ENVIRONMENTAL & PHYSIOLOGICAL INFLUENCES ON THE BEHAVIOUR AND SURVIVAL OF ADULT SOCKEYE SALMON DURING THEIR COASTAL MIGRATION

by

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ABSTRACT

The reproductive migration of anadromous salmon through coastal waters is among the most challenging phases of their life cycle, yet our understanding of the mechanisms underlying this migration is limited. Thus, the objective of this thesis was to develop a better understanding of how environmental conditions and fish physiologic state influence behaviour and survival of homing anadromous salmon in coastal waters.

Using a literature synthesis, I identified consistent behaviours across anadromous salmon species and life stages in marine waters including diel patterns and consistent swimming speeds. I further identified critical knowledge gaps, including a need for synchronized study of both environmental and physiological conditions. In field studies, I combined thermal data loggers, biotelemetry and physiological sampling and found that homing sockeye salmon exhibited diel and variable thermal experiences (8.4 °C to 20.5 °C) in coastal waters, potentially related to gaining cues for navigation. Sockeye salmon tended to follow coastlines and migration rate was related to wind patterns, salinity and fish physiological state. I propose that wind-induced currents exposed sockeye salmon entering the estuary to stronger olfactory cues associated with freshwater, which resulted in faster migration rates due to increased navigation ability or advanced reproductive maturity through a neuroendocrine response. Once migration neared freshwater, sockeye salmon used wind-induced currents to aid in movements, which may be associated with energy conservation.

I further identified a genomic signature related to marine survival, which was associated with stress, immune response, metabolic processes, protein biosynthesis and osmoregulation. This genomic signature was similar to that identified in a previous study

examining freshwater survival, but with an opposite relationship with survival, which I attribute to the attenuation of disease resistance of fish upon exposure to elevated river temperatures.

Through the use of multiple research approaches, this thesis advances the biological understanding of the marine homing migration of sockeye salmon by empirically establishing novel relationships between environmental conditions, physiological state and sockeye salmon behaviour and survival in marine waters. In addition, this thesis is broadly applicable to other anadromous salmon, as well as to studies invoking a similar approach of physiological biotelemetry for studying animal movements.

PREFACE

Chapter 2: A synthesis of tagging studies examining the behaviour and survival of anadromous salmonids in marine environments

Authors: SM Drenner, TD Clark, CK Whitney, EG Martins, SJ Cooke, SG Hinch.

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Chapter 4: Environmental conditions and physiological state influence estuarine behaviour of homing sockeye salmon.

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Chapter 5: Transcriptome patterns and blood physiology associated with homing sockeye salmon fate during final stages of marine migration

Authors: SM Drenner, SG Hinch, NB Furey, TD Clark, S Li, T Ming, KM Jeffreis, DA Patterson, SJ Cooke, D Robichaud, DW Welch, AP Farrell, KM Miller

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	alpha
°C	degrees Celsius
χ^2	chi square
π	pie
μ	micro
AIC	Akaike information criterion
AIC_c	Akaike information criterion corrected for small sample sizes
AlSh	along shore wind stress
AR1	auto-regressive correlation structure of order 1
BC	British Columbia
bl	body length
CA	catecholamine
cDNA	complementary deoxyribonucleic acid
Cl^-	chloride
cm	centimeter
cos	cosine
cRNA	amino-allyl aaRNA
CrSh	cross shore wind stress
CST	capture/sampling/tagging
CWT	coded wire tags
d	day
df	degrees of freedom
DFO	Department of Fisheries and Oceans Canada
DOR	date of release
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FL	fork length
g	gram
GC	glucocorticoid
GLM	generalized linear model
GLS	generalized least squares
GnRH	gonadotrophic releasing hormone
GSE	gross somatic energy
h	hour
HPG	hypothalmus-pituitary-gonadal
HPI	Hypothalmus-Pituitary-Interrenal
k	thousand
K^+	potassium
km	kilometer
KMO	Kaiser-Mayer-Olkin
m	meter
MANOVA	multivariate analysis of variance

min	minute
ml	milliliter
mm	millimeter
mRNA	messenger ribonucleic acid
MRS	mortality related signature
n	sample size
N	newton
N^2	nitrogen
Na^+	sodium
NASCO	North Atlantic Salmon Conservation Organization
NKA	Na^+K^{+-}ATPase
NPAFC	North Pacific Anadromous Fish Commission
NO_2	liquid nitrogen
NSOG	Northern Strait of Georgia
OTN	Ocean Tracking Network
PC	Principle component
PCA	Principle component analysis
PIT	passive integrated transponder
POST	Pacific Ocean Shelf Tracking
PSAT	pop-off satellite tags
PST	Pacific Standard Time
qRT-PCR	quantitative real-time polymerase chain reaction
RNA	ribonucleic acid
s	second
SD	standard deviation
sGnRH	salmon gonadotrophic releasing hormone
sin	sine
SoG	Strait of Georgia
SPOT	smart position or temperature transmitting
ST3m	sea temperature at 3m depth
TOPP	Tagging of Pelagic Predators
VIF	variance inflation factor

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DEDICATION

This thesis is dedicated to my wonderful family, Kim, Pam, Ray and Bryan Drenner.

CHAPTER 1: INTRODUCTION

1.1 Animal migrations

Migrations are among the world's most spectacular forms of animal behaviour. Animal migrations occur across a wide range of taxonomic groups and range in scale and complexity. For instance, migratory birds are known to fly astonishing distances, in some cases covering tens of thousands of kilometers between continents during their migrations, while salamanders may crawl a mere few meters from winter burrows to breeding pools. Although there is a great deal of diversity among animal migrations, in all cases, migrations involve challenging abiotic and biotic conditions that can have profound effects on an individual's fitness, the results of which expand to populations, communities and ecosystems.

Animal migrations have intrigued scientists and natural historians for thousands of years, even appearing in the early writings of Aristotle, who mused about the annual disappearance of Redstarts and Garden Warblers, attributing their seasonal absence to transmutations to other bird species (Thompson, 1910). Since then, migration has been defined and described by a number of sources with some debate [see Dingle (1996), Dingle & Drake (2007), Milner-Gulland et al. (2011) and refs therein], but generally can be characterized as the directed movements of individuals between separate and distinct environments within a reasonably predictable time frame (Dingle, 1996; Endler, 1977; Milner-Gulland et al., 2011; Northcote, 1978). The evolutionary basis for migration is that the fitness benefits and costs associated with residing in a location change over a life cycle such that fitness advantages are gained by migrating between habitats (Dingle,

1996). While our understanding of animal migration has greatly advanced since the early days of study, the ecological, energetic and physiologic mechanisms that underlie migration remain poorly understood for many species.

Recently, Nathan et al. (2008) introduced a unifying framework for studying movement ecology, which can be readily applied to the study of animal migrations. Based on their framework, migration can be divided into two basic components: the focal individual and external factors (Fig. 1.1). The focal individual is comprised of an individual's navigation capacity (i.e., the ability to orient towards and navigate between habitats), their motion capacity (i.e., biomechanics of movement), and their internal state (i.e., physiological and energetic state). These individual based processes interact with themselves through feedback loops and with external factors (e.g. the abiotic and biotic environment), eliciting behavioural responses, such as migratory route, or migration timing that ultimately influences migration success (Nathan et al., 2008) (Fig. 1.1). Below I introduce and expand on internal and external processes in the context of their importance for understanding animal migrations.

A fundamental element of all animal migrations, and perhaps the most impressive feat, is the ability to orient towards and navigate between discrete locations (i.e., navigation capacity), or simply put 'where' to migrate. To accomplish this, animals are able to detect and respond to a variety of environmental stimuli including magnetic fields, polarized light, celestial cues, the Sun, odors, temperature, salinity, wind, and currents to name a few [reviewed in Dingle (1996), Milner-Gulland et al. (2011)]. These external cues can vary widely among taxa, and in many cases, combinations of external cues are used (Chapman et al., 2015; Dingle, 1996; Gwinner, 1990; Leggett, 1977; Milner-

Gulland et al., 2011). For example, migratory bird species may rely on magnetic fields (Walcott et al., 1979), celestial cues, and the sun as cues for orientation (Alerstam et al., 2001) in a hierarchical manner, while both red-bellied newts (Grant et al., 1968) and teleost fish (Hara, 1986) rely heavily upon odors for orientation. Regardless of the specific cue, their detection results in a cascade of internal events, ultimately inducing behavioural and physiological responses that allow animals to successfully migrate between habitats. In general, the mechanisms behind migratory orientation and navigation are extremely complex, and perhaps remain the least understood aspect of animal migrations (Alerstam, 2006).

'When' an animal migrates is just as essential for migration success as 'where' an animal migrates because animals must synchronize their physiology and behaviour to meet the demands of migration. For this reason, migration events and timing are typically synchronized with various external stimuli [reviewed in (Dingle, 1996; Milner-Gulland et al., 2011)]. Photoperiod is perhaps the most common (especially in temperate regions) environmental cue associated with the initiation of migration because it is highly predictable. However, there are a variety of other cues migrants rely upon depending on the modes of migration (i.e., flight, swimming, walking). For example, flying migrants such as birds and insects are known to respond to wind patterns (Åkesson & Hedenström, 2007; Chapman et al., 2015), whereas swimming migrants such as migratory fish species, marine turtles and marine mammals respond to water flow (i.e., river and ocean currents) (Åkesson & Hedenström, 2007; McCleave et al., 1984). Temperature is also a key environmental cue especially for migratory ectotherms, whose internal temperatures fluctuate with that of the external environment (McCleave et al., 1984). The ability to use

and respond to supplemental information from the environment during the course of migration are important adaptations necessary for navigation, timing, maximizing energy efficiency, and making physiological preparations.

In terms of physiology, migration timing and behaviour are influenced by the endocrine system with some similarities across migratory taxa. Namely, stimulation of a neurosensory organ (i.e., corpora allata in insects and hypothalamus in vertebrates) results in the mobilization of hormones (either through primary release or downstream cascades), which ultimately influence migratory physiology and behaviours. In many cases, physiological changes occur in anticipation of changing environmental conditions migrants will experience. The processes of metamorphosis in urodele amphibians and smoltification in anadromous salmon exemplify such preparatory endocrine responses. In both cases, changes in photoperiod result in the release of a number of hormones (corticosteroids, prolactin) that are associated with multiple internal processes including increased resistance to desiccation, which promotes movements of amphibians and salmon smolts from hypoosmotic environments (i.e., freshwater habitats) to hyperosmotic environments (i.e., land and seawater for amphibians and salmon smolts, respectively) (Hirano et al., 1987; McCormick & Saunders, 1987; Rankin, 1991). Interestingly, the same hormones associated with preparations for movement into a drying environment are also associated with reversal of these processes during migrations back into hydrating environments. These types of physiological changes are essential for migratory success, but they can also come at a substantial energetic cost.

Migrations typically involve intense exercise over large distances, and migrants not only use energy to fuel movements but also to prepare physiologically, and in some

cases, morphologically for changing environments (Dingle, 1996). Because of this, energy mobilization prior to the initiation of migration is crucial, which is exemplified in migratory birds that alter their metabolic pathways to favor fat storage well before initiating migration (Ramenofsky, 1990; Wingfield et al., 1990). In fact, an optimal fuel load must be reached before many migratory animals are able to initiate migration, and this amount of fuel storage, which is in many cases fat, is directly related to migration distance (Alerstam & Lindström, 1990; Crossin et al., 2004). Metabolic shifts may also occur during migrations to account for increased activity and physiological requirements. For these reasons, many species are known to employ strategies to mitigate energetic costs during migration such as insects and birds adjusting flight patterns in relation to wind patterns (Alerstam & Lindström, 1990; Fontaine, 1976), or diadromous fish and marine turtles using ocean currents to aid in movements (Luschi et al., 2003; Thomson et al., 2007). Indeed, energetic state may be tied to migratory distance, behavioural strategies, and migration timing. Thus, anything that raises energetic costs during migration can have impacts on an individual's fitness.

Migrations are inherently stressful events because they typically involve changes to life history stages, physiologic state and the environment as outlined above. A stressor is defined as any stimuli that displace an organism from homeostasis (Wendelaar Bonga, 1997). Stressors can be physical (i.e., escape from predation events, fighting, temperature, flow), chemical (i.e., pollutants, salinity, oxygen), or perceived, but all invoke a similar stress response across vertebrates [reviewed in Sapolsky et al. (2000), Wendelaar Bonga (1997)]. After encountering a stressor, two main classes of hormones are released, catecholamines (CAs: epinephrine and norepinephrine), which are an

immediate response; and glucocorticoids (GCs), which are more prolonged over time (Sapolsky et al., 2000; Wendelaar Bonga, 1997). Once released, GCs (e.g., corticosterone in amphibians, reptiles and birds and cortisol in teleost fish and mammals) interact with other components of the endocrine system and have a number of secondary effects such as facilitating energy mobilization, affecting immune and reproductive physiology, increasing metabolic rate, and changing the hydromineral balance (Barton, 2002; Mommsen et al., 1999; Sapolsky et al., 2000; Schreck, 2010). Overall, the stress response is meant to enable an organism to cope with a stressor, and is hence considered adaptive (Romero, 2004). However, if a stressor becomes chronic, or if the magnitude of a stressor pushes an organism to its physiologic limit such that it cannot maintain homeostasis, the stress response can become maladaptive, causing physiologic or behavioural impairments that can lead to mortality in extreme cases (Barton, 2002).

As evidenced from above, migrations are some of the most energetically demanding and physiologically challenging phases in an animal's entire life cycle. It is perhaps not surprising then that migration events are associated with elevated levels of mortality. Mortality itself is a natural feature of migrations and can act as a powerful selection mechanism, resulting in populations that are adapted to migration conditions, such as historic norms in environmental conditions (Dingle, 1996). However, deviations in the environment through climate change processes could greatly impact migratory populations, which in many cases are already experiencing declines globally (Wilcove & Wikelski, 2008). Hence, it is becoming even more important to increase our understanding of the internal and external processes underlying migration that will ultimately aid in the conservation of populations, communities, and ecosystems.

