

Friedland, K.D., Ward, B.R., Welch, D.W., and Hayes, S.A. 2014. Postsmolt growth and thermal regime define the marine survival of steelhead from the Keogh River, British Columbia. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 6:1–11.

Fuey, N.B., Hinch, S.G., Bass, A.L., Middleton, C.T., Minke-Martin, V., Lotto, A.G. 2016. Predator swamping reduces predation risk during nocturnal migration of juvenile salmon in a high-mortality landscape. Journal of Animal Ecology 85:948-959

Furey, N.B., Vincent, S.P., Hinch, S.G., Welch, D.W. 2015. Variability in migration routes influences early marine survival of juvenile salmon smolts. PLOS ONE. 10:e0139269

Fuss, H.J., and Hopley, C.W. 2003. Gill Na+,K+-ATPase activity of hatchery chum salmon fry during freshwater rearing and acclimation to brackish water and its relationship to marine survival. North American Journal of Aquaculture, 65:134–140

Ganz, T. 2012. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 102:783–788

Genovart, M., Negre, N., Tavecchia, G., Bistuer, A., Parpal, L. and Oro, D. .2010. The young, the weak and the sick: evidence of natural selection by predation. PLoS ONE 5:1–5

Goetz, F.A., Jeanes, E., Moore, M.E., and Quinn, T.P. 2015. Comparative migratory behavior and survival of wild and hatchery steelhead (*Oncorhynchus mykiss*) smolts in riverine, estuarine, and marine habitats of Puget Sound, Washington. Environmental Biology of Fishes 98:357-375

Gonzalez, S.F., Chatziandreou, N., Nielsen, M.E., Li, W., Rogers, J., Taylor, R., and Cossins, A. 2007. Cutaneous immune responses in the common carp detected using transcript analysis. Molecular Immunology 44:1664–1679

Groot, C, Margolis, L. 1991. Pacific Salmon Life Histories. UBC Press, Vancouver British Columbia

Gscheng, M., Kalko, E.K.V., Querner, U., Fiedler, W., and Berthold, P. 2008. All across Africa: highly individual migration routes of Eleonora's falcon. Proceedings of the Royal Society B-Biological Sciences 275:2887–2896

Gunnarsson, G.S., Karlsbakk, E., Blindheim, S., Plarre, H., Imsland, A.K., and Handeland, S.

2017. Temporal changes in infections with some pathogens associated with gill disease in farmed Atlantic salmon (*Salmo salar* L). Aquaculture 468:126–134
Gunnerod, T.B., Hvidsten, N.A. and Heggberget, T. 1988. Open sea releases of Atlantic salmon smolts, *Salmo salar*, in central Norway, 1973-83. Canadian Journal of Fisheries and Aquatic Science 45:1340–1345
Hanson, K.C., Gale, W.L., Simpson, W.G., Kennedy, B.M., and Ostrand, K.G. 2011. Physiological characterization of hatchery-origin juvenile steelhead *Oncorhynchus mykiss* adopting divergent life-history strategies. Journal of Fish and Wildlife Management *2*:61–71
Hartman, K., Howell, J., and Semmens, K. 2012. Habitat use, survival, and site fidelity of rainbow trout stocked into an Appalachian river. Journal of Applied Aquaculture 24:299–315
Hartt, A.C., and Dell, M.B. 1986. Early oceanic migrations and growth of juvenile Pacific salmon and steelhead trout. Bulletin number 46. International North Pacific Fisheries Commission, Vancouver
Harvey, B.C., White, J.L., and Nakamoto, R.J. 2005. Habitat-specific biomass, survival, and growth of rainbow trout (*Oncorhynchus mykiss*) during summer in a small coastal stream. Canadian Journal of Fisheries & Aquatic Sciences 62:650–658
Hasch, S.J., and Melnychuk, M.C. 2012. Residualization of hatchery steelhead: a meta-analysis of hatchery practices. North American Journal of Fisheries Management 32:905–921
Havird, J.C., Henry, R.P., and Wilson, A.E. 2013. Altered expression of Na+/K+-ATPase and other osmoregulatory genes in the gills of euryhaline animals in response to salinity transfer: a meta-analysis of 59 quantitative PCR studies over 10 years. Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics 8:131–140
Hays, G.C., Kennedy, H., and Frost, B.W. 2001. Individual variability in diel vertical migration of a marine copepod: why some individuals remain at depth when others migrate. Limnology and Oceanogrraphy 46:2050–2054
Healy, S.J., Hinch, S.G., Porter, A.D., Rechisky, E.L., Welch, D.W., Eliason, E.J., Lotto, A.G., and Furey, N.B. 2017. Route-specific movements and survival during early marine migration of hatchery steelhead *Oncorhynchus mykiss* smolts in coastal British Columbia. Marine Ecology Progress Series 577:131–147
Hedger, R.D., Martin, F., Hatin, D., Caron, F., Whoriskey, F.G., and Dodson, J.J. 2008. Active migration of wild Atlantic salmon *Salmo salar* smolt through a coastal embayment. Marine Ecology Progress Series 355:235–246
Heggberget, T.G., Hvidsten, N.A., Gunnerød, T.B. and Møkkelgjerd, P.I. 1991. Distribution of adult recaptures from hatchery-reared Atlantic salmon (*Salmo salar*) smolts released in and off-shore of the River Surna, western Norway. Aquaculture 98:89–96
Held, M.B.E., and Harley, C.D.G. 2009. Responses to low salinity by the sea star *Pisaster ochraceus* from high- and low-salinity popoulations. Invertebrate Biology2 128:381–390
Henriksen, M.M.M., Kania, P.W., Buchmann, K., and Dalsgaard, I. 2015a. Effect of hydrogen peroxide and/or *Flavobacterium psychrophilum* on the gills of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 38:259–270
Henriksen, M.M.M., Kania, P.W., Buchmann, K., and Dalsgaard, I. 2015b. Evaluation of the immune response in rainbow trout fry, *Oncorhynchus mykiss* (Walbaum), after waterborne exposure to *Flavobacterium psychrophilum* and/or hydrogen peroxide. Journal of Fish Diseases 38:55–66