1.2 Reproductive migrations of anadromous salmon

1.2.1 Anadromous salmon background

One of the most notable examples of an animal migration is the reproductive migration of anadromous salmon (from genuses *Salmo* and *Oncorhynchus*, hereafter referred to collectively as 'salmon') from marine feeding grounds to freshwater spawning sites. An anadromous life cycle means that salmon are born in freshwater environments (and in some cases intertidal areas) and migrate to the ocean to feed and mature. Depending on the species, salmon spend anywhere from 1-4 years feeding in the ocean before they embark on migrations back to natal freshwater (or intertidal) sites to reproduce [for further details on variability in life history stages see Groot & Margolis, (1991), Quinn (2005), Verspoor et al. (2007)]. In doing so, salmon play an important ecological role by serving as important prey items and nutrient sources, and by acting as vectors for transporting marine-derived nutrients to freshwater and terrestrial ecosystems (Gende et al., 2004). In addition, salmon provide significant social and cultural value to local and native peoples, and are also commercially exploited through fisheries. However, over the last century, many populations of wild salmon have declined in abundance creating conservation and management concerns (Boisclair, 2004; Bradford, 1995; Lichatowich et al., 1999; Parrish et al., 1998). While there are numerous possible factors acting over multiple life stages that may contribute to declines in abundance (Lichatowich et al., 1999; Parrish et al., 1998), the reproductive migration presents unique physiological and environmental challenges for migrating salmon, and thus may contribute to population declines. Below, I introduce the physiological, energetic and

environmental aspects involved in the reproductive migration of salmon, with emphasis on how these factors are thought to influence behaviour and migration success.

1.2.2 Reproductive maturation

The initiation of the reproductive migration occurs while fish are in the ocean and is tied to changing photoperiod (Hinch et al., 2006), which activates the Hypothalmus-Pituitary-Gonadal (HPG) axis (Fukaya et al., 1998; Kitahashi et al., 1998; Mylonas & Zohar, 2001) triggering sexual maturation. After activation, the hypothalamus releases gonadotrophic releasing hormone (GnRH) triggering increases in circulating concentrations of sex steroids testosterone, 11-keto testosterone (males) and estradiol (females), which fuel gamete development (Ueda & Yamauchi, 1995). Once salmon embark on their reproductive migration, the timing of migration events and physiological changes are paramount in determining reproductive success. In fact, in several key studies on Fraser River sockeye salmon (*Oncorhynchus nerka*), researchers successfully related reproductive maturity of individual fish samples in the ocean to migration timing and success (Cooke et al., 2008a; Cooke et al., 2006b; Crossin et al., 2009a; Crossin et al., 2007; Crossin et al., 2009b), providing evidence of the overall importance of the maturation process on migration.

1.2.3 Homing

Salmon exhibit remarkable home site fidelity, although some straying does occur, which represents an important evolutionary bet-hedging strategy (Candy & Beacham, 2000; Eriksson & Eriksson, 1991; Keefer & Caudill, 2014). The ability of salmon to

navigate back to their natal spawning sites is referred to as ‘homing’. The mechanisms behind homing remain poorly understood and likely change over the duration of migration. While homing in the ocean towards freshwater, it is largely thought that salmon use some combination of cues including magnetic fields, light, chemical odors, currents and celestial cues to aid in homing (Dittman & Quinn, 1996; Hansen et al., 1993; Keefer & Caudill, 2014; Quinn & Dittman, 1990; Stabell, 1984). In a recent study, the migration route used by sockeye salmon for locating coastal waters from the open-ocean was found to be dependent on geomagnetic fields and sea surface temperature (Putman et al., 2013). However, after salmon reach coastal waters and especially as salmon near freshwater entry points, it likely becomes more difficult for salmon to detect changes in geomagnetic cues over such fine scales. Therefore, during the coastal stage of migration, salmon may begin to use additional cues such as coastlines and chemical cues emitted into estuaries from freshwater entry points to assist in location of freshwater entry points (Quinn & Dittman, 1990).

Among potential navigational cues, olfactory homing is believed to be the predominant mechanism salmon use for homing in estuaries and especially once in freshwater environments (Hasler & Scholz, 1983a; Keefer & Caudill, 2014; Quinn & Dittman, 1990). The principle behind olfactory homing is that salmon are able to detect chemical odors through sensory glands (e.g. olfactory bulbs) located in the brain (Hara, 1992). These chemical odors ‘imprint’ on juvenile salmon while they are rearing in freshwater habitats and can then be detected by returning adults to locate their natal spawning sites (Hasler & Scholz, 1983a). Olfaction in homing salmon has largely been studied during the freshwater phase of migration, while the marine phase is relatively

understudied (Keefer & Caudill, 2014). However, studies have found evidence of olfactory driven reproductive maturation via elevated levels of gonadotropin-releasing hormone (sGnRH) in the olfactory bulb of homing chum salmon (*Oncorhynchus keta*) in estuaries (Ueda, 2011).

In addition to olfactory cues potentially influencing maturation, salmon may also directly use olfactory cues while in estuaries to locate freshwater entry locations. In one study, Atlantic salmon (*Salmo salar*) migration pathway through a Norwegian fjord was related to wind patterns, presumably because the salmon were following olfactory cues from freshwater that were dispersed by winds (Davidsen et al., 2013). Indeed, environmental conditions such as river discharge, wind patterns and tides might influence the concentration, gradient and spatial distribution of olfactory cues in estuaries, and therefore the ability of homing salmon to navigate during coastal and estuarine migrations. However, very few existing studies have examined these relationships.

1.2.4 Osmoregulation

The ability to maintain internal homeostasis in the face of changing osmotic conditions is a fundamental feature of all euryhaline fish including anadromous salmon that migrate between seawater and freshwater environments (Baldisserotto et al., 2007). While in full strength seawater, the internal ion concentrations of fish are approximately one third that of the external environment. Thus, seawater acclimated fish actively drink seawater to balance net water loss to the external environment and actively excrete excess ions through the gill. In contrast, while in freshwater environments, the internal ion concentrations of fish are greater than that of the external environment. Thus, freshwater

acclimated fish excrete excess water gained from the environment through the kidneys and in urine and actively take up ions through the gill.

For reproductively migrating salmon, the transition from seawater to freshwater environments requires drastic changes to the osmoregulatory system that begins well in advance of reaching freshwater (Flores et al., 2012; Shrimpton et al., 2005). This osmoregulatory transition is accomplished via stimulation of the Hypothalmus-Pituitary-Interrenal (HPI) axis, which produces an increase in plasma cortisol concentrations along with changes in other compounds associated with freshwater entry (Clarke & Hirano, 1995; Makino et al., 2007; Mancera & Fuentes, 2006; McCormick & Saunders, 1987; Norris & Hobbs, 2006). Among the physiological responses are a shift in the number, distribution, and function of gill chloride cells (Uchida et al., 1997), the decline of gill $Na^{+}K^{+-}$ATPase (NKA) enzyme activity, and increased mRNA expression of a freshwater specific NKA isoform (α1a) (Flores et al., 2012; Shrimpton et al., 2005).

Similar to the maturation process, the osmoregulatory transition towards freshwater acclimation occurs progressively over the course of migration (Flores et al., 2012; Shrimpton et al., 2005). Although salmon can encounter lower salinities far offshore due to diffusion of freshwater from coastal waters (Thomson, 1989), the changes in salinity are not as abrupt as in coastal areas. Indeed, salinity levels in coastal systems may further influence osmoregulatory state and thus migration behaviour. For example, homing sockeye salmon that were captured in coastal marine waters and then experimentally held in full-strength seawater had lower survival compared to sockeye salmon held in freshwater (Cooperman et al., 2010). Furthermore, once sockeye salmon from this study were released back into the estuary, those held in freshwater migrated

faster into the river (Hinch, 2009). Consistent with this trend, a separate study found sockeye salmon migrated earlier into freshwater in years when they encountered lower salinity levels in coastal waters (Thomson & Hourston, 2011). Based on these studies, it would appear that the osmoregulatory transition that takes place during coastal migration cannot be easily reversed, and exposure to lower salinity levels can have significant effects on the migration behavior and survival of homing salmon.

1.2.5 Energetics

Energetic state is yet another internal factor that must be managed during reproductive migrations. Anadromous salmon are capital breeders, meaning their reproductive migrations are fueled by endogenous energy reserves. Energy is stored as lipids in muscle tissue and catabolized for use in energetically expensive physical activities and physiological processes that occur during the reproductive migration (Hinch et al., 2006). For these reasons, the amount of energy stored prior to migration is thought to be adaptive and thus directly related to migratory difficulty (i.e., distance, elevation gain) of spawning migrations (Crossin et al., 2004). In addition, migration timing likely evolved in response to meeting energetic constraints associated with migration (Burgner, 1991; Dingle, 1996), and therefore, energy may play a role in migration success. In one study, sockeye salmon with lower energy content in their dorsal musculature when sampled in the ocean had lower survival to reaching spawning grounds (Crossin et al., 2009a). Owing to this delicate balance of energy, it is perhaps not surprising that salmon have been observed using currents (Thomson et al., 2007), selective tidal transport (Levy & Cadenhead, 1995) and behavioural thermoregulation

(Tanaka et al., 2000), potentially as adaptive strategies for conserving energy during homing migrations. Overall, anything that depletes energy reserves during the reproductive migration, such as elevated stress, could have implications towards an individual's reproductive fitness.

1.2.6 Stress

Homing salmon have been shown to have increasing stress hormone concentrations as migration proceeds towards river entry (Hinch et al., 2006). Increased levels of stress hormones such as cortisol are related to a number of biological processes including ionoregulatory abilities, reproductive maturation, olfaction and energy use (Barton, 2002; Carruth et al., 2002; Mommsen et al., 1999), all of which are necessary for successful completion of the reproductive migration. However, salmon can encounter a variety of abiotic and biotic stressors in coastal areas such as predation events, elevated temperature, variable salinities, fisheries, and pollutants, which could push stress hormone levels beyond baseline values. Regardless of the stressor type, elevated stress levels in salmon can have secondary effects such as depress reproductive development, immune function and osmoregulatory function (Barton, 2002; Schreck et al., 2001), and reduce aerobic scope (Eliason et al., 2011; Pörtner, 2007; Pörtner & Farrell, 2008), which can lead to mortality in extreme cases. For example, elevated stress levels have been associated with lower survival in the marine environment (Cooke et al., 2006a; Crossin et al., 2009a), likely a result of stress induced physiological and behavioural impairments that make individuals more susceptible to disease and predation.

1.2.7 Coastal and estuarine environment

Coastal and estuarine environments present unique challenges for migrating salmon and may represent the most complex phase of the homeward migration. Not only are salmon having to gain cues for navigation and prepare physiologically for reproduction and the transition to freshwater, but there are also biotic pressures such as increased presence of predators and novel pathogens not encountered in previous life stages. Furthermore, salmon can begin to encounter variable abiotic conditions in coastal environments (Quinn et al., 1989). Variable abiotic conditions in coastal environments are typically associated with freshwater discharge, which creates a vertically stratified environment with a layer of less dense, less saline and generally warmer water that rests on top of more dense, more saline and generally cooler water below. This variable structure of coastal water presents a number of tradeoffs for homing salmon. On one hand, freshwater influenced surface waters may contain olfactory cues necessary for navigation (Døving & Stabell, 2003), but surface waters may also expose fish to above optimal temperatures, lower salinity levels (Quinn et al., 1989) and visually oriented predators such as seals, all of which can result in elevated stress levels.

Temperature in particular has been implicated as a major factor limiting migration success in freshwater migrating adult salmon (Crossin et al., 2008; Keefer et al., 2008a; Keefer et al., 2008b; Mathes et al., 2010). In coastal marine waters, homing salmon appear to select a very narrow range of temperatures (Quinn et al., 1989; Walker et al., 2000), which seems to fall within a temperature range that maximizes aerobic and cardiac scope [based on freshwater experiments; (Brett, 1971; Farrell et al., 2008)]. However, homing salmon in coastal waters also appear to make periodic vertical movements into

surface waters that expose salmon to above optimal temperatures (Quinn et al., 1989). Exposure to above optimal temperatures can result in more rapid energy use (Brett, 1971) and an elevated stress response (Jeffries et al., 2012), which can have a number of secondary consequences outlined above. In addition to high temperatures, surface waters are also typically lower in salinity, which can result in osmotic stress depending on an individual's osmoregulatory state. Furthermore, elevated temperatures and lower salinity can increase pathogenicity of microbes fish are carrying from previous life stages (Miller et al., 2014). However, the role that water temperature and salinity have on the behavior and success of homing adult salmon remains poorly understood.

In summary, there are a number of challenges facing migrating salmon in coastal environments, and research has shown that, in some cases, up to 50% of salmon that reach coastal waters do not successfully enter freshwater (Cooke et al., 2006a; Crossin et al., 2009a). Certainly these relatively low survival rates alone, which are comparable to rates during the much more extensive in-river migration phase, provides impetus for the study of this phase of the reproductive migration. Moreover, in addition to low survival rates, the coastal ocean environment is predicted to experience drastic shifts in the future due to climate related changes such as earlier spring freshet and altered wind patterns that would impact river discharge into and oceanography within coastal systems (IPCC, 2007). Deviations in environmental conditions from historic norms could have profound effects on anadromous salmon (Crozier et al., 2008), which are highly adapted to historical environmental conditions they experienced during migrations (Eliason et al., 2011; Hodgson & Quinn, 2002). It is therefore becoming ever more important to

understand the mechanisms underlying anadromous salmon migration so that predictions can be made for how populations will respond to future climate related changes.

1.3 Physiological biotelemetry

Migration is most optimally studied at the level of the individual because this is the primary level upon which natural selection acts (Dingle & Drake, 2007). However, there are numerous logistical challenges associated with studying free-swimming fish, especially in marine environments. Historically, visual ID tags or visual markings were used, but the amount of information gained was limited because individuals had to be recaptured. The recent development of biotelemetry technology, such as acoustic and radio transmitters, allows researchers to monitor movements, behaviour and migration success of individuals in the wild without the need to recapture tagged individuals (Cooke et al., 2008b; Cooke et al., 2004b). These types of technologies have been used to successfully study free-swimming salmon in coastal marine environments, providing useful information on migration rates, swim speeds, swim depth, orientation and survival to name a few (Cooke et al., 2008a; Cooke et al., 2006a; Cooke et al., 2006b; Crossin et al., 2009a; Crossin et al., 2007; Quinn, 1988; Quinn & terHart, 1987; Quinn et al., 1989). Furthermore, biotelemetry can also be used in conjunction with multiple tag types [for examples see Donaldson et al. (2009) and Hasler et al. (2011)] and physiological sampling of individuals to provide novel insights into the biology of salmon migrations (Cooke et al., 2008b).

1.4 Thesis objectives and chapter overview

The broad objective of my thesis is to examine how environmental and physiological conditions influence anadromous salmon behaviour and survival during their reproductive migration through marine environments. In order to understand the mechanistic basis of the spatial ecology of homing salmon, this thesis integrates tools across multiple disciplines (i.e., physiology, genomics, behavioral ecology, telemetry, oceanography, statistical modeling and fisheries management). The chapters hereafter are presented in a way such that each successive chapter uses information gained from previous chapters to build towards my overarching objective. Information from this thesis will be crucial for the conservation, economic development and management of anadromous salmon fisheries, particularly in the face of climate change.