Hertz, E., Trudel, M., Tucker, S., Beacham, T.D., Parken, C., Mackas, D., and Mazumder, A. 2016. Influences of ocean conditions and feeding ecology on the survival of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*). Fisheries Oceanography 25:407– 419

Hewson, C.M., Thorup, K., Pearce-Higgins, J.W., Atkinson, P.W. 2016. Population decline is linked to migration route in the Common Cuckoo. Nature Communications 7:12296

Hoenig, J.M. Groner, M.L., Smith, M.W., Vogelbein, W.K., Taylor, D.M., Landers, D.F., Swenarton, J., Gauthier, D.T., Sadler, P., Matsche, M., Haines, A., Small, H.J., Pradel, R., Choquet, R., and Shields, J.D. 2016. Impact of disease on the survival of three commercially fished species. Ecological Applications 38:42–49

Holyoak, M., Casagrandi, R., Nathan, R., Revilla, E., and Spiegel, O. 2008. Trends and missing parts in the study of movement ecology. Proceedings of the National Academy of Sciences 105:19060–19065

Hostetter, N. J., Evans, A. F., Roby, D. D., and Collis, K. 2012. Susceptibility of juvenile steelhead to avian predation: the influence of individual fish characteristics and river conditions. Transactions of the American Fisheries Society 141:1586–1599

Hostetter, N.J., Evans, A.F., Roby, D.D., Collis, K., Hawbecker, M., Sandford, B.P., Thompson, D.E., and Loge, F.J. 2011. Relationship of external fish condition to pathogen prevalence and out-migration survival in juvenile steelhead. Transactions of the American Fisheries Society 140:1158–1171

Igota, H., Sakuragi, M., Uno, H., Kaji, K., Kaneko, M., Akamatsu, R., and Maekawa, K. 2004. Seasonal migration patterns of female sika deer in eastern Hokkaido, Japan. Ecological Research 19:169-178

Irvne, J.R., and Akenhead, S.A. 2013. Understanding smolt survival trends in sockeye salmon. Marine and Coastal Fisheries 5:303–328

Irvie, J.R., and Fukuwaka, M.A. 2011. Pacific salmon abundance trends and climate change. ICES Journal of Marine Science 68:1122–1130

Jeffies, K.M., Hinch, S.G., Gale, M.K., Clark, T.D., Lotto, A.G., Casselman, M.T., Li, S., Rechiky, E.L., Porter, A.D., Welch, D.W., and Miller, K.M. 2014. Immune response genes and pathogen presence predict migration survival in wild salmon smolts. Molecular Ecology 23:5803–5815

Jolly, G.M. 1965. Explicit estimates from capture-recapture data with both death and immigration-stochastic model. Biometrika 52:225–47

Kendall, N.W., Marston, G.W., and Klungle, M.M. 2017. Declining patterns of Pacific Northwest steelhead trout (*Oncorhynchus mykiss*) adult abundance and smolt survival in the ocean. Canadian Journal of Fisheries and Aquatic Sciences 16:1–16

Kendall, N.W., Mcmillan, J.R., Sloat, M.R., Buehrens, T.W., Quinn, T.P., Pess, G.R.,Kuzischin, K.V., McClure, M.M., and Zabel, R.W. 2015. Anadromy and residency in steelhead and rainbow trout (*Oncorhynchus mykiss*): a review of the processes and patterns, Canadian Journal of Fisheries and Aquatic Sciences 72:319–342

Khimmakthong, U., Deshmukh, S., Chettri, J.K., Bojesen, A.M., Kania, P.W., Dalsgaard, I., and Buchmann, K. 2013. Tissue specific uptake of inactivated and live *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*): Visualization by immunohistochemistry and in situ hybridization. Microbial Pathogenesis, 59–60:33–41

Komatsu, K., Tsutsui, S., Hino, K., Araki, K., Yoshiura, Y., Yamamoto, A., Nakamura, O., and Watanabe, T. 2009. Expression profiles of cytokines released in intestinal epithelial cells of the rainbow trout, *Oncorhynchus mykiss*, in response to bacterial infection.

Developmental and Comparative Immunology 33:499-506
Krasnov, A., Skugor, S., Todorcevic, M., Glover, K. A., & Nilsen, F. (2012). Gene expression in Atlantic salmon skin in response to infection with the parasitic copepod Lepeophtheirus salmonis, cortisol implant, and their combination. *BMC Genomics*, *13*(1), 130. http://doi.org/10.1186/1471-2164-13-130
Kusakabe, M., Ishikawa, A., Ravinet, M., Yoshida, K., Makino, T., Toyoda, A., Fujiyama, A., and Kitano, J. 2017. Genetic basis for variation in salinity tolerance between stickleback ecotypes. Molecular Ecology 26:304–319
Laake, J. 2013. RMark: an R interface for analysis of capture-recapture data with MARK. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center, Seattle WA.
La, V.T., and Cooke, S.J. 2011. Advancing the science and practice of fish kill investigations. Reviews in Fisheries Science 19:21–33
Lafferty, K.D., Harvell, C.D., Conrad, J.M., Friedman, C.S., Kent, M.L., Kuris, A.M., Powell, E.N., Rondeau, D., and Saksida, S.M. 2015. Infectious diseases affect marine fisheries and aquaculture economics. Annual Review of Marine Science *7*:471–496
Legendre, P., Oksanen, J., and ter Braak, C.J.F. 2011. Testing the significance of canonical axes in redundancy analysis. Methods in Ecology and Evolution 2:269–277
Lewis, J., Hori, T., and Rise, M. 2010. Transcriptome responses to heat stress in the nucleated red blood cells of the rainbow trout (*Oncorhynchus mykiss*). Physiol Genomics 42:361–373
Livak, K. J., and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods 25:402–408
Madetoja, J., Dalsgaard, I., Wiklund, T., and Hou, C.T. 2002. Occurrence of *Flavobacterium psychrophilum* in fish-farming environments. Diseases of Aquatic Organisms 52:109–118
Marnelli-Liedtke, T.L., Shively, R.S., Holmberg, G.S., Sheer, M.B., and Schrock, R.M. 1999. Nonlethal gill biopsy does not affect juvenile Chinook salmon implanted with radio transmitters. North American Journal of Fisheries Management 19:856–859
McCormick, S., and Saunders, R.L. 1987. Preparatory physiological adaptations for marine life in salmonids: osmoregulation, growth and metabolism. American Fisheries Society Symposium 1:211–229.
McCormick, S.D., Lerner, D.T., Monette, M.Y., Nieves-Puigdoller, K., Kelly, J.T., and Björnsson, B.T. 2009. Taking it with you when you go: how perturbations to the freshwater environment, including temperature, dams, and contaminants, affect marine survival of salmon. American Fisheries Society Symposium 69:195–214
Melnychuk, M.C., Christensen, V., Walters, C.J. 2013. Meso-scale movement and mortality patterns of juvenile coho salmon and steelhead trout migrating through a coastal fjord. Environmental Biology of Fishes 96:325–339
Melychuk, M.C., Welch, D.W., Walters, C.J. 2010. Spatio-temporal migration patterns of Pacific salmon smolts in rivers and coastal marine waters. PLOS ONE 5:e12916
Melnychuk, M.C., Welch, D.W., Walters, C.J., Christensen, V. 2007. Riverine and early ocean migration and mortality patterns of juvenile steelhead trout (*Oncorhynchus mykiss*) from the Cheakamus River, British Columbia. Hydrobiologia 582:55–65
Mesa, M.G. 1994. Effects of multiple acute stressors on the predator avoidance ability and physiology of juvenile Chinook Salmon. Transactions of the American Fisheries Society

123:786–793.
Metcalfe, J.D., Arnold, G.P. 1997. Tracking fish with electronic tags. Nature 387:665-666
Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Pihl, T.P.B., Klitgaard, K., Bodker, R., Fach, P., and Moutailler, S. 2014. High-throughput screening of tick-borne pathogens in Europe. Frontiers in Cellular and Infection Microbiology 4:1–13
Miller, K.M., Gardner, I.A., Vanderstichel, R., Burnley, T., Angela, D., Li, S., Tabata, A., Kaukinen, K.H., Ginther, N.G. 2016. Report on the Performance Evaluation of the Fluidigm BioMark Platform for High- Throughput Microbe Monitoring in Salmon. Fisheries and Oceans Canada Canadian Sciene Advisory Secretariat, Nanaimo, British Columbia
Miller, K.M., Li, S., Kaukinen, K.H., Ginther, N., Hammill, E., Curtis, J.M.R., Patterson, D.A., Sierocinski, T., Donnison, L., Pavlidis, P., Hinch, S.G., Hruska, K.A., Cooke, S.J., English, K.K., and Farrell, A.P. 2011. Genomic signatures predict migration and spawning failure in wild Canadian salmon. Science 331:214–217
Miller, K.M., Gunther, O., Li, S., Kaukinen, K., and Ming, T.J. 2017. Molecular indices of viral disease development in wild migrating salmon. Conservation Physiology 5:1–67
Miller, K.M., Schulze, A.D., Ginther, N., Li, S., Patterson, D.A., Farrell, A.P., and Hinch, S.G. 2009. Salmon spawning migration: metabolic shifts and environmental triggers. Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics 4:75–89
Miller, K.M., Teffer, A., Tucker, S., Li, S., Schulze, A.D., Trudel, M., Juanes, F., Tabata, A., Kaukinen, K.H., Ginther, N.G., Ming, T.J., Cooke, S.J., Hipfner, M., Patterson, D.A., and Hinch, S.G. 2014. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evolutionary Applications 7:812–855
Moore, M., Berejikian, B.A., and Tezak, E.P. 2012. Variation in the early marine survival and behavior of natural and hatchery-reared hood canal steelhead. PLOS ONE 7:e49645
Moore, M.E., Berejikian, B.A., and Tezak, E.P. 2010. Early marine survival and behavior of steelhead smolts through Hood Canal and the Strait of Juan de Fuca. Transactions of the American Fisheries Society 139:49–61
Mork, K.A., Gilbey, J., Hansen, L.P., Jensen, A.J., Jacobsen, J.A., Vikebø, F., Mcginnity, P., Holm, M., Holst, J.C., Niall, O., Melle, W., Thomas, K., Verspoor, E., and Wennevik, V. 2012. Modelling the migration of post-smolt Atlantic salmon (*Salmo salar*) in the Northeast Atlantic. ICES Journal of Marine Science 69:1616-1624
Nathan, R., Getz, W.M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., and Smouse, P.E. 2008. A movement ecology paradigm for unifying organismal movement research. Proceedings of the National Academy of Sciences of the United States of America 105:19052–19059
Nematollahi, A., Decostere, A., Pasmans, F., and Haesebrouck, F. (2003). *Flavobacterium psychrophilum* infections in salmonid fish. Journal of Fish Diseases, 26:563–574.
Neville, C.M., Beamish, R.J., Chittenden, C.M. 2015. Poor survival of acoustically-tagged juvenile Chinook Salmon in the Strait of Georgia, British Columbia, Canada. Transactions of the American Fisheries Society 144:25–33
Newman, K.B., and Brandes, P.L. 2010. Hierarchical modeling of juvenile Chinook Salmon survival as a function of Sacramento–San Joaquin Delta water exports. North American Journal of Fisheries Management 30:157–169