The use of tagging as an approach for studying behaviour, movement and survival of individuals is a common theme throughout all chapters of my thesis. This becomes immediately apparent in chapter 2 of this thesis, which sought to synthesize the greater body of literature that used tagging as an approach for studying anadromous salmon behaviour and survival in marine environments. From this synthesis, I was able to identify knowledge gaps and generate recommendations for how to address these gaps in future research, many of which were addressed in empirical studies presented in chapters 3, 4 and 5 of my thesis.

Each of chapters 3, 4, and 5 focus on sockeye salmon from the Fraser River, British Columbia (BC), Canada as a model organism for studying anadromous salmon migration. There are hundreds of geographically and genetically distinct populations of sockeye salmon that spawn within the Fraser River watershed, all with distinct life

history traits that are highly conserved among populations (Groot & Margolis, 1991). Homeward migrating adults return to the Fraser River through the Strait of Georgia (SoG), which is essentially a large estuary due to significant freshwater influence from the Fraser River. The estuarine-like conditions in the SoG are extremely useful for studying how varying environmental conditions influence homing behaviour. Indeed, some of the best information on homing migrations of anadromous salmon comes from studies on Fraser River sockeye salmon [for examples see Hinch et al. (2006), Hinch et al. (2012) and references therein], providing extensive background to build upon in my thesis.

In chapter 3, I sought to characterize the environment sockeye salmon experienced while homing in the Fraser River estuary. For this objective I focus on temperature as an environmental variable because it is commonly thought of as the most important abiotic factor for salmon. In chapter 3, I also test for environmental and physiological influences on sockeye salmon thermal experience. Once I established what environmental conditions sockeye salmon experience, in chapter 4, I sought to relate environmental conditions and individual fish physiologic state to homing sockeye salmon migration behaviour (e.g. migration rate, timing and route) in the SoG. In chapter 5, I use genomic techniques including microarray analysis and quantitative real-time PCR (qRT-PCR) to examine gene expression in gill tissue combined with physiological analysis of blood plasma and biotelemetry to examine the physiological mechanism underlying marine survival of homing sockeye salmon to entry into the Fraser River. Finally, in chapter 6, I bring together results from previous chapters to form broad conclusions and make recommendations for future research.

Due to the observational nature of chapters 3, 4 and 5, coupled with the large degree of biological complexity occurring during this phase of migration, I use a common approach that incorporates multiple explanatory variables into statistical models. By using models that simultaneously account for effects of multiple factors, I am able to test numerous hypotheses outlined in more detail within respective chapters below.

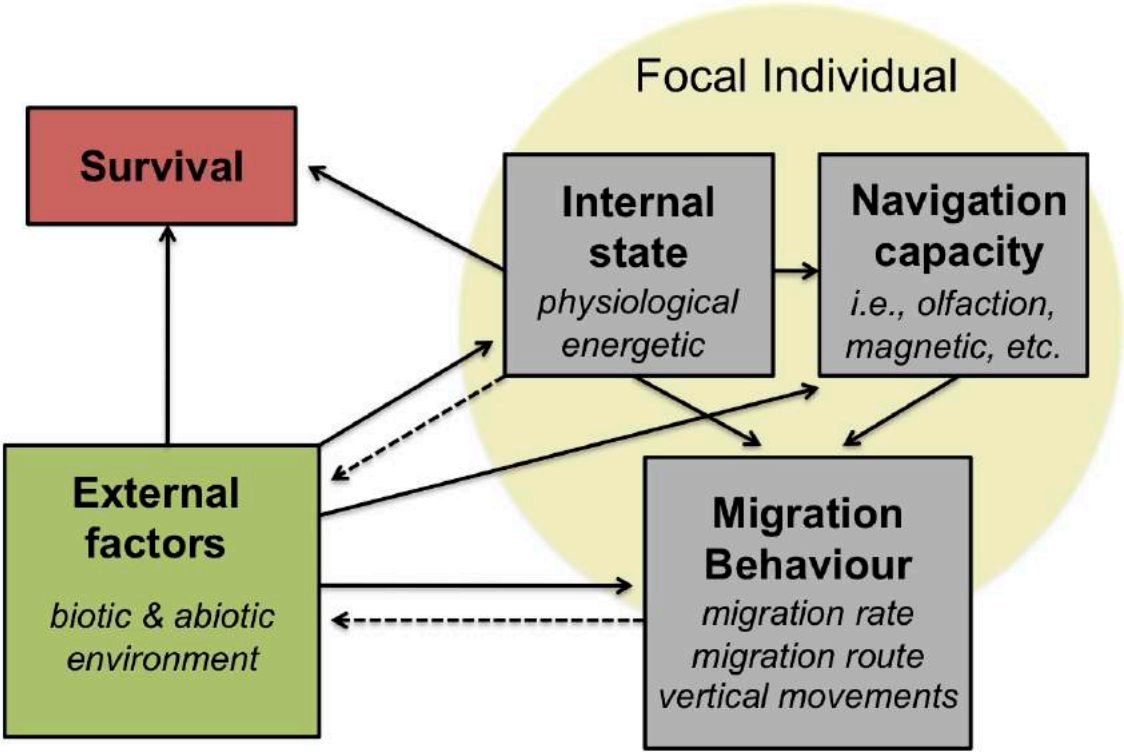

Figure 1.1 Conceptual framework for the study of animal migrations as adapted from Nathan et al. (2008). Solid arrows indicate relationships among processes while dashed arrows represent relationships for feedback mechanisms, such as how an individual's migration route influences the environmental conditions they encounter or choose to encounter based on their physiological state.

CHAPTER 2: A SYNTHESIS OF TAGGING STUDIES EXAMINING THE BEHAVIOUR AND SURVIVAL OF ANADROMOUS SALMON IN MARINE ENVIRONMENTS

2.1 Introduction

2.1.1 Importance of salmon, and recent population trends

Anadromous salmon are important ecologically, culturally, and economically across the globe, as a critical aspect of their ecological systems, as a significant commercial and artisanal fishery, and as a sensitive environmental indicator. They provide cultural and social value to local and native peoples (Augerot & Smith, 2010), and form a multi-million dollar global fishery. While at sea and in freshwater, salmon are important prey items and nutrient sources, and they continue to provide such benefits after death by supplying enrichment to terrestrial systems as they decay on the riverbed (Gende et al., 2004; Quinn, 2005). An anadromous life history means that salmon can be affected by changes in both freshwater and marine ecosystems, including widespread habitat degradation, altered ecosystem productivity, overharvest, and climate change (Beamish et al., 1997; Boisclair, 2004; Bryant, 2009; Crozier et al., 2008; Fleming & Jensen, 2002; Lichatowich, 1999; Parrish et al., 1998).

Over the last century, many populations of wild salmon have declined in abundance (Lackey et al., 2006; Nehlsen et al., 1991; Parrish et al., 1998; Quinn et al., 2005; Ward, 2000; Welch et al., 2000). In recent years, some populations have been threatened with extinction and extirpation (COSEWIC, 2003; Irvine et al., 2005) resulting in many areas that are either devoid of salmon (ICES, 2000; Lichatowich, 1999) or are

reliant on hatchery-raised salmon populations (Lackey et al., 2006). In many regions, enhancement programs such as hatcheries and fish farms (aquaculture) have been introduced in an attempt to supplement wild populations and to meet the global demands for human consumption of salmon. However, hatchery and aquaculture enhancement may have inadvertently introduced a new suite of concerns for wild populations, such as interbreeding risk resulting in a loss of genetic variation, increased competition for scarce resources and habitat, and an increase in disease prevalence and dispersal (Kallio-Nyberg & Koljonen, 1997; Pedersen et al., 2007; Sweeting et al., 2003; Unwin, 1997). The 'crisis' of declining salmon populations is currently considered one of the major issues in fisheries biology (Lichatowich, 1999), and extensive management efforts are being applied in an attempt to conserve at-risk populations. In general, population declines seem to be more drastic in southern latitudes, and are less apparent at higher latitudes (Chaput & Prevost, 1999; Coronado & Hilborn, 1998; ICES, 2005; Peterman et al., 1998). Perhaps the most alarming aspect is that the causes for the declines remain largely unknown.

The majority of research and management efforts on anadromous salmon have historically focused on the freshwater phase of the lifecycle (including outmigrating juveniles, and upriver migrating adults) (Crozier et al., 2008; Quinn, 2005). The reason for this is largely technical, as there are inherent difficulties with studying salmon in the marine environment. Consequently, current knowledge of the marine phase of the lifecycle (i.e., both juveniles and adults in saline environments including estuaries, coastal waters and open-ocean) is still quite limited, despite it being generally acknowledged as a critical stage related to low survival caused by both abiotic and biotic

factors (Bradford, 1995; Friedland, 1998; Parrish et al., 1998; Pearcy, 1992; Peterman et al., 1998; Ruggerone & Nielsen, 2004).

2.1.2 Objectives of this review

In light of the declining abundance of many wild salmon populations, and the knowledge gap relating to the behaviour and survival of salmon in the marine environment, this chapter has three main objectives. First, I reviewed the scientific literature to quantify the number and range of studies that have investigated aspects of salmon behaviour and survival in the marine environment. Owing to the impressive, informative and long-term data sets that have been generated through tagging studies, I focused this literature review on studies that utilized some form of tagging to investigate aspects of salmon biology in marine waters. Second, I sought to synthesize the current state of knowledge concerning salmon behaviour and survival in the marine environment. Third, I highlight particular knowledge gaps that require further attention and suggest some approaches, both technological and methodological, from which future studies could benefit in order to improve our understanding of salmon biology. The review is broken into various life history phases that occur within the marine environment, namely the out-migration of juveniles [plus Atlantic salmon kelts and adult steelhead (*Oncorhynchus mykiss*)], sub-adults and adults in the open-ocean, and mature adults on their return spawning migration towards freshwater. To fully appreciate the complexity of the 'salmon crisis', with an aim to target key factors that may be responsible for the global decline in abundance of wild salmon, I first examine the complex life histories of the salmon, followed by a brief review of tag types that are commonly applied to salmon.

2.1.3 Life histories of salmon

There is only one species of Atlantic salmon (family Salmonidae), while the Pacific salmon (family Salmonidae; genus *Oncorhynchus*) comprise eight species, including Chinook (*O. tshawytscha*), chum, coho (*O. kisutch*), pink (*O. gorbuscha*), sockeye, masu *(O. masou*), amago *(O. rhodurus*), and steelhead. In addition, there are anadromous forms of brown trout and sea trout (*S. trutta*), and cutthroat trout (*O. clarkii*). Various species of Pacific salmon are found on both sides of the northern Pacific Ocean (Western Canada and the U.S. from California to Alaska, Japan, Russia, and Korea), whereas Atlantic salmon are found in the north-western (Spain north to the British Isles, Greenland, Norway and Finland) and the north-eastern (eastern Canada and the U.S.) Atlantic Ocean. Both Pacific and Atlantic salmon are considered anadromous, but in many of the species there are minorities of non-anadromous forms that remain in freshwater for the duration of their lives, however the latter are not included in this review.

There is a tremendous amount of variation in the timing of different life stages between and within anadromous salmon species [see for Pacific salmon and trout: (Groot & Margolis, 1991; Quinn, 2005); for Atlantic salmon: (Mills, 1999; Verspoor et al., 2007)]. However, most anadromous salmon can be characterized by a generalized life cycle. Adults of both Atlantic and Pacific salmon spawn in freshwater streams or lakes (and some in intertidal areas; pink and chum salmon) and either die soon after (semelparous species of Pacific salmon), or have the ability to survive the spawning period (i.e., iteroparous species). Eggs deposited in substrate hatch to produce alevins,

which remain under gravel and use a yolk sac for nutrition until they emerge as fry four to six weeks later. At this point, some species migrate directly to the ocean, while others remain in freshwater as parr and feed on small aquatic organisms typically for one to two years before migrating to the ocean. In the spring of a subsequent year, fish still in freshwater become smolts and migrate to the sea to forage and mature for a number of years before returning to natal spawning grounds to reproduce. The return spawning migration is among the most spectacular in the animal kingdom, with some species traversing entire oceans before entering freshwater and migrating up to 1,500 km upriver to spawn (Crozier et al., 2008; Groot & Margolis, 1991).

2.1.4 Overview of tagging technologies and techniques

Various types of tags have historically, and are currently used for research on salmon. Tags can be grouped into three main categories: passive, electronic, and biological. Passive tags are those which do not have an inbuilt battery, they often involve a visual marking of the fish, and they are primarily used for identification of individuals or groups once they are recaptured or within sight. Passive tags include external marks (e.g. adipose fin clips), external visual tags (= t-bar anchor tags [e.g. Carlin, Floy, Peterson Disk, cinch tags]) and internally injected tags such as coded wire tags (CWT) and passive integrated transponder (PIT) tags. While PIT tags are characterised as passive, they use radio frequency energy from an antenna or a closely held scanner to power the tag circuits and allow a unique identifying signal to be transmitted.

Electronic tags [reviewed in Cooke et al. (2012)] were characterized as those that possess an inbuilt battery and may either store acquired data to an onboard memory chip

[e.g. archival tags (= data loggers)] or transmit the data, typically via acoustic or radio transmission, to a nearby receiver (e.g. standard acoustic and radio tags). There exist combined technology tags, such as pop-off satellite tags (PSATs) and smart position or temperature transmitting tags (SPOTs), which first archive and then transmit data to a satellite. Electronic tags have been used to measure a great range of environmental, behavioural and physiological information from fish, including temperature, depth, light, global or local position, acceleration, swimming muscle contractions, and heart rate (Block, 2005; Block et al., 1998; Clark et al., 2010; Clark et al., 2008; Cooke et al., 2004b; Kawabe et al., 2003; Tanaka et al., 2001). Radio signals attenuate rapidly in seawater, so radio tags are typically restricted to freshwater environments or when the radio signal can transmit through air such as with PSATs or SPOT tags. Acoustic tags, whether manually tracked by boat or automatically by an array of installed receivers, have proven useful in both marine and freshwater environments, although signal transmission can be affected by water depth and extraneous acoustic noise. Electronic tags are typically several orders of magnitude larger and more expensive than passive tags, which can both lower sample sizes within a study and restrict tagging to large individuals. Electronic tags that transmit allow for tracking along a migration route, meaning that tag recovery is not necessary to obtain data. Archival tags can acquire data even when fish are not within range of a receiver, but they must be recovered to download stored data.

Biological tags, or ‘natural tags’, include natural distinguishable markings, scale measurements, parasite identification, otolith (earbone) analysis, and DNA identification, many of which can provide information on factors such as fish age and habitats traversed.

Biological tags are used without prior capture of the fish, thus eliminating any potential effects of capture and handling [for reviews see (Bakke & Harris, 1998; Campana et al., 1995; MacKenzie, 2002)]. Methods such as otolith sampling necessitate that the fish be killed prior to sampling, while other methods can be performed non-lethally. Although methods such as parasite and DNA identification may not be considered 'tagging' in a classical sense, such methods have been used to provide detailed information concerning the origin and movement patterns of the fish.

2.2 Methods

Literature searches were carried out using two commercial academic search engines, ISI Web of Knowledge and Aquatic Sciences and Fisheries Abstracts, with a focus on peer-reviewed journal articles published in the English language as early as 1900 and extending to September 2011. I used combinations of key terms to focus search results on literature that used tagging as a method to study movement, behaviour, or survival in marine ecosystems of anadromous salmon within the genera *Oncorhynchus* and *Salmo*. Specifically, I focused on research of anadromous forms of Pacific salmon (pink, sockeye, Chinook, coho, chum, amago, masu), Atlantic salmon, as well as anadromous brown and sea trout, steelhead, and cutthroat trout (see Appendix A for exact Boolean search terms).

Search results from both academic search engines were pooled and duplicates removed. All abstracts from resulting papers in the search databases were read in order to eliminate any papers that did not meet the criteria for inclusion in the literature review; the study had to involve some form of tagging of free-living salmon (i.e. salmon released

into the natural environment) and results had to include information on behaviour or survival in the marine environment. A descriptive review was performed on the papers meeting my criteria.

For the descriptive review, a spreadsheet was first constructed with predetermined variables to be queried of each paper. The variables were chosen as a means to address the author's objectives, methods, and results. Examples of variables that were queried of papers include the year of study, author's motivation (i.e. basic biology, conservation, enhancement, fisheries management), geographic location, fish natal origin, species, life stages, tag types, author's inferred variables from tags (i.e. swim speed, travel behaviour, location, survival), handling/tagging effects (i.e. measured, acknowledged, not mentioned), tag loss/failure (i.e. measured, acknowledged, not mentioned), tag detection efficiency (i.e. measured, acknowledged, not mentioned), hatchery/farmed vs. wild fish, environmental variables tested, and physiological variables tested. Although I limited the descriptive review to peer-reviewed articles from directed searches, information from relevant government and non-government agency reports were incorporated into the review where appropriate, but not into the numerical results.

2.3 Results & discussion

2.3.1 General observations

I identified 207 peer-reviewed articles published in the English language that met my criteria of using tagging in free-living fish to address anadromous salmon behaviour or survival in the marine environments. The earliest publication resulting from the literature review appeared in 1940 (Belding, 1940). As expected, the number of

publications continuously increased since then (Figs. 2.1, 2.2), reflecting an increasing use of tagging for gathering information on salmon in marine environments. The main motivation for research was primarily the pursuit of basic biological information (75.4%; n=156), followed by fisheries management (30.4%; n=63), achievement of broad conservation goals (23.2%; n=48), development or testing of tagging methodologies (14.5%; n=30), assessment of salmon enhancement (14.5%; n=30), assessment of habitat degradation (5.3%; n=11), and climate change (2.9%; n=6). Given the widespread recognition of the impacts that global warming is having and will probably continue to have on aquatic systems (IPCC, 2007), I would expect that climate change, which was the least identified motivation of any of the research I reviewed, to increase in study over coming years.

Irrespective of fish species or study origin, the majority of research in which geographic location was specifically defined (n=207) was performed in the northeast Pacific Ocean (45.9%; n=95) and the northeast Atlantic Ocean (35.7%; n=74). Other locations included the northwest Atlantic Ocean (9.2%; n=17), northwest Pacific Ocean (6.3%; n=13), Bering Sea (3.9%; n=8), and southern Pacific Ocean near New Zealand (1.9%; n=4). Out of 206 studies that defined fish natal origin, fish stocks from Norway/Finland (24.6%; n=46) and British Columbia/Puget Sound (24.1%; n=55) have been the most studied, followed by the continental U.S. west coast (19.3%; n=44), British Isles (9.2%; n=21), eastern Canada/U.S. (8.8%; n=20), Japan/Russia (6.6%; n=15), Alaska (5.7%; n=13) and New Zealand (1.8%; n=4). Furthermore, the majority of studies examined fish of hatchery origin (37.7%; n=60) compared to wild origin (12.6%; n=20), ranched (sea cage) origin (4.7%; n=7), a combination of wild and hatchery origin (30.0%;

n=47), or a combination of ranched and hatchery origin (3.1%; n=5). Out of the total occurrences of species within the research [i.e. (n=245) because some studies examined more than one species], Pacific salmon were the most frequently studied (58.4%; n=143), whereas Atlantic salmon and anadromous trout comprised 38% (n=93) and 3.7% (n=9) of studies, respectively (Fig. 2.1). Within the Pacific salmon, Chinook was the most studied (27.3%; n=39), followed by coho (21.7%; n=31), sockeye (19.6%; n=28), steelhead (11.9%; n=17), chum (11.2%; n=16), pink (7.0%; n=10), and masu (1.4%; n=2) salmon. Overall, these results indicate very skewed distributions of research in terms of geographic location, species, and stock origins.

Various forms of tag technologies have been employed throughout the last half-century. Passive tag use has increased in recent decades, and out of the total number occurrences of tags [i.e. (n=255) because some studies use more than one tag type], this was the most common tagging approach that I identified (57.3%; n=146) (Fig. 2.2**)**. In regard to the total number of occurrences of tags in the literature (n=255), acoustic tags were the single most dominant tagging method (27.0%; n=69), followed by CWTs (19.2%; n=49), external visual tags (t-bar anchor) (19.0%; n=48), external markings (12.9%; n=33), radio tags (9.4%; n=24), PIT tags (6.3%; n=16), data loggers (4.7%; n=12), and various forms of biological tags (e.g. otoliths, parasites, scales) (1.6%; n=4). When external markings were used (n=33) they were primarily combined with another form of tagging (75.8%; n=25). When acoustic transmitters were used (n=102), they were applied primarily to study juveniles (Tables 2.1, 2.2). In contrast, when data loggers were used (n=13), they were applied primarily to study adults in the open-ocean during or prior to their spawning migration to freshwater (Tables 2.1, 2.2), likely reflecting tag size, and

efforts to maximize tag retrievals by relocating fish once they arrive at spawning grounds. Biological tags are relatively new techniques and were used in only four (1.6%) studies. The low number of studies using biological tags may have been an artefact of the literature search terms being too narrow to locate more of these studies.

Nearly 40% of studies examined multiple life stages (36.2%; n = 75), whereas the majority only examined a single life stage (63.8%; n = 132). External tags and CWTs were used most frequently in studies that examined multiple life stages beginning at the juvenile stage (Tables 2.3, 2.4), likely because they can be applied to large numbers of juvenile fish at a low cost, there is a publicly available database of CWT data (RMPC), and because early marine juvenile survival is thought to be important when considering lifetime fitness. Acoustic transmitters were employed most frequently when the research objectives were to examine just one life stage (Tables 2.3, 2.4), an issue largely related to limited transmitter battery life.

The most frequent variable authors inferred from tagging studies was survival (59.0%; n=122), various travel behaviours (e.g. holding, vertical migrations) (44.0%; n=91), assessments of fish position or location (37.2%; n=77), swim speed (26.6%; n=55), migration route (23.7%; n=49) and origin (9.7%; n=20). A large proportion of the studies did not directly assess potential mechanisms influencing survival or behaviour; less than half of the studies (45.0%; n=93) reported on linking environmental variables to tagging results, and even fewer (13.5%; n=28) looked for associations between individual physiology and tagging results. Temperature was the most common environmental variable found to be associated with behaviour (17.9%; n=27) and survival (9.1%; n=6) (Table 2.5). Among physiological variables the authors tested, energetic state of the fish

was most commonly associated with behaviour (2.0%; n=3), whereas ionoregulatory state of the fish was most commonly associated with survival (4.5%; n=3) (Table 2.5). Other variables commonly found to be associated with behaviour or survival included fish size and stock effects (i.e. populations, wild versus hatchery) (Table 2.5). However, this does not necessarily mean that these particular variables are the most important in affecting salmon behaviour or survival, as the variables were not equally tested for among studies.

2.3.2 Life-stage specific observations

2.3.2.1 Juvenile salmon and Atlantic salmon kelts

Most tagging studies focused on the juvenile portion of the salmon life cycle (65.7%; n=136), likely because this life stage exhibits high and variable mortality rates, as well as a result of the ease of capture of fish, relatively high abundance, proximity to research institutions during outmigration (near river mouths and urban areas), and availability from hatchery programs. In contrast, research on the kelt life stage of Atlantic salmon was the least common focus, comprising only 5.0% (n=6) of studies on iteroparous species (n=119). Although less studied than juvenile out-migrations, kelt out-migration behaviour and survival patterns mirrored that of outmigrating smolts (Hedger et al., 2009) so will be discussed in combination.

Juvenile pink, sockeye, steelhead, Atlantic salmon and Atlantic salmon kelts tend to move actively and rapidly through coastal (continental shelf) waters during out-migration to the ocean (Hedger et al., 2009; Lacroix et al., 2005; Lacroix & McCurdy, 1996; Martin et al., 2009; Melnychuk et al., 2010; Thorstad et al., 2004; Welch et al., 2011). Chum, coho and Chinook tend to migrate at a much slower rate and can remain in

coastal waters for longer periods of time (Bond et al., 2008; Healey & Groot, 1987; Melnychuk et al., 2010; Welch et al., 2011). Apart from differences between species, movement rates through estuarine and coastal environments vary between population, fish origin [e.g. hatchery vs. wild; (Chittenden et al., 2008; Melnychuk et al., 2010; Thorstad et al., 2007)] and body size (Bond et al., 2008; Chittenden et al., 2008).

Juvenile salmon in coastal waters tend to migrate during ebb tides and at night (Aprahamian & Jones, 1997; Lacroix & McCurdy, 1996; Martin et al., 2009), swimming actively within tides (Chamberlin et al., 2011; Lacroix et al., 2004; Moore et al., 1998). While estimates of swimming speed show some variability [e.g. from 0.53 body lengths per second (bl s^{-1}) (Thorstad et al., 2007) up to 4 bl s^{-1} (Lacroix & McCurdy, 1996)], an average routine rate of 1 bl s^{-1} is common (Finstad et al., 2005). Laboratory swimming respirometry studies have found that a speed of 1 bl s^{-1} is associated with a minimum gross cost of transport (Brett, 1995). Juveniles and kelts often exhibit clear diel vertical and horizontal movement patterns. Nocturnal migration tends to be more rapid than movement during the day (Aprahamian & Jones, 1997; Martin et al., 2009). Swimming depth during the day tends to be quite shallow, within 1-3 m of the ocean surface, and even less (< 0.5 m) during the night (Davidsen et al., 2008). Changes in swimming depth and migration speed may be strongly related to temperature and salinity (Manel-La et al., 2009), or light conditions (Davidsen et al., 2008), the latter perhaps being a strategy related to predator avoidance (Reddin et al., 2006). Indeed, vertical movement trends may be closely linked to the feeding patterns of avian predators, resulting in movement downward in the water column during daylight hours (Reddin et al., 2006).

Mortality during the juvenile out-migration stage is higher than during other

marine life history stages, even when compared to the lengthy adult open-ocean stage (Moore et al., 2010). Using acoustic telemetry, mortality of juveniles departing coastal waters has been shown to be very high (Thorstad et al., 2007; Welch et al., 2011), although recent research has shown that juvenile mortality in the open ocean may be even higher (Welch et al., 2011). Estimates of survival for early ocean migrating salmon have been made for Atlantic salmon (Dempson et al., 2011; Lacroix et al., 2004; Thorstad et al., 2007; Thorstad et al., 2011), Chinook (Duffy & Beauchamp, 2011; Welch et al., 2011), coho (Chittenden et al., 2008; Welch et al., 2011), chum (Fukuwaka & Suzuki, 2002), sockeye (Welch et al., 2011; Welch et al., 2009), steelhead (Balfry et al., 2011; Melnychuk, 2009; Moore et al., 2010; Welch et al., 2011; Welch et al., 2004) and anadromous brown trout (Thorstad et al., 2007). Juvenile survival can be affected by a multitude of factors including predation (Collis et al., 2001; Dieperink et al., 2002; Hvidsten & Mokkelgjerd, 1987; Kennedy et al., 2007), competition (Beamish et al., 2008), parasites (Hvidsten et al., 2007; Sivertsgard et al., 2007), inability to osmoregulate (Chittenden et al., 2008; Fuss & Hopley, 2003; Kennedy et al., 2007), pollution (Heintz et al., 2000), marine entry timing (Beamish et al., 2008; Staurnes et al., 1993; Thorstad et al., 2007; Zabel & Williams, 2002), adverse ocean conditions (temperature, salinity, oxygen, pH, productivity) (Farmer, 1994; Lyse et al., 1998; Serrano et al., 2009), dams encountered in freshwater (Welch et al., 2008), and smolt size (Duffy & Beauchamp, 2011; Farmer, 1994).

Furthermore, survival rates have been shown to differ between hatchery and wild fish. Survival estimates for wild fish tend to be higher than those for hatchery juveniles (Beamish et al., 2008; Buchanan et al., 2010; Chittenden et al., 2010a; Chittenden et al.,

2008; Kallio-Nyberg et al., 2004; Melnychuk, 2009; Moore et al., 2010; Siira et al., 2006; Unwin, 1997; Welch et al., 2004). In one study, survival of wild steelhead smolts during migration away from inshore waters ranged from 18-39%, while hatchery smolt survival was 3% (Melnychuk, 2009). Trends such as this suggest a discrepancy in fitness between the two groups, possibly due to differences in physiology (Chittenden et al., 2008), behaviour (Hvidsten & Johnsen, 1993), and size (Farmer, 1994; Heggberget et al., 1993; Jutila et al., 2003; Kallio-Nyberg et al., 2007).

2.3.2.2 Open-ocean

The open-ocean migration of salmon has been studied the least frequently, being the primary focus of only 8.7% (n=18) of tagging studies. This is likely due to the difficulty of accessing fish within this environment, technological constraints, and associated financial costs. In fact, much of what is known about salmon migration in the open-ocean comes from early research by fisheries capture and the use of external tags. This type of research was performed by international organizations such as the North Atlantic Salmon Conservation Organization (NASCO) (Atlantic salmon), and the North Pacific Anadromous Fish Commission (NPAFC) (Pacific salmon), which provided some of the first scientific insights into the open-ocean behaviour and ecology of anadromous salmon at sea. This early research revealed that salmon populations are often highly mixed at sea. For example, Pacific salmon stocks from Japan, Russia, Canada, and the United States utilize several of the same marine feeding grounds (Burgner et al., 1992; French et al., 1976; Godfrey et al., 1975; Major et al., 1978; Neave et al., 1976; Takagi et al., 1981).