Nichols, K.M., Edo, A.F., Wheeler, P.A., and Thorgaard, G.H. 2008. The genetic basis of smoltification-related traits in *Oncorhynchus mykiss*. Genetics 179:1559–1575

Nilsen, T.O., Ebbesson, L.O.E., Madsen, S.S., McCormick, S.D., Andersson, E., Bjornsson, B., Prunet, P.T., and Stefansson, S.O. (2007). Differential expression of gill Na+,K+-ATPase - and -subunits, Na+,K+,2Cl- cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon *Salmo salar*. Journal of Experimental Biology 210:2885–2896

Niu, C.J., Rummer, J.L., Brauner, C.J., and Schulte, P.M. 2008. Heat shock protein (Hsp70) induced by a mild heat shock slightly moderates plasma osmolarity increases upon salinity transfer in rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology - C Toxicology and Pharmacology, 148:437–444

Oksanen, J., Kindt, R., Legendre, P., O'Hara, and Stevens, M.H. (2008). The vegan package. Community Ecology Package, 190. Retrieved from https://bcrc.bio.umass.edu/biometry/images/8/85/Vegan.pdf

Olesiuk, P.F. 1999. An assessment of the status of harbour seals (*Phoca vitulina*) in British Columbia. Fisheries and Oceans Canada, Nanaimo.

Osterback, A.M.K., Frechette, D.M., Hayes, S.A., Bond, M.H., Shaffer, S.A., and Moore, J.W. 2014. Linking individual size and wild and hatchery ancestry to survival and predation risk of threatened steelhead (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 71:1877–1887

Peña, M.A., Masson, D., and Callendar, W. 2016. Annual plankton dynamics in a coupled physical–biological model of the Strait of Georgia, British Columbia. Progress in Oceanography 146:58–74

Perry, R.W., Brandes, P.L., Burau, J.R., Klimley, A.P., MacFarlane, B., Michel, C., and Skalski, J.R. 2013. Sensitivity of survival to migration routes used by juvenile Chinook salmon to negotiate the Sacramento-San Joaquin River Delta. Environmental Biology of Fishes 96:381–392

Perry, R.W., Skalski, J.R., Brandes, P.L., Sandstrom, P.T., Klimley, A.P., Ammann, A., and MacFarlane, B. 2010. Estimating Survival and Migration Route Probabilities of Juvenile Chinook Salmon in the Sacramento–San Joaquin River Delta. North American Journal of Fisheries Management 30:142–156

Peterman, R.M., Marionone, S.G., Thomson, K.A., Jardine, I.D., Crittenden, R.N., Leblond, P.H., and Walters, C.J. 1994. Simulation of juvenile sockeye salmon (*Oncorhynchus nerka*) migrations in the Strait of Georgia, British Columbia. Fisheries Oceanography 3:221-235

Porter, A.D., Rechisky, E.L., Winchell, P.M. and Welch, D.W. 2016. Final report on the comparative marine survival of Seymour River steelhead and testing the performance of 180 kHz small acoustic tags in the Salish Sea, 2015. Report to the Pacific Salmon Foundation and the Salish Sea Marine Survival Project. Nanaimo, British Columbia:

Project, S. S. M. S. (2017). Salish Sea Marine Survival Project: The Project. Retrieved May 20, 2015 from www.marinesurvivalproject.com

Putman, N.F., Meinke, A.M., Noakes, D.L.G. 2014a. Rearing in a distorted magnetic field disrupts the "map sense" of juvenile steelhead trout. Biology Letters 10:20140169

Putman, N.F., Scanlan, M.M., Billman, E.J., O'Neil, J.P., Couture, R.B., Quinn, T.P., Lohmann, K.J., Noakes, DLG 2014b. An inherited magnetic map guides ocean navigation in juvenile pacific salmon. Current Biology 24:446–450

Raida, M.K., and Buchmann, K. 2008. Bath vaccination of rainbow trout (*Oncorhynchus mykiss* Walbaum) against *Yersinia ruckeri*: effects of temperature on protection and gene expression. Vaccine 26:1050–1062

Raida, M.K., and Buchmann, K. 2009. Innate immune response in rainbow trout (*Oncorhynchus mykiss*) against primary and secondary infections with *Yersinia ruckeri* O1. Developmental and Comparative Immunology 33:35–45

Raida, M.K., Holten-Andersen, L., and Buchmann, K. 2011. Association between *Yersinia ruckeri* infection, cytokine expression and survival in rainbow trout (*Oncorhynchus mykiss*). Fish and Shellfish Immunology 30:1257–1264

Richards, J.G. 2003. Na+/K+-ATPase -isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. Journal of Experimental Biology 206:4475–4486

Robertson, L.S., and Mccormick, S.D. 2012. Transcriptional profiling of the parr–smolt transformation in Atlantic salmon. Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics 7:351–360

Sawyer, H., Kauffman, M.J., Nielson, R.M., and Horne, J.S. 2009. Identifying and prioritizing ungulate migration routes for landscape-level conservation. Ecological Applications 19:2016-2025

Schmidt-Posthaus, H., Bernet, D., Wahli, T., and Burkhardt-Holm, P. 2001. Morphological organ alterations and infections diseases in brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* exposed to polluted river water. Diseases in Aquatic Organisms 44(3):161–170