A small number of recent studies have utilized recovery of data loggers and manual tracking of fish tagged with acoustic transmitters to assess fine scale movements of salmon in the open-ocean. Salmon migrating in the open-ocean tend to swim at speeds of 1 bl s^{-1} on average (Ogura & Ishida, 1992; Ogura & Ishida, 1995; Quinn et al., 1998), which is similar to average swim speeds observed in other life stages (see above). Vertical distribution in the water column varies diurnally, seasonally, and by species [e.g. Chinook dive below 50 m whereas most other species remain within the upper 20 m of the water column (Ogura & Ishida, 1995)], as determined by acoustic tracking of tagged individuals (Ogura, 1999; Ogura & Ishida, 1992; Ogura & Ishida, 1995) and data logger recoveries (Azumaya & Ishida, 2005; Friedland et al., 2001; Ishida et al., 2001; Walker et al., 2000). Vertical migrations are most likely related to maximizing foraging efficiency (Ishida et al., 2001), predator avoidance, and for navigational purposes (Quinn, 1991).

There are several factors that are thought to influence salmon survival in the open-ocean, including migration routes, timing, food availability, predator levels, ocean conditions (Chittenden et al., 2009a; Friedland et al., 2000; Hinch et al., 1995; Kallio-Nyberg et al., 1999; Salminen & Kuikka, 1995) and carry-over effects from earlier life stages (Rechisky et al., 2009). However, relatively few tagging studies have estimated survival in the open-ocean and the limited results suggest that survival rates can vary considerably among species and populations, and the causes remain poorly understood. For example, to investigate trends in survival across the northeast Pacific over a long time scale, a study using CWT data from coho salmon found that ocean survival of northern stocks (northern BC and Alaska) increased from the 1980s to 1990, whereas survival of southern stocks has been declining over the same time period (Coronado &

Hilborn, 1998). This inverse-covariablity between northern and southern latitude salmon production has been similarly shown in other salmon species (Mueter et al., 2002), and is thought to be associated with changing ocean regimes (Mantua & Hare, 2002; Mantua et al., 1997). However, using CWT data, a more recent study found no significant inverse-covariability on interannual timescales between northern and southern stocks of coho salmon (Teo et al., 2009), which demonstrates our lack of understanding on the processes influencing population dynamics of salmon in the open-ocean.

2.3.2.3 Return migration

Although they can travel thousands of kilometers in high seas, most maturing salmon have the ability to navigate back to natal freshwater streams upon reaching maturity. Nevertheless, straying behaviours (e.g. individuals spawning in non-natal waters) are present in several species (Candy & Beacham, 2000; Eriksson & Eriksson, 1991; Labelle, 1992; Larsson, 1984; Pedersen et al., 2007; Schroeder et al., 2001) and may represent an important evolutionary survival strategy. Even though some populations in certain watersheds (e.g. the Fraser River) have recently exhibited variable river entry timing (Cooke et al., 2004a), upriver spawning migrations by mature adults usually commence within reasonably predicable time frames (Gilhousen, 1990; Woodey, 1987). Such predictability certainly facilitates the study of this life stage, which ranked second in the analysis (22.2%; n=46).

Timing and location of arrival of salmon to the continental shelf from ocean feeding grounds is based on environmental factors in the ocean (Groot & Quinn, 1987; Healey & Groot, 1987; Quinn & Dittman, 1990; Thomson et al., 2007) and physiological

state of the fish (Cooke et al., 2008a; Crossin et al., 2009a). Swim speed for returning adults has been determined simplistically using manual tracking of individuals (Ogura & Ishida, 1995; Quinn et al., 1989), and by more sophisticated means using data loggers that directly measure swim speed (Tanaka et al., 2005). Again it emerges that adult salmon are observed to routinely swim at average speeds around 1 bl s^{-1} (Ogura & Ishida, 1995; Quinn et al., 1989; Tanaka et al., 2001). Migration rates and timing are influenced by a range of environmental factors [reviewed in Hinch et al. (2006)], some of which are tidal currents, wind-generated currents, salinity levels and temperature (Karppinen et al., 2004; Levy & Cadenhead, 1995; Olson & Quinn, 1993; Smith et al., 1994; Smith & Smith, 1997; Solomon & Sambrook, 2004; Stasko et al., 1976). As in the open-ocean, vertical position in the water column in coastal areas can vary among species and even within species between relatively short distances on continental shelves. For example, manually tracked sockeye salmon were observed to choose different depths when swimming in well mixed coastal waters versus stratified coastal waters, preferring deeper water when they encountered a stratified water column created by river discharge (Quinn et al., 1989). Vertical movements may be related to species preferring narrow ranges of temperature (Hinke et al., 2005). Several species continue to exhibit diel vertical movement patterns during this portion of their life (Candy & Quinn, 1999; Ishida et al., 2001; Karppinen et al., 2004; Quinn et al., 1989), which may be a behaviour used to conserve energy prior to river migration, avoid predators, prepare osmotically for freshwater entry, or aid in navigation (Hinch et al., 2006; Hinke et al., 2005; Olson & Quinn, 1993).

Though only a few studies have focused on aspects of salmon physiology, the

role of physiological state as a key driver of return migration behaviour and survival is highlighted by a series of studies conducted on Fraser River sockeye salmon. Specifically, fish with advanced reproductive preparedness (e.g. elevated plasma concentrations of reproductive hormones, including testosterone, 11-ketotestosterone, and 17β-estradiol) migrated fastest coastally and entered the river earlier (Cooke et al., 2006b; Crossin et al., 2009a; Crossin et al., 2007). Marine survival was related to physiological stress such that fish with elevated plasma ion, glucose and lactate levels perished in coastal waters before entering the river (Cooke et al., 2006a; Cooke et al., 2006b; Crossin et al., 2009a). Survival was also lower in fish that were less physiologically prepared for freshwater entry [i.e., higher plasma chloride and total osmolality (Crossin et al., 2009b)]. These studies provide examples of how telemetry can be combined with physiological measurements to address research questions.

2.3.3 Knowledge gaps and future directions

2.3.3.1 Core knowledge

This review identified several priority areas for research due to inadequate investigation to date. I believe these knowledge gaps constrain the current understanding of salmon in marine environments, and potentially limit the application of contemporary tagging technologies for management and conservation purposes. Below, I discuss each area and give recommendations to address these concerns wherever possible.

Globally, knowledge of the impact of climate change on salmon behaviour and survival in the marine environment is limited. Less than half of tagging studies analyzed in this review attempted to link abiotic factors such as temperature, salinity, oxygen, and

productivity to salmon behaviour or survival [except see Azumaya & Ishida (2004), Logerwell et al. (2003), Pyper & Peterman (1999), Ryding & Skalski (1999), Welch et al. (1995); Welch et al. (1998); Welch et al. (2000)]. Major climatic changes have already occurred (Bond et al., 2008), and shifts in ocean temperatures, salinity, oxygen concentration, pH, and prey abundance are expected to intensify (Caldiera & Wickett, 2003; Meehl et al., 2007), with profound compounding effects on salmon distribution and survival (Crozier et al., 2008). Tagging can be a powerful tool to increase our understanding of the impacts of environmental change on salmon, particularly if studies are long-term and combined with effective environmental monitoring (e.g. through the use of data loggers). Furthermore, experimental studies that manipulate temperature or salinity can be combined with biopsy and telemetry techniques to further contribute to the knowledge base [e.g. Cooperman et al. (2010)].

In addition, certain regions (e.g. Bering Sea, northwest Pacific Ocean, New Zealand), populations (e.g. those from Alaska, Japan/Russia, New Zealand), life stages (e.g. open-ocean, kelts) and species (e.g. pink, masu) are underrepresented in the literature. Most of the research I analyzed examined hatchery fish rather than wild fish, and relatively few tagging studies compared the two [except see Beamish et al. (2008), Buchanan et al. (2010), Chittenden et al. (2010a), Chittenden et al. (2008), Johnson et al., (2010), Kallio-Nyberg et al. (2004), Kallio-Nyberg et al. (2011), Melnychuk (2009), Melnychuk et al. (2010); Moore et al. (2010), Siira et al. (2006), Unwin (1997), Welch et al. (2011), Welch et al. (2004)], despite known differences in behaviour and survival. For example, wild populations commonly display adaptive plasticity in migration timing due to environmental variation and as a means of avoiding interspecies competition (Beamish

et al., 2008), while hatchery raised fish are manually released according to a hatchery schedule (Chittenden et al., 2010a). Hatchery fish often have lower fitness and subsequent survival in natural environments than wild stocks (Chittenden et al., 2010a). This suggests that conclusions from tagging studies using hatchery fish should perhaps not be applied broadly to wild populations. Tagging studies among populations, as well as between hatchery and wild fish, could provide insights into key differences among such groups.

There are also limited data on full life cycle (i.e., outmigrating smolts to returning adults) analyses, as very few studies assess more than one life stage at one time, a method that does not account for any cumulative effects throughout the life history. For instance, juvenile growth rates can affect fitness and survival in all remaining life stages, and successful development at sea may have cascading effects on subsequent reproductive maturation and spawning success. Tagging juveniles and assessing the entire life cycle while monitoring abiotic factors may provide powerful insights into which environmental effects have the greatest impact on lifetime fitness. Various technologies exist that could be implemented on a large scale relatively inexpensively, such as external visual tags or PIT tags, however, more expensive acoustic tags or data loggers could provide more detailed information on both biotic and abiotic factors.

Finally, I identified a definite lack of research on salmon survival and mortality at sea. Although some research has looked at lifetime survival through tagging, these studies were unable to determine exactly where and why mortality occurs. Understanding lifetime survival rates is critical to understanding population viability, yet there is no conclusive data to date to suggest which life stage is associated with the highest

mortality. This has made it challenging to relate environmental variables to mortality across life stages. While current technologies cannot yet provide precise estimates of location and cause of mortality, this may change in the near future. For example, to control the problem of limited battery life, acoustic transmitters have now been designed that can 'turn off' while salmon are at sea, and then power-up 2-3 years later upon return migration to freshwater where they can be tracked with acoustic arrays (Welch et al., 2009).

2.3.3.2 Tagging models, procedures and technologies

A common feature of studies designed to estimate survival from tagged animals in the wild is the potential for imperfect (i.e. < 100%) encounter (i.e. detection or recapture of electronic and passive tags, respectively) probabilities. When researchers do not account for encounter probabilities that are < 100%, survival estimates will be biased low, and erroneous interpretations of results can occur in cases where encounter probabilities vary among tagged fish belonging to different strata (e.g. sex) or assigned to different experimental treatments (Lebreton et al., 1992). Capture-recapture models for open populations have been developed since the 1960's to deal explicitly with imperfect encounter probabilities in the estimation of survival and other demographic parameters from tagged animals (Amstrup et al., 2005). However, despite the long-standing availability and continued development of capture-recapture models and specialized computer software for their implementation, only 20.9% (n=23 out of 110) of the studies where capture-recapture models were applicable have accounted for imperfect encounter probabilities in the estimation of survival for anadromous salmon. Indeed, in general

there seems to be little appreciation and use of capture-recapture models in fisheries research (Pine et al., 2003). Encounter probabilities have been measured for multiple species of salmon smolts using the Pacific Ocean Shelf Tracking (POST) array in coastal waters [reviewed in Welch et al. (2011)].

A related class of models allows researchers to estimate survival from tag recoveries of harvested animals (Brownie & Robson, 1983) or from both live encounters and tag recoveries (Burnham, 1993). Tag recovery, whether by commercial fisheries or by other means, was used in 63.5% (n=129) of studies reporting how tag data were retrieved. The use of models based on tag-recovery data to estimate survival could be applied to these studies. An interesting application of models based on tag recovery is the possibility to separate fishing from natural mortality if an estimate of tag reporting probability is available (Pine et al., 2003). Several experiments have been proposed to estimate the probability that tags are reported. For example, reporting probability can be estimated as the recovery of standard (i.e. no- or low-reward) tags relative to high-reward tags (assuming these are 100% reported if encountered) (Pollock et al., 2001); or by planting tags into fisheries catches and calculating the ratio between planted tags reported and the known number of tags that were planted (Hearn et al., 2003).

Both capture-recapture and tag-recovery models are based on the assumption that tags are not lost or shed and, in the case of electronic tags, that they do not fail. If this assumption is violated, survival will be underestimated (Amstrup et al., 2005). However, tag loss/shed was measured in only 7.7% (n=16) of studies and was not even acknowledged in 69.0% (n=143) of studies where it could possibly have occurred. Double-tagging individuals could minimize the impacts of tag loss/shed on survival

estimates (Kendall et al., 2009), an approach that was employed in 28.5% (n=59) of the studies. Assuming that loss/shed of the two tags are independent, information on the number of fish recaptured with one or both tags could be used to estimate the probability of tag loss/shed and then used to adjust survival estimates (Kendall et al., 2009). Alternatively, multistate capture-recapture models could be used to jointly estimate survival and tag/shed loss (Conn et al., 2004). When looking exclusively at studies using electronic tags (n=69), 11.6% (n=8) measured tag failure. These measures are important as they allow researchers to construct time to failure curves for the electronic tags. This information, along with fish detection times, can be incorporated into the likelihood function of a capture-recapture model to account for tag failure into estimates of survival (Cowen & Schwarz, 2005; Townsend et al., 2006). A similar approach could also be used to account for the loss/shed of passive tags into survival estimates (Cowen & Schwarz, 2005).

Another important assumption of capture-recapture and tag-recovery models is that tagging does not affect survival; otherwise survival estimates will be biased low (Amstrup et al., 2005). Capture methods (Chopin & Arimoto, 1995), tag types (Bailey et al., 1998), tagging methods (e.g. external attachment, surgical application, gastric insertion, injection) (Cooke et al., 2011b; Hall et al., 2009), the use of anesthetics, handling time, tag size and release technique (e.g. recovery period) can all impact survival of the tagged fish. Capture-recapture models can be modified to account for short-term tagging effects on survival of newly tagged individuals (Brownie & Robson, 1983). In fact, tagging effects are not only issues in studies of survival but also of movement and behaviour (Bridger & Booth, 2003; Cooke et al., 2011b). However, only

10.6% (n=22) of studies assessed tagging/handling effects, and an acknowledgment of potential tagging/handling effects was made in only 33.8% (n=70) of studies. Tag size is a major limitation in salmon research, especially in studies of juvenile fish (McMichael et al., 2010), and very few of the studies I reviewed assessed survival costs or tag burdens on juveniles [except see Chittenden et al. (2009b), Chittenden et al. (2010b), Chittenden et al. (2008), Hall et al. (2009), Melnychuk (2009), Moffett et al. (1997), Voegeli et al. (1998)]. While there have been a number of studies performed under laboratory settings to assess tag effects to supplement field studies or to model tag limits for certain species (Cooke et al., 2005; Finstad et al., 2005; Hall et al., 2009; Thorstad et al., 2009; Welch et al., 2007), few studies conducted these trials under field conditions [except see Cooke et al. (2005), Halttunen et al. (2010), Welch et al. (2011)].