Schönherz, A.A., Hansen, M.H.H., Jørgensen, H.B.H., Berg, P., Lorenzen, N., and Einer-Jensen, K. 2012. Oral transmission as a route of infection for viral haemorrhagic septicaemia virus in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 35:395–406

Schreck, C. B., Stahl, T. P., Davis, L. E., Roby, D. D., and Clemens, B.J. 2006. Mortality estimates of juvenile spring–summer Chinook Salmon in the lower Columbia River and estuary, 1992–1998: evidence for delayed mortality? Transactions of the American Fisheries Society 135:457–475

Seber, G.A.F. 1965. A note on the multiple-recapture census. Biometrika 52:249–259

Sicurella, B., Musitelli, F., Rubolini, D., Saino, N., and Ambrosini, R. 2016. Environmental conditions at arrival to the wintering grounds and during spring migration affect population dynamics of barn swallows *Hirundo rustica* breeding in Northern Italy. Populaion Ecology 58:135–145

Singh, N.J., Börger, L., Dettki, H., Bunnefeld, N., Singh, N.J., Börger, L., Dettki, H., Bunnefeld, N., and Ericsson, G. 2012. From migration to nomadism : movement variability in a northern ungulate across its latitudinal range. Ecological Applications 22:2007–2020

Skalski, J.R., Townsend, R., Lady, J., Giorgi, A.E., Stevenson, J.R., and McDonald, R.D. 2002. Estimating route-specific passage and survival probabilities at a hydroelectric project from smolt radiotelemetry studies. Canadian Journal of Fisheries and Aquatic Sciences 59:1385–1393

Skov, C., Chapman, B.B., Baktoft, H., Brodersen, J., Brönmark, C., Hansson, L.A., Hulthén, K., and Nilsson, P.A. 2013. Migration confers survival benefits against avian predators for partially migratory freshwater fish. Biology Letters 9:20121178

Society, S.S. 2015. Seymour Salmonid Society: History. Retrieved May 20, 2016, from

www.syemoursalmon.com
Spurgeon, S. L., Jones, R. C., and Ramakrishnan, R. 2008. High throughput gene expression measurement with real time PCR in a microfluidic dynamic array. PLoS ONE, 3:e1662
Steel, A.E., Sandstrom, P.T., Brandes, P.L., and Klimley, A.P. 2013. Migration route selection of juvenile Chinook salmon at the Delta Cross Channel, and the role of water velocity and individualmovement patterns. Environmental Biology of Fishes 96:215–224
Stefansson, S.O., Nilsen, T.O., Ebbesson, L.O.E., Wargelius, A., Madsen, S.S., Bjornsson, B.T., and McCormick, S. D. (2007). Molecular mechanisms of continuous light inhibition of Atlantic salmon parr-smolt transformation. Aquaculture 273: 235–245
Steinum, T., Sjåstad, K., Falk, K., Kvellestad, A., and Colquhoun, D.J. 2009. An RT PCR-DGGE survey of gill-associated bacteria in Norwegian seawater-reared Atlantic salmon suffering proliferative gill inflammation. Aquaculture 293:172–179
Stich, D.S., Zydlewski, G.B., Kocik, J.F., and Zydlewski, J.D. 2015. Linking behavior, physiology, and survival of Atlantic salmon smolts during estuary migration. Marine and Coastal Fisheries 7:68–86
Sutherland, B.J.G., Hanson, K.C., Jantzen, J.R., Koop, B.F., and Smith, C.T. 2014. Divergent immunity and energetic programs in the gills of migratory and resident *Oncorhynchus mykiss*. Molecular Ecology 23:1952–1964
Teffer, A.K., Hinch, S.G., Miller, K.M., Patterson, D.A., Farrell, A.P., Cooke, S.J., Bass, A.L., Szekeres, P., and Juanes, F. 2017. Capture severity, infectious disease processes and sex influence post-release mortality of sockeye salmon bycatch. Conservation Physiology 5.1
Tengs, T., and Rimstad, E. 2017. Emerging pathogens in the fish farming industry and sequencing- based pathogen discovery. Developmental and Comparative Immunology, 75:109-119
Thomas, A.C., Nelson, B.W., Lance, M.M., Deagle, B.E., and Trites, A.W. 2016. Harbour seals target juvenile salmon of conservation concern. Canadian Journal of Fisheries and Aquatic Sciences 74:907-921
Thorstad, E.B., Økland, F., Finstad, B., Sivertsgård, R., Bjørn, P.A., McKinley, R.S. 2004. Migration speeds and orientation of Atlantic salmon and sea trout post-smolts in a Norwegian fjord system. Environmental Biology of Fishes 71:305–311
Tipping, J., Gannam, A., Hillson, T., and Poole, J. 2003. Use of size for early detection of juvenile hatchery steelhead destined to be precocious males. North American Journal of Aquaculture 65:318–323
Tobback, E., Hermans, K., Decostere, A., Van Den Broeck, W., Haesebrouck, F., and Chiers, K. 2010. Interactions of virulent and avirulent *Yersinia ruckeri* strains with isolated gill arches and intestinal explants of rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 90:175–179
Toenshoff, E.R., Kvellestad, A., Mitchell, S.O., Steinum, T., Falk, K., Duncan, J., and Horn, M. 2012. A Novel betaproteobacterial agent of gill epitheliocystis in seawater farmed Atlantic salmon (*Salmo salar*). PLoS ONE 7:e32696
Tucker, S., Trudel, M., Welch, D.W., Candy, J.R., Morris, J.F.T., Thiess, M.E., Wallace, C., Teel, D.J., Crawford, W., Farley, E.V., and Beacham, T.D. 2009. Seasonal stock-specific migrations of juvenile sockeye salmon along the west coast of North America: implications for growth. Transactions of the American Fisheries Society 138:1458–1480