Remarkable advancements have been made in the field of fish tagging throughout the last few decades. Movement towards electronic rather than passive tags has enabled researchers to more thoroughly investigate the movement and survival patterns of individual salmon in the marine environment. Nevertheless, the historic (and ongoing) studies that utilized passive tags (primarily CWT, and/or adipose fin clip) remain some of the most enlightening due to their large sample sizes across multiple years (cost effectively), and their applicability to very small juveniles. Clearly, this is an area where current electronic tagging technologies require further advancement to minimize costs, decrease tag sizes, and thus allow long-term studies to be conducted with an aim to more comprehensively examine the interannual variability in salmon biology.

Indeed, some manufacturers have concentrated on miniaturizing electronic tags such that they are of use in very small fish, such as the recently developed JSAT tags

(McMichael et al., 2010). At present, many of these miniaturized tags emit an acoustic or radio signal such that the fish can be detected when they swim within range of particular receivers. Owing to these tags, an excellent database is accumulating regarding the early marine phase of the lifecycle of salmon smolts, including aspects of behaviour and survival (Chittenden et al., 2008; Fukuwaka & Suzuki, 2002; Melnychuk, 2009; Moore et al., 2010; Welch et al., 2011; Welch et al., 2009; Welch et al., 2004). A limitation of these studies is that fish must be presumed dead if they are not detected on subsequent receivers following their detection on a prior receiver. This results in areas between receivers where many fish may have disappeared for reasons that cannot be ascertained with current technologies and infrastructure.

Though not frequently used yet, multi-sensor tags are one future development that holds considerable promise as they allow detailed insight into the behaviour (e.g. acceleration, tail beat frequency, dive patterns) and physiology (e.g. heart rate, blood oxygen status) of individual fish in the natural environment (Block, 2005; Block et al., 1998; Clark et al., 2010; Clark et al., 2008; Kawabe et al., 2003; Tanaka et al., 2001). While many adult salmon can accommodate certain multi-sensor tags, miniaturization of the tags to the point where they can be used in smolts is some distance into the future. Multi-sensor tags are typically archival due to the inherent difficulties of transmitting data from multiple sensors to a receiver during the transient period when the fish is in range. This limitation is guiding engineering research to develop archival tags that transmit stored data intermittently to receivers whenever the fish is in range. The transmission will continue where it left off once the fish is in range of a subsequent receiver. An exciting prospect is that other animals may ultimately act as ‘receivers’.

That is, large animals (e.g. sharks, whales) that are capable of carrying a PSAT, for example, could receive data from nearby smaller animals (e.g. juvenile and adult salmon) and transmit the data both from themselves and from the smaller animals to satellite receivers. Termed 'business card' tags, these technologies promise exciting avenues for salmon research in the future (Holland et al., 2009).

2.4 Conclusions

Tagging and telemetry are tools that have the potential to integrate research and researchers across disciplines to advance our knowledge of salmon behaviour, physiology and survival. By combining passive and newly emerging electronic and biological tagging approaches, incorporating environmental, physiological and behavioural observations into tagging studies, and utilizing broad-scale telemetry arrays and curtains (e.g. Pacific Ocean Shelf Tracking Project – POST, Ocean Tracking Network – OTN, Tagging of Pelagic Predators – TOPP), multi-life stage and multi-trophic level investigations are within reach [see Cooke et al. (2008b)]. Finally, international collaboration, as is occurring in projects such as OTN and TOPP, will greatly benefit salmon research in the marine environment.

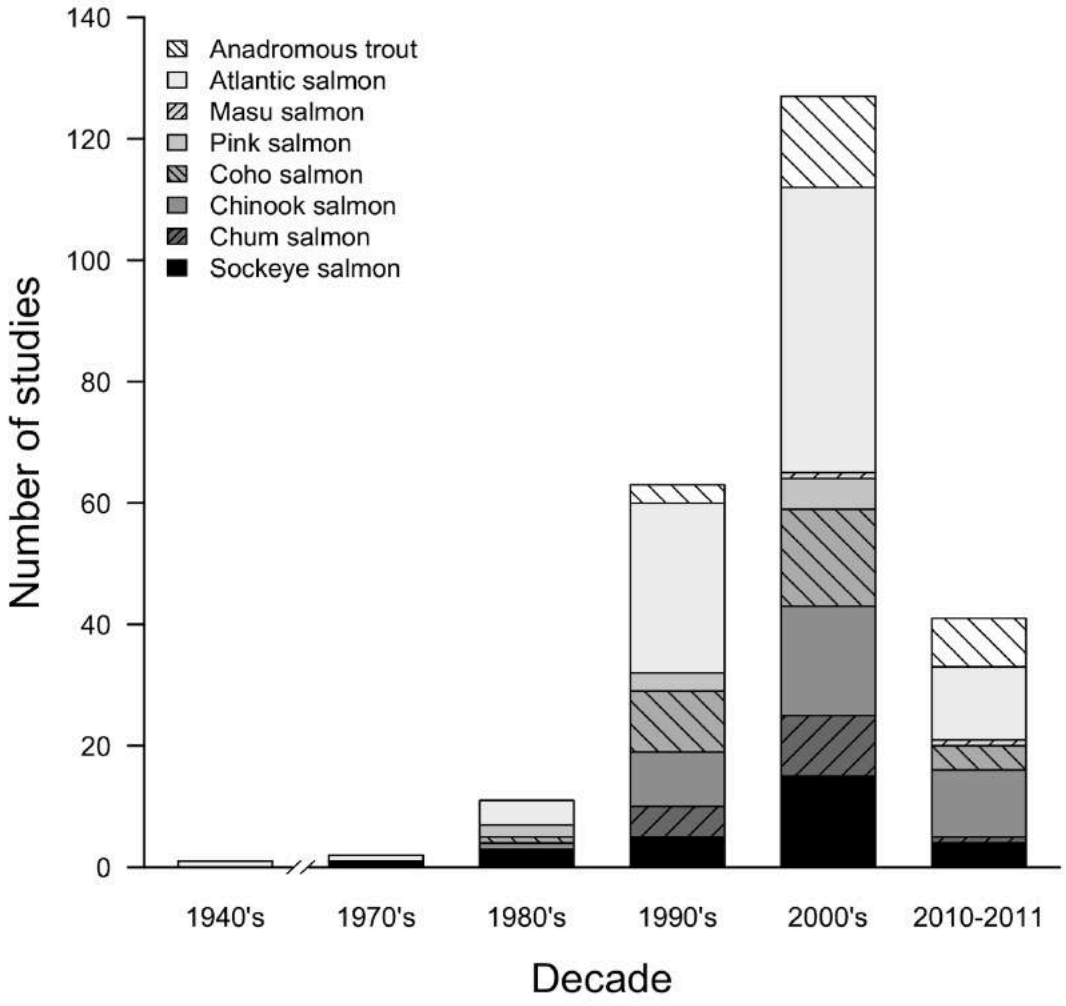

Figure 2.1 Number of studies of particular species by publication decade. Total number of studies (n=245) exceeds that of reviewed papers (n=207) because many studies investigated more than one species. Steelhead, cutthroat, brown and sea trout were combined into "Anadromous trout".

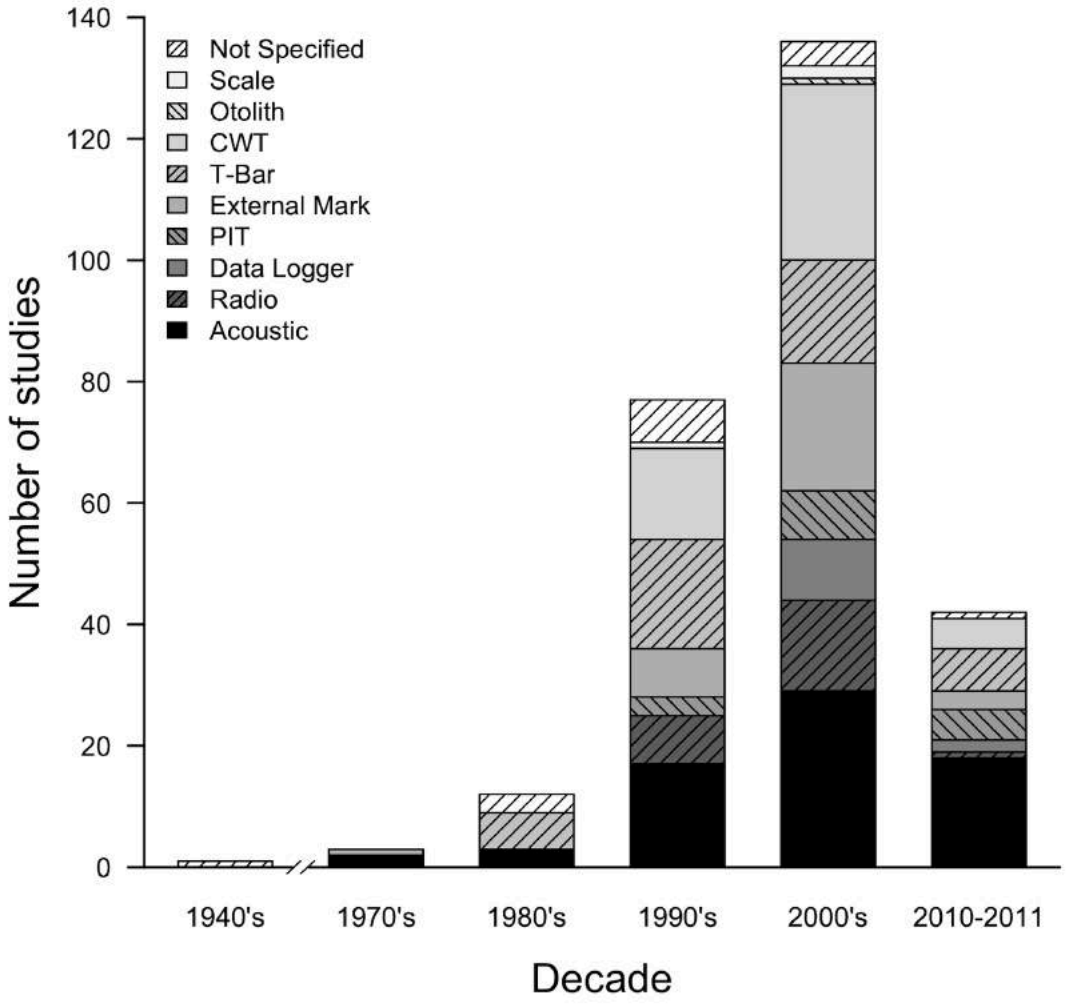

Figure 2.2 Number of use of specific tag types by publication decade. Total number of tag use (n=271) exceeds that of reviewed papers (n=207) because many studies used more than one tag type. The category "T-Bar" includes carlin, cinch, spaghetti, Floy, and Petersen disk tags. The plot does not include data from a paper published in 1940 because the tag type used was not specified by the author.

Table 2.1 Frequency (% within parentheses) of use of different tag types to study the life stages of anadromous salmon in the marine environment for studies focusing on survival (i.e. those focusing on only survival and both on survival and behaviour). The first % value within parentheses shows the relative frequency of use of a given tag type across life stages. The second % value shows the relative frequency of use of different tag types to study a particular life stage. The total frequency of tag use (n=342) exceeds that of reviewed papers (n=207) because many studies encompassed more than one life stage. The category "Biological" includes otoliths and scales, whereas the category "External" includes Carlin, cinch, Floy, Petersen disk tags and external markings.

	Tag type							
Life stage	Acoustic	Radio	Data Logger	PIT	CWT	External	Biological	Row Total
Out-migration (juveniles)	23 (63.9/42.6)	3 (20/5.6)	0 (0/0)	7 (53.8/13)	10 (25.6/18.5)	11 (19.3/20.4)	0 (0/0)	54 (NA/100)
Out-migratin (juveniles) to open ocean	2 (5.6/20)	0 (0/0)	0 (0/0)	0 (0/0)	2 (5.1/20)	6 (10.5/60)	0 (0/0)	10 (NA/100)
Out-migration (kelts)	3 (8.3/42.9)	0 (0/0)	1 (100/14.3)	1 (7.7/14.3)	0 (0/0)	2 (3.5/28.6)	0 (0/0)	7 (NA/100)
Return migration	4 (11.1/15.4)	11 (73.3/42.3)	0 (0/0)	0 (0/0)	2 (5.1/7.7)	9 (15.8/34.6)	0 (0/0)	26 (NA/100)
Open-ocean	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	1 (2.6/33.3)	2 (3.5/66.7)	0 (0/0)	3 (NA/100)
Open-ocean to return migration	1 (2.8/100)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	1 (NA/100)
Entire life cycle (juvenile-return adult)	3 (8.3/4.9)	1 (6.7/1.6)	0 (0/0)	5 (38.5/8.2)	24 (61.5/39.3)	27 (47.4/44.3)	1 (100/1.6)	61 (NA/100)
Column Total	36 (100/NA)	15 (100/NA)	1 (100/NA)	13 (100/NA)	39 (100/NA)	57 (100/NA)	1 (100/NA)	162 (100/100)

Table 2.2 Frequency (% within parentheses) of use of different tag types to study the life stages of anadromous salmon in the marine environment for studies focusing on behaviour (i.e. those focusing on only behaviour and both on behaviour and survival). The first % value within parentheses shows the relative frequency of use of a given tag type across life stages. The second % value shows the relative frequency of use of different tag types to study a particular life stage. The total frequency of tag use (n=342) exceeds that of reviewed papers (n=207) because many studies encompassed more than one life stage. The category "Biological" includes otoliths and scales, whereas the category "External" includes Carlin, cinch, Floy, Petersen disk tags and external markings.

	Tag type							
Life stage	Acoustic	Radio	Data Logger	PIT	CWT	External	Biological	Row Total
Out-migration (juveniles)	36 (54.5/60)	3 (13.6/5)	1 (8.3/1.7)	6 (66.7/10)	4 (21.1/6.7)	9 (18.8/15)	1 (25/1.7)	60 (NA/100)
Out-migratin (juveniles) to open ocean	2 (3/20)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	8 (16.7/80)	0 (0/0)	10 (NA/100)
Out-migration (kelts)	4 (6.1/40)	0 (0/0)	2 (16.7/20)	1 (11.1/10)	0 (0/0)	3 (6.3/30)	0 (0/0)	10 (NA/100)
Return migration	16 (24.2/32)	17 (77.3/34)	3 (25/6)	0 (0/0)	1 (5.3/2)	13 (27.1/26)	0 (0/0)	50 (NA/100)
Open-ocean	3 (4.5/27.3)	0 (0/0)	2 (16.7/18.2)	0 (0/0)	4 (21.1/36.4)	1 (2.1/9.1)	1 (25/9.1)	11 (NA/100)
Open-ocean to return migration	1 (1.5/25)	0 (0/0)	3 (25/75)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	4 (NA/100)
Entire life cycle (juvenile-return adult)	4 (6.1/11.4)	2 (9.1/5.7)	1 (8.3/2.9)	2 (22.2/5.7)	10 (52.6/28.6)	14 (29.2/40)	2 (50/5.7)	35 (NA/100)
Column Total	66 (100/NA)	22 (100/NA)	12 (100/NA)	9 (100/NA)	19 (100/NA)	48 (100/NA)	4 (100/NA)	180 (100/100)

Table 2.3 Frequency (% within parentheses) of use of different tag types to study single and multiple life stages of anadromous salmon in the marine environment for studies focusing on survival (i.e. those focusing on only survival and both on survival and behaviour). The first % value within parentheses shows the relative frequency of use of a given tag type across type of study. The second % value shows the relative frequency of use of different tag types to study one or multiple life stages. The total frequency of tag use (n=340) exceeds that of reviewed papers (n=207) because some studies used more than one type of tag. The category "Biological" includes otoliths and scales, whereas the category "External" includes carlin, cinch, Floy and Petersen disk tags and external markings.

	Tag type							
Type of study	Acoustic	Radio	Data Logger	PIT	CWT	External	Biological	Row Total
Single stage	33 (91.7/37.9)	14 (93.3/16.1)	1 (100/1.1)	8 (61.5/9.2)	10 (25.6/11.5)	21 (36.8/24.1)	0 (0/0)	87 (NA/100)
Multiple stages	3 (8.3/4)	1 (6.7/1.3)	0 (0/0)	5 (38.5/6.7)	29 (74.4/38.7)	36 (63.2/48)	1 (100/1.3)	75 (NA/100)
Column Total	36 (100/NA)	15 (100/NA)	1 (100/NA)	13 (100/NA)	39 (100/NA)	57 (100/NA)	1 (100/NA)	162 (100/100)

Table 2.4 Frequency (% within parentheses) of use of different tag types to study single and multiple life stages of anadromous salmon in the marine environment for studies focusing on behaviour (i.e. those focusing on only behaviour and both on behaviour and survival). The first % value within parentheses shows the relative frequency of use of a given tag type across type of study. The second % value shows the relative frequency of use of different tag types to study one or multiple life stages. The total frequency of tag use (n=340) exceeds that of reviewed papers (n=207) because some studies used more than one type of tag. The category "Biological" includes otoliths and scales, whereas the category "External" includes carlin, cinch, Floy and Petersen disk tags and external markings.

	Tag type							
Type of study	Acoustic	Radio	Data Logger	PIT	CWT	External	Biological	Row Total
Single stage	61 (93.8/47.7)	19 (90.5/14.8)	8 (66.7/6.3)	7 (77.8/5.5)	7 (36.8/5.5)	24 (50/18.8)	2 (50/1.6)	128 (NA/100)
Multiple stages	4 (6.2/8)	2 (9.5/4)	4 (33.3/8)	2 (22.2/4)	12 (63.2/24)	24 (50/48)	2 (50/4)	50 (NA/100)
Column Total	65 (100/NA)	21 (100/NA)	12 (100/NA)	9 (100/NA)	19 (100/NA)	48 (100/NA)	4 (100/NA)	178 (100/100)

Table 2.5 Number and frequency (% within parentheses) of a variable being found significant out of the total number of significant findings for behaviour (n=151) or survival (n=66). Note that the table is based on studies focusing solely on behaviour or survival, but not both.

		Study focus	
Category	Variable	Behaviour	Survival
Environmental	Temperature	27 (17.9)	6 (9.1)
	Depth	16 (10.6)	1 (1.5)
	Diel Effects	16 (10.6)	0 (0)
	Tide	15 (9.9)	0 (0)
	Current	8 (5.3)	0 (0)
	Salinity	7 (4.6)	2 (3)
	Productivity	2 (1.3)	3 (4.5)
	River Discharge	4 (2.6)	3 (4.5)
Physiological	Reproductive State	2 (1.3)	2 (3)
	Stress Hormones	0 (0)	1 (1.5)
	Ionoregulatory State	0 (0)	3 (4.5)
	Energetic Status	3 (2)	1 (1.5)
Other	Fish Size	16 (10.6)	15 (22.7)
	Stock	16 (10.6)	14 (21.2)
	Sex	2 (1.3)	1 (1.5)
	Release Date	4 (2.6)	4 (6.1)
	Release Location	2 (1.3)	3 (4.5)
	Trophic Effects	5 (3.3)	1 (1.5)
	Fisheries	1 (0.7)	4 (6.1)
	Predation	5 (3.3)	2 (3)
Total		151 (100)	66 (100)

CHAPTER 3: VARIABLE THERMAL EXPERIENCE AND DIEL THERMAL PATTERNS OF HOMING SOCKEYE SALMON IN COASTAL MARINE WATERS

3.1 Introduction

Reproductive migrations are challenging life history events often associated with the convergence of physiological and environmental transitions (Dingle, 1996). This is certainly true for anadromous salmon that encounter variable environmental conditions during their migration from ocean feeding grounds to freshwater spawning sites. Among environmental variables, temperature is thought to be the 'master' abiotic factor for fish (Fry, 1968). Anadromous salmon have a narrow range of temperatures that they routinely feed and rear in (Brett, 1952; Elliott, 1976; Elliott, 1991; Larsson & Berglund, 2005), and temperature is known to influence their distribution, migratory behaviour, physiology, growth, bioenergetics, and survival across all life stages (Crozier et al., 2008; Friedland, 1998; Jonsson & Jonsson, 2009; Martins et al., 2012a; Reddin & Shearer, 1987; Richter & Kolmes, 2005; Welch et al., 1995). Changes to ocean temperatures are predicted under climate change scenarios (IPCC, 2007), making it important to understand current thermal experience of anadromous salmon in the ocean in order to predict their response to future changes in ocean temperatures. However, little is known about the thermal experience of anadromous salmon during their reproductive migration in marine waters, especially in relation to biotic and abiotic factors (Drenner et al., 2012).

In particular, the reproductive migration of anadromous salmon through coastal marine waters is a critical phase due to salmon encountering variable environmental

conditions (e.g. salinities and temperatures) and high predator densities, while altering their mechanisms for orientation and their physiological state (Hinch et al., 2006; Thorstad et al., 2010). Fine scale movement patterns of homing salmon in coastal marine waters have been previously studied using ultrasonic tracking, acoustic telemetry and thermal data loggers [reviewed in Drenner et al. (2012)]. Studies revealed that behaviour is highly variable among species, but homing salmon typically migrate in the upper 50 m of the water column (Davidsen et al., 2013; Døving et al., 1985; Quinn et al., 1989; Ruggerone et al., 1990) [except chum salmon and Chinook salmon that have been found to migrate up to depths > 200 m (Candy & Quinn, 1999; Tanaka et al., 2000)], orient themselves with the thermocline (Døving et al., 1985; Quinn et al., 1989; Westerberg, 1982), exhibit diel patterns (Candy & Quinn, 1999; Madison et al., 1972; Quinn et al., 1989; Ruggerone et al., 1990; Walker et al., 2000), show a preference for a narrow range of temperature (Quinn et al., 1989; Walker et al., 2000), and undertake vertical migrations that expose fish to variable temperatures [e.g., 5 to > 20 °C (Døving et al., 1985; Olson & Quinn, 1993; Quinn et al., 1989; Walker et al., 2000)] and salinities [e.g., 7.8 – 33.6 ppt (Olson & Quinn, 1993; Quinn et al., 1989)]. Although movement patterns of coastal migrating salmon have been used to infer thermal experience, there are few direct measures of thermal experience or the factors associated with this during this life stage.

It is speculated that vertical positioning in the water column (and thus environmental experience) during return migrations may be related to a number of factors including olfactory homing (Døving et al., 1985; Quinn et al., 1989; Ruggerone et al., 1990), behavioural thermoregulation (Tanaka et al., 2000), predator avoidance and fish

physiological state (Hansen & Quinn, 1998; Hinch et al., 2006; Olson & Quinn, 1993). Olfactory homing is thought to be the major mechanism used by salmon for navigation during this stage of migration (Døving & Stabell, 2003; Hasler & Scholz, 1983b; Quinn, 1990; Ueda, 2011). Surface waters in coastal marine areas likely contain olfactory cues from natal streams that salmon must "sample" to aid in navigation (Døving & Stabell, 2003). However, the choice of utilizing a particular depth or temperature could depend on trade-offs involved in minimizing energy use (Tanaka et al., 2000), maintaining physiologic homeostasis, and reducing encounters with predators. For example, homing salmon in coastal waters are undergoing physiological preparations for freshwater entry and reproduction which involves reconfiguring ion exchange systems and developing gonads, both of which are associated with elevated physiological stress (Cooke et al., 2006b; Crossin et al., 2007; Hinch et al., 2006; Høgåsen, 1998). Management of energy reserves is also crucial during this phase, especially for semelparous Pacific salmon that are relying on finite energy reserves to fuel the remainder of migration (Crossin et al., 2009a). Exposure to above optimal temperatures, as can occur in surface waters, could cause increases in stress hormone concentrations (Jeffries et al., 2012), more rapid energy use (Brett, 1971; Lee et al., 2003), alteration of reproductive maturation (Pankhurst & King, 2010), and osmoregulatory failure (Jeffries et al., 2012). Further complicating such 'trade-offs', in laboratory experiments with salmon held in full strength seawater, chum salmon and sockeye salmon that are more 'freshwater-prepared' in terms of their osmoregulatory systems experienced higher levels of stress and lower survival (Cooperman et al., 2010; Hirano et al., 1990). Thus one might expect more freshwater-prepared migrants to spend more time in surface waters that are warmer and less saline.

In general, I would expect that homing salmon should take advantage of natal stream olfactory cues, which would potentially exist in warmer, less saline surface waters, under appropriate physiological conditions (i.e., when fish are less stressed or more freshwater-prepared) and/or when there are less visual oriented predators in the vicinity (i.e., during night).

The concept of species-specific thermal optima is well established for freshwater rearing and migrating salmon (Brett, 1952; Jonsson & Jonsson, 2009; Richter & Kolmes, 2005). Recently, investigators have discovered that thermal tolerance can vary substantially among populations within a single species (e.g. Fraser River sockeye salmon) and between sexes within a population, but this research has only focused on freshwater environments (Eliason et al., 2011; Farrell et al., 2008; Martins et al., 2012b). There has been little research into whether population- or sex-specific differences in thermal optima exist in marine environments, although there is evidence of population-dependent responses to changing ocean conditions (e.g., salinity, wind-generated currents) in marine waters (Thomson & Hourston, 2011).

To further our understanding of the factors associated with thermal experience of homing anadromous salmon in coastal marine waters, I analyzed data from recovered thermal data loggers that were attached to sockeye salmon during their coastal ocean return migration through the SoG to the Fraser River. The SoG is well studied in terms of its oceanography (Thomson, 1981; Thomson, 1994), and the linkage between salmon migratory behaviour and physiology is well examined in this region (Hinch et al., 2006). However, there has been no research linking behaviour and physiology of homing salmon in the marine environment to thermal experience. My objectives were first to characterize

the thermal experience of tagged sockeye salmon migrating through the SoG, and second to test whether thermal experience of sockeye salmon was associated with physiological state of fish, diel patterns, abiotic ocean conditions, meteorological conditions, migration timing, sex and population. This paper represents the first attempt to directly link fish physiological state and environmental conditions to the thermal experience of homing anadromous salmon in the marine environment using telemetry.

3.2 Methods

3.2.1 Study site

The SoG is a deep inland basin located between mainland BC and Vancouver Island (Fig. 3.1) that is approximately 200 km long, 40 km wide, and has an average and maximum water depth of 155 and 400 m, respectively. The southern part of the SoG is strongly influenced by freshwater discharge from the Fraser River. This warmer, less dense water enters the SoG creating a unique spatial habitat. Fraser River discharge dilutes seawater in the surface layer, causing a strongly stratified interface between shallow (<10 m) brackish surface layer and the deeper (>10 m) more saline seawater (Thomson, 1981). Tidal currents and wind-generated circulation also strongly influence the environment within the SoG (Thomson, 1981). Currents in the northern SoG maintain a slow counterclockwise rotation driven mainly by winds from the northwest. Currents in the southern part of the SoG are driven by a combination of winds and river discharge with currents circulating in a clockwise direction. These slower currents (~ 0.1 to 0.2 m/s) are also affected by the mixed, mainly semidiurnal tidal currents, which are stronger in the south (~1 m/s), decreasing in strength northward. Rising tides result in flood

currents entering the strait from the south and north (the tides meet in the northern sector of the SoG), while falling tides produce ebb currents that exit the SoG to the south and north.

3.2.2 Fish capture, biopsy and tagging

All tagging and sampling was conducted with the approval of the Animal Care Committee of the University of British Columbia, in accordance with the Canadian Council on Animal Care. As part of a larger project monitoring sockeye salmon movements and survival, ~1,000 adult sockeye salmon returning to the Fraser River were captured by commercial purse seine (2006) or commercial troll (2010) fishery in northern Discovery Passage ~215 km from the mouth of the Fraser River (Fig. 3.1) during August 11-26 of 2006 and August 11 – Sep 2 of 2010. After capture, individual fish were brought on board the vessel and transferred to a holding tank that was flushed with free-flowing ambient seawater. Pre-sampling holding durations for troll and purse seine caught fish were < 15 min and < 30 min respectively. Non-lethal handling and sampling of unanaesthetized (Cooke et al., 2005) sockeye salmon followed one of two procedures. The full procedure started by moving an individual fish from the holding tank onto a foam-padded, v-shaped trough with a constant supply of cold seawater. A 3 ml blood sample was taken from the caudal vein to assess blood plasma concentrations for stress parameters (glucose, cortisol, lactate), osmolality (an overall measure of physiological condition), sex hormones (testosterone, estradiol) and ion concentrations (i.e., Na^+, K^+, Cl^-, indicative of osmoregulatory state). The blood sample was stored on ice until the rest of the procedure was completed. Next, a small (< 4 mm) gill tissue sample was taken

from gill filament tips for a microarray analysis that was part of a separate research project, the details of which will be presented elsewhere. An adipose fin punch (0.5 g) was then taken for DNA population identification and stored in 95% ethanol prior to analysis. Fork length (FL) was measured to the nearest cm. Lastly, a transmitter ("tag") with an attached thermal logger was inserted gastrically using a plastic applicator and fish were immediately released overboard. The reduced procedure, performed on approximately 50% of fish, was the same as described above, but without blood and gill tissue sampling. The full sampling procedure took less than three minutes (Cooke et al. 2005). After the fish was released, the blood samples were centrifuged and the blood plasma transferred into liquid NO_2.