Tucker, S., Hipfner, J.M., and Trudel, M. 2016. Size- and condition-dependent predation: a seabird disproportionately targets substandard individual juvenile salmon. Ecology, 97:461–471

Ueda, H. 2012. Physiological mechanisms of imprinting and homing migration in Pacific salmon *Oncorhynchus* spp. Journal of Fish Biology 81:543–558

Verleih, M., Borchel, A., Krasnov, A., Rebl, A., Korytář, T., Kühn, C., and Goldammer, T. 2015. Impact of Thermal Stress on Kidney-Specific Gene Expression in Farmed Regional and Imported Rainbow Trout. Marine Biotechnology 17:576–592

Wang, T., Holland, J.W., Carrington, A., Zou, J., and Secombes, C.J. 2007. Molecular and functional characterization of Il-15 in rainbow trout *Oncorhynchus mykiss*: a potent inducer of IFN- expression in spleen leukocytes. Journal of Immunology 179(3):1475–1488

Welch, D.W., Batten, S.D., and Ward, B.R. 2007. Growth, survival, and tag retention of steelhead trout (*O. mykiss*) surgically implanted with dummy acoustic tags. Hydrobiologia 582:289–299

Welch, D.W., Boehlert, G.W., and Ward, B.R. 2003. POST - the Pacific Ocean salmon tracking project. Oceanological Acta 25:243–253

Welch, D.W., Melnychuk, M.C., Payne, J.C., Rechisky, E.L., Porter, A.D., Jackson, G.D., Ward, B.R., Vincent, S.P., Wood, C.C., and Semmens, J. 2011. In situ measurement of coastal ocean movements and survival of juvenile Pacific salmon. Proceedings of the National Academy of Sciences of the United States of America 108:8708–8713

Welch, D.W., Melnychuk, M.C., Rechisky, E.R., Porter, A.D., Jacobs, M.C., Ladouceur, A., McKinley, R.S., and Jackson, G.D. 2009. Freshwater and marine migration and survival of endangered Cultus Lake sockeye salmon (*Oncorhynchus nerka*) smolts using POST, a large-scale acoustic telemetry array. Canadian Journal of Fisheries and Aquatic Sciences 66:736–750

Welch, D.W., Ward, B.R., Batten, S.D. 2004. Early ocean survival and marine movements of hatchery and wild steelhead trout (*Oncorhynchus mykiss*) determined by an acoustic array: Queen Charlotte Strait, British Columbia. Deep Sea Research II 51: 897–909

White, G.C., Burnham, K.P. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study, 46:S120–S139

Yurk, H., Trites, A.W. 2000 Experimental attempts to reduce predation by Harbor Seals on out-migrating juvenile salmonids. Transactions of the American Fisheries Society 129:1360–1366

Zou, J., and Secombes, C. 2016. The Function of Fish Cytokines. Biology 5:23.

Appendix

A.1 Acoustic tagging

Following surgery procedures described in Collins et al. (2013) and Furey et al. (2016), fish were randomly selected from raceways and anaesthetized in a solution of buffered tricane methanesulfonate (MS-222*;* 100 mg L^{-1}; 200 mg L^{-1} $NaHCO_3$), measured for mass and FL (total air exposure <1 minute), and placed ventral side up on a V-shaped surgery trough. Water from a maintenance bath of MS-222, (50 mg L^{-1} MS-222, 100 mg L^{-1} $NaHCO_3$) which was oxygenated using air stones and monitored for consistent temperature, was irrigated across the gills for the duration of each surgery. A small ~8-10 mm midventral incision was made just posterior of the pelvic fins. VEMCO V7-*2L* acoustic transmitters (7 mm x 18 mm, ~0.7 g in water; 69 kHz, VEMCO Ltd., Bedford, NS; www.vemco.com) were inserted through the incision and positioned lengthwise inside the body cavity. The incision was closed using two absorbable monofilament sutures (Ethicon monocryl 5-0 monofilament, www.ethicon.com) then fish were placed in an aerated bucket of ambient river water to monitor recovery prior to returning to hatchery raceways.

A.2 Survival analyses

I used a mark-recapture approach to estimate survival of acoustic-tagged smolts, where detection at each acoustic receiver subarray 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. I used R (R Core Team 2014) with the package RMark (Laake 2013) 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.

A.3 Data screening

Prior to beginning survival analyses, I screened the raw detection data from all 273 tagged smolts for false detections, which could occur because of environmental conditions or collisions between multiple acoustic-tag transmissions. Two or more detections of the same tag along a subarray within 0.5 hours and with more detections spaced with short intervals (<0.5 hour spacing) than with long intervals (>0.5 hours spacing) were considered real. Detections that failed to meet these criteria were assessed individually and were passed if the migration sequence was reasonable and if travel time for the segment was within the 10^{th} - 90^{th} percentiles of either segment or cumulative travel times. Of >12,000 steelhead detections recorded across all acoustic subarrays, only six were considered false and removed from subsequent analyses.

A.4 Capture history sequencing

A capture history is a sequence of 1's and 0's that indicates whether an individual smolt was detected at each acoustic sub-array during their migration. The capture history sequence for the river-release group began with release in the Seymour River followed by detection at the subarrays deployed at the Seymour River Mouth, Northern Strait of Georgia (NSOG), Discovery Islands (DI), Johnstone Strait (JS), and Queen Charlotte Strait (QCS). The sequence for the marine-release fish was the same except that they were released in Burrard Inlet so that NSOG

was the first detection site. Detections of the marine-release fish at the Seymour River Mouth (n = 5), were not included in the capture history, but these fish were otherwise retained in the analysis. Finally, I removed the two fish that migrated south after river exit and were detected on the Juan de Fuca (JDF) subarray since these fish would otherwise appear to have died in the Strait of Georgia.

A.5 Goodness-of-fit

We assessed goodness of fit (GOF) with the median ĉ test within Program MARK. The variance inflation factor ($\hat{c}$) estimate for the most highly parameterized model in our model set [φ(release x segment$_{\text{Release to QCS}}$) p (release x segment)] was 1.64 (SE = 0.03) indicating that there was minor overdispersion. We adjusted the likelihood term for the model and inflated (multiplied) the standard errors on the estimates by the ĉ value to account for overdispersion in the data.

A.6 Effect of release location

I investigated the effect of release location to assess if it was reasonable to pool the two groups in the areas where their migration routes overlapped (NSOG to QCS) to increase sample sizes to the furthest marine subarrays, and to test if release location had an impact on survival post-Northern Strait of Georgia (NSOG). For this test, I modeled survival (φ) and detection probability (p) both with and without effects for release location and used AIC to compare the performance of the resulting model set. Groups were kept separate for migrations from release to NSOG because their migration segments differed. Only smolts released in freshwater had detections on Seymour River estuary receivers prior to NSOG, so only river-released smolts

were used to estimate detection probability [i.e. *p*(site)] for estuary receivers. I hypothesized that φ was the same for both release groups at each of the subarrays in the common migration corridor from NSOG to Queen Charlotte Strait (QCS) [φ (release x $segment_{Release\ to\ NSOG}$ + $segment_{NSOG\ to\ QCS}$)], and φ varied by release group at all subarrays [φ (release x $segment_{Release\ to\ QCS}$)]. I used the same strategy to test if detection probability varied by release location [*p*(site) versus *p*(release x segment)] resulting in a set of four models across all combinations of φ and *p*. Although all tagged fish were implanted with the same model of acoustic tag and all tags were programmed identically, it was reasonable to test if release location affected detection probability because the migration timing between the groups was statistically different at NSOG, Discovery Islands (DI), and Johnstone Strait (JS) (Wilcoxin $p \leq 0.001$; differences of ~10 days in mean arrival dates), but not at QCS (Wilcoxin p=0.24; difference of 2.5 days in mean arrival). Migration timing can potentially affect detection probability through temporal changes in the level of background noise (e.g. weather events).

Since there was no evidence that survival or detection probability varied by release group (the summed weight across the models where a single parameter was estimated for both release groups was 95% and 98% for survival and detection probability respectively) I pooled all tagged smolts in the common migration corridor for all subsequent analyses. This model where the release location groups were pooled in common migration segments was used as the base model for further hypothesis tests described below (base model: φ(release x $segment_{Release\ to\ NSOG}$ + $segment_{NSOG\ to\ QCS}$) *p*(site); Table 2.3).

A.7 Effects of fork length, tag burden, and gill sampling on survival

I assessed if body size, tag burden, and gill sampling 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). To test these effects, I used AIC to compare the performance of the base model with three other models that were parameterized the same as the base model, but that also included an additive effect for one of the three covariates of interest (i.e. Table 2.2).

A.8 Final estimates of survival and detection probability

To account for model selection uncertainty in top candidate models including the effects of fork length, tag burden, and gill sampling (i.e. Table 2.3), I obtained the final estimates of survival and detection probability by model-averaging across the four models. The CJS models return the survivals for each migration segment and detection probabilities for each subarray. To calculate cumulative survival estimates from release to QCS, I multiplied survival probabilities for each consecutive migration segment. The cumulative survivals for the two release location groups differ since their segment survivals for the initial migration segments to NSOG were estimated independently. Beyond NSOG, the release location groups were pooled and only one survival estimate was made for both groups. I derived the variance for the cumulative survival estimates using the Delta Method.

A.9 Survival rates

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 subarray, and then from departure from one subarray 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 subarray. For the subarrays that spanned multiple channels at NSOG and Discovery Islands, I measured the distance to the central point of each channel.

Since both survival and travel time are random variables with associated error, I used bootstrapping to calculate the variance around the estimates of daily survival rate. I first sampled the fish 1000 times with replacement and calculated survival using the CJS model as described above for each sample. It was not possible to calculate the travel times for all of these samples because not all fish that survive have travel times (fish have to be detected on both sides of the segment in order to have a travel time calculation). Rather than discard samples without travel times, I calculated the travel times for each segment from separate samples that were drawn only from fish with travel times in that segment. To reduce the probability of inappropriate pairings (i.e. fast travel times with poor survivals), I calculated the survival rates by matching each survival sample with all 1000 travel time samples. I used the mean of these estimates as the final estimate of survival rate per day, and the distribution to calculate standard deviations and confidence intervals on the mean.

For consistency, I also used bootstrapping 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 actual distance swum is unknown. I 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 ($\hat{c}$). Currently, there is no clear method for handling over dispersed data when employing bootstrap techniques. As the $\hat{c}$ was only 1.64 for this data set, the effect was minimal.

As a final step in the assessment of route-specific survival, I calculated survival rates per day and per km for each route through the Discovery Islands because the migration distance was ~1/3 longer through SC than through DP. I used the same methods for this calculation as for the segment-specific survival rates described above, but substituting the multi-state model for the CJS model.

A.10 Route-specific survival

I assessed route-based movements and survival through the Discovery Islands region (Fig. 2.1) using a spatial multi-state mark-recapture model where migration routes functioned as 'states'. Similar to CJS models, multi-state models estimate survival (defined as S as opposed to φ for CJS models) and detection probability (p), but they also estimate the probability of movement between states (migration routes; ψ). A key assumption of multi-state models is that survival is modeled with the survival probability for the state where the animal was captured, and then movement to a new state takes place (i.e. survival in segment i to i+1 does not depend on state in segment i+1). Thus, this model was appropriate to assess route selection and route-

specific survival through the Discovery Islands since the DI subarray was placed at the south end (entrance) of this area. In addition to this assumption, CJS model assumptions (see A2 Survival Analysis) also apply to multi-state models.

For this analysis, tagged steelhead were assigned to state A until they reached the DI subarray. At this point, those that migrated through Discovery Passage (DP) remained in state A, but those that used Sutil Channel (SC) transitioned to state B. I could not include Desolation Sound (DS, the third route through the Discovery Islands area) in this assessment because only one smolt was detected using this route. For each fish, I defined the migration route based on the location of its last detection on the Discovery Islands subarray (i.e. a fish that was detected on SC and then DP was assumed to have migrated through DP). At the JS subarray, all fish were assigned to state A and remained there until QCS. Six fish that were detected on DI were removed from the analysis because they were subsequently detected on NSOG (i.e. they probably did not migrate north).

To test if survival to JS varied by route through the Discovery Islands, I parameterized S with and without a route parameter and compared model performance using AIC [base model: $S(\text{release} \times \text{segment}_{\text{Release to NSOG}} + \text{segment}_{\text{NSOG to QCS}})$ versus differing-by-route model: $S(\text{release} \times \text{segment}_{\text{Release to NSOG}} + \text{route} \times \text{segment}_{\text{DI to JS}} + \text{segment}_{\text{NSOG-DI and JS to QCS}})$]. For these models, I assumed the transition probability would vary by route [ψ(route)] since the detection data showed the proportion of fish taking each path was quite different. I was unable to estimate detection probability for each route because we could not determine which route was initially taken by those smolts detected at JS but not DI. Therefore, I assumed detection probability to be the same for each route [p(site)], which is reasonable because the receiver configurations were very similar in both channels. Multi-state models don't perform well near the boundaries of 0

and 1, so I used bootstrapping of to gain a more robust estimate of survival though these channels. I was not able to include $\hat{c}$ goodness-of-fit with bootstrapped results, though this parameter was low at 1.64, indicating a minor lack of fit.

A similar approach was used to assess whether survival to the DI subarray was influenced by route selection at NSOG. Because the NSOG subarray is located at the north end of Texada Island, route (state) for this analysis was assigned based on the channel where smolts were first detected (east or west of Texada Island). The lack of a subarray at the south end of Texada Island (entrance) prevented me from assessing S and ψ within the actual channels around this island. Models were parameterized as for the test of route selection through the Discovery Islands, but with the route parameter for S shifted to the NSOG-DI segment (Table A1). Additionally, because S was modelled separately by release group until smolts reached the common migration corridor at NSOG, I had to consider if ψ would vary by release group in addition to route. However, I constrained ψ to be the same for both release locations (but allowed them to vary by route) because only two river-release smolts were detected to the east of Texada Island.

As a final step in my assessment of route-specific survival, I calculated survival rates per day and per km for each route through the Discovery Islands because the migration distance was ~1/3 longer through SC than through DP. I used the same methods for this calculation as for the segment-specific survival rates described above (see Survival rates), but substituting the multi-state model for the CJS model.

A.11 Appendix tables

Table A1: Ranking of multi-state models using QAICc to test if survival to the Discovery Islands subarray was influenced by route selection at the Northern Strait of Georgia subarray (i.e. east or west of Texada Island).

	Model	No. of parameters	QAICc	ΔQAICc	weight	QDeviance
Survival the same between routes	S(release * segment$_{\text{Release to NSOG}}$[a] + segment$_{\text{NSOG to QCS}}$) *p* (site[b]) ψ (route)	11	625.029	0	0.737	13.611
Survival separate between routes	S(release * segment$_{\text{Release to NSOG}}$[a] + route[c] * segment$_{\text{NSOG to DI}}$ + segment$_{\text{DI to QCS}}$) *p* (site[b]) ψ (route)	12	627.093	2.064	0.263	13.586

a - Segment length to NSOG differed by release group
b - Only river-released smolts were used to estimate *p* for the estuary receivers
c – The route parameter was used to provide independent estimates for the channels around Texada Island (Strait of Georgia and Malaspina Strait).

Table A2: Raw detection counts of acoustic tagged steelhead (*Oncorhynchus mykiss*) smolts at each subarray (when applicable) through the study system, as well as number detected based on routes through the Northern Strait of Georgia and Discovery Islands subarrays. 'NSOG' = Northern Strait of Georgia subarray, 'DI' = Discovery Islands subarray, 'JS' = Johnstone Strait subarray, 'QCS' = Queen Charlotte Strait subarray. Two smolts were also detected at the Juan De Fuca subarray, and were removed from survival analyses. These counts are not necessarily reflective of those used in route-specific multi-state models.

Array	Sub-array (route)	Number Detected (marine-release/river-release)	Number of smolts detected based on routes along NSOG and DI			
			NSOG	DI	JS	QCS
Seymour River	N/A	5/66				
Northern Strait of Georgia	East of Texada	41/3		36	19	14
	West of Texada	51/12		48	29	17
Discovery Islands	Discovery Passage	60/12			48	30
	Sutil Channel	34/3			13	11
	Desolation Sound	2/0			1	0
Johnstone Strait	N/A	51/9				27
Queen Charlotte Strait	N/A	33/5				

A.12 Appendix figures

Figure A1: Frequency distribution histograms of first (top) and last (bottom) detections of steelhead smolts (*Oncorhynchus mykiss*) on the Seymour River estuary receivers by time of day (in hours). Grey and white regions in the background indicate times of local night and day (sundown and sunrise), respectively. Fish movements in the estuary were predominantly nocturnal.