The transmitters applied to fish were either Vemco acoustic tags (various models of V16 tags; all 16 mm diameter, and <70 mm length, Vemco Inc., Shad Bay, NS, Canada) or Lotek radio tags (model MCFT-3A-3V; 16 mm diameter, 51 mm length, Lotek Wireless Inc., Newmarket, ON, Canada). Thermal data loggers (iButton, DS1921Z; factory stated resolution= ±0.1 °C, accuracy= ±1 °C; Maxim Integrated Products, Inc., Sunnyvale, California) were fixed to the transmitters with Plasti Dip® (PlastiDip International, Blaine, MN). In a separate study by our team that tested thermal data logger accuracy and precision under laboratory conditions, mean iButton accuracy was reported as 0.4 ± 0.3 °C and mean precision was reported as 0.2 ±0.0 °C, which was more accurate than values reported by the manufacturer (Donaldson et al., 2009). Thermal data loggers were programmed to measure and store hourly temperature readings and associated date/time.

After release, individual fish movements were monitored using fixed telemetry arrays positioned along the sockeye salmon migration route at the lower Fraser River and Mission (Fig. 3.1). Acoustic tags could be detected at the lower Fraser River and at Mission, whereas radio tags could only be detected at Mission because radio signals are attenuated in seawater. Tags were recovered by means of capture in commercial, aboriginal or sport fisheries, or by recovery on the spawning grounds by Fisheries and Oceans Canada (DFO) stock assessment crews. Rewards were provided to encourage return of thermal loggers.

3.2.3 Laboratory assays

Individual population origin was determined from DNA analysis of adipose fin clips [mean percentage error <1% (Beacham et al., 2004)]. Plasma osmolality, ions (Na^+, K^+, Cl^-), glucose and lactate were measured using the procedures outlined in (Farrell et al., 2001). Plasma cortisol, testosterone and 17β-oestradiol were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Neogen Corporation; Lexington, KY, USA). Testosterone and 17β-oestradiol samples were extracted in ethyl ether according to manufacturer's protocols. Cortisol, testosterone and 17β-oestradiol samples were all run in duplicate at appropriate dilutions. Additional details on assays are provided in Farrell et al. (2001).

3.2.4 Tag recovery and oceanographic data collection

I used data from 50 recovered thermal data loggers representing the marine thermal experience of 50 individual sockeye salmon from 2006 (n = 19) and 2010 (n =

31). Out of the 50 recovered thermal data loggers, two were fixed to acoustic tags and 48 were fixed to radio tags. Acoustic tags are less likely than radio tags to be noticed by persons handling fish due to lack of the radio antenna that protrudes from the mouth of a fish. In total, 35 of these fish were physiologically sampled, including 21 males and 14 females. Thermal data loggers were downloaded and paired with telemetry data by matching the date/time from the data loggers to date/time of radio or acoustic tags registering at detection sites (Fig. 3.1). This enabled us to extract fish thermal experience from the point of release in northern Discovery Passage to detection in the Fraser River either at the mouth of the Fraser River (for acoustic tags) or at Mission (for acoustic and radio tags) (Fig. 3.1). However, the Mission detection site is located approximately 75 km up-river from the SoG, and for this study I was only concerned with fish thermal experience within the SoG (i.e., prior to Fraser River entry). To determine point of river entry for radio tags, I used migration rate data from detection of acoustic tags (n = 365) on receivers at river entry and at Mission combined with visual inspection of individual fish temperature plots to look for evidence of river entry based on abrupt increases in temperature when fish entered the Fraser River. Based on this, I determined that there was a thermal signature associated with river entry and discarded temperature observations that followed past this point from the data set for all radio tags. The final data set included 14,690 hourly temperature readings. The study duration (date from when the first fish was released and the last fish entered the Fraser River) ranged from August 11 to September 6 in 2006 and August 11 to September 21 in 2010.

Oceanographic and meteorological data (i.e., water temperature, sea surface salinity, tide height, and various wind velocity and wind stress variables) from the SoG

were compiled from DFO coastal lighthouse monitoring sites (Chrome Island, Nanoose Bay, and Point Atkinson lightstations), and Environment Canada meteorological Buoy 46146 (Fig. 3.1) for 2006 and 2010. Oceanographic and meteorological variables were chosen based on biological knowledge of the factors influencing oceanography in the SoG (see study site description above). I compared oceanographic trends between monitoring sites to verify that the sites I selected were representative of conditions in the SoG. Vertical profile data taken from the Nanoose Bay monitoring site was used to characterize the vertical temperature and salinity structure of the water column. To match oceanographic/meteorological variables with individual fish temperature observations taken on an hourly scale, I needed data measured on hourly scale. Therefore, I used data from two monitoring sites (tide height measured at Point Atkinson and all other oceanographic/meteorological variables measured at Buoy 46146) that measured variables on hourly basis to match with individual fish temperature observations based on matching date and time to the nearest hour. Buoy 46146 recorded near surface water temperatures at ~ 3 m depth, and wind variables were measured 10 m above the sea surface. Although of interest, sea surface salinity was not available as mean hourly data, and was therefore not paired with fish temperature observations. Fraser River hourly temperature data was gathered from DFO Environmental Watch Program monitoring buoys in the lower Fraser River (Whonnock; Fig. 3.1).

3.2.5 Data analysis

Data exploration followed protocols presented in (Zuur et al., 2010) and was applied separately for oceanographic variables and physiological variables. Examination

of multi-panel scatterplots indicated possible co-linearity between environmental variables (e.g., between sea surface temperature and Fraser River temperature) and between physiological variables (e.g., between lactate and cortisol). Therefore, I used Pearson correlation coefficient > 0.8 and variance inflation factor (VIF) > 3 to identify and remove collinear variables (Zuur et al., 2010). Variables that I selected to include in my analysis are presented below (variables chosen to be removed are indicated in parenthesis and were closely related to one or more other variable included in the analysis): sea temperature at 3m depth (Fraser River temperature), along-shore wind stress (along-shore wind velocity, wind stress magnitude), cross-shore wind stress (cross-shore wind velocity, wind stress magnitude), lactate (glucose, cortisol, osmolality), testosterone (estradiol), Na^+ and Cl^- ions (osmolality, K^+ ion).

I used a mixed-effects model analysis because the data consisted of multiple temperature measurements of the same fish. Initial analysis fitting mixed-effects models to the raw time-series data for individual fish revealed substantial residual variability. Therefore, to increase the signal to noise ratio I aggregated fish body temperature and environmental data by computing the median values for each hour of the day [i.e., 24 median values for each individual; (Murtaugh, 2007)]. Two models were fit separately for oceanographic/meteorological and physiological variables. The first model (referred to as "ocean" model) was fit using oceanographic/meteorological variables, and the second model (referred to as "physio" model) incorporated significant variables from the "ocean" model with physiological variables. The reasoning behind a separate analysis of oceanographic/meteorological and physiological variables, rather than incorporating all variables in the same model, was because not all fish for which I had temperature data

were physiologically sampled, and therefore the sample sizes for physiological data were lower than for oceanographic/meteorological dataset (n = 1,200 for "ocean" model, and n = 816 for "physio" model).

Predictor variables included as fixed effects in the "ocean" model were sea temperature at 3m depth (ST3m), along-shore wind stress (AlSh), cross-shore wind stress (CrSh), tide height (tide), and study year. To test for the effects of sampling blood and gill tissue on subsequent thermal experience, I also included a variable indicating whether the fish was sampled or not sampled (referred to as "sampled"). For each fixed variable in the "ocean" model (except study year, tide and sampled), I included two variables representing a within-subject effect (centered predictor variables within an individual) and a between-subject effect (mean of the predictor variable for an individual). Including both a within- and a between-individual component for predictor variables accounts for not all fish experiencing the same conditions while migrating due to differences in migration timing between individuals [see van de Pol & Wright (2009) and Dingemanse & Dochtermann (2013) for details on the importance of incorporating within and between individual predictors when predictor values within individual vary between individuals]. To test for diel patterns in fish thermal experience, I included periodic terms [$\sin(2\pi\times hour/24)$, $\cos(2\pi\times hour/24)$] into the "ocean" model as fixed effects. Within-subject centering was not applied to periodic terms as all individuals in the data set "experienced" each of the 24 hours. Visual inspection of hourly fish temperature plots indicated possible differences in diel patterns of fish thermal experience between study years. Therefore I included interaction terms for study year with the periodic terms.

Predictor variables included as fixed effects in the "physio" model were any significant variables from "ocean" model together with a centered variable for lactate (indicates stress), testosterone (indicates reproductive maturity), Na^+ (indicates freshwater preparedness), Cl^- (indicates freshwater preparedness), and sex. Using interaction plots I identified possible interactions between sex and testosterone. Therefore I included interactions for sex with testosterone in the "physio" model.

I determined appropriate random effects to include in my models by comparing models that included all fixed effect terms with all possible combinations of random effect groups (all models included the individual level) using Akaike information criterion (AIC) (Burnham & Anderson, 2002). AIC selection revealed that the most parsimonious model included random effects for (from lowest to highest level): individual fish, stock complex (population or groups of populations of sockeye salmon), and run-timing group (aggregates of stocks complexes). Run-timing groups are management-derived distinctions based on the biology of the populations [i.e., dates they migrate into the Fraser River (Beacham et al., 2004)]. In my data set, there were three run-timing groups (from earliest migration date to latest): early-summer (n = 8), summer-run (n = 9), and late-run (n = 33). Late-run fish historically exhibited a "milling" period prior to river entry, but since 1995, portions of late-run fish began entering the river 3-6 weeks earlier than historic norms. Late-run fish that enter the river early are called "early" migrating late-run fish. This behavioral phenomenon has been the focus of numerous studies because "early" migrants experience high levels of mortality in freshwater (Cooke et al., 2004a; Hinch et al., 2012). Based on this interesting biological phenomenon, I subdivided the late-run group into two groups: "early" late-runs (n = 15),

and “normal” late-runs (n = 18). “Early” migrating late-run fish were those that migrated earlier into the Fraser River than historic norms [i.e., September 12 (Cooke et al., 2005a)], and “normal” migrating late-run fish were those that migrated at historic norms [i.e., later than September 12 (Cooke et al., 2005a)].

Median fish temperatures values were transformed to their reciprocal to reduce heterocedasticity identified with plots of residuals by fitted values. In addition, variance structures for the variables “study year” and “sampled” were subsequently included to reduce heterocedasticity associated with these predictors (Pinheiro & Bates, 2002). Autocorrelation plots for the residuals indicated temporal correlation for fish temperature observations. To account for temporal correlation, I fit both the “ocean” and “physio” models with an auto-regressive correlation structure of order 1 (AR1). Backwards model selection was done using marginal F-tests to select a reduced model containing only significant terms. Variables were removed when $p > 0.01$, a conservative threshold that was chosen to account for multiple testing (Zuur et al., 2009). I computed marginal (based on significant fixed effects) and conditional (based on fixed and random effects) R^2 values as described in (Nakagawa & Schielzeth, 2013). All data exploration and statistical analysis were performed in R version 2.15.2 (R Core Development Team, 2012), using packages “nlme” (Pinheiro et al., 2012) and “AICcmodavg” (Mazerolle, 2012).

3.3 Results

3.3.1 Characterization of fish thermal experience and ocean conditions

Visual inspection of individual fish hourly temperature plots showed common patterns related to initial thermal experience after release and subsequent diel thermal patterns. The marine thermal experience for eight individual sockeye salmon (Fig. 3.2) from the release location in Discovery Passage to Fraser River entry reveals the range of thermal habitats encountered. Individual fish were selected from my larger dataset from both years of study based on patterns that are described hereafter being visually apparent. Hourly individual fish temperature plots for all 50 individual fish reveal patterns similar to those shown here (see Appendix B).

I identified two "stages" of fish thermal experience from individual fish hourly temperature plots. The first stage was immediately following release, in which fish experienced a period of relatively consistent temperature around approximately 10-12 °C. The duration of time a fish spent in the "consistent" thermal experience stage varied between-individuals. This is exemplified in hourly temperature plots for the fish represented by tag IDs 150.32_160 and 150.46_99, where the former remained in the "consistent" stage after release for less than a day, and the later remained in the "consistent" stage after release for approximately six days (Fig. 3.2). I performed a changepoint analysis in R version 2.15.2 (R Core Development Team, 2012), using package "changepoint" (Killick & Eckely 2013) to determine the point when the mean temperature begins to vary for individual fish after release. Based on the changepoint analysis, the mean (±SD), minimum and maximum number of days spent in "consistent" thermal experience stage after release was 2.0 ± 1.4 days, 0.3 days, and 6.0 days,

respectively. The coefficient of variation, mean (±SD), minimum and maximum hourly thermal experience during "consistent" stage was 0.04, 10.6 ± 0.4 °C, 9.0 °C, and 13.4 °C, respectively. All hourly temperature readings from the "consistent" temperature stage were excluded from my subsequent data analysis that focused on the remaining period of fish thermal experience due to the possibility of the "consistent" temperature stage being associated with a capture/tagging effect or the environment at the site of release (see discussion). This reduced the overall sample size from 14,690 to 12,232 hourly temperature observations.

Following the stage of "consistent" thermal experience, individual fish hourly thermal experience became more variable and this trend continued throughout the remainder of the marine migration to freshwater entry. Within the "variable" thermal experience stage, there was a large amount of variability in hourly thermal experience both within-individuals and between-individuals. For example, within the 2010 study year, the fish represented by tag ID 150.36_42 experienced a wide range of temperatures (i.e., 8.6-17.9 °C) with frequent temperature variations, whereas the fish represented by tag ID 150.44_189 experienced a slightly narrower range of temperatures (i.e., 9.0-16.4 °C) and less frequent temperature variations (Fig. 3.2). The coefficient of variation, mean (±SD), minimum and maximum hourly thermal experience during the "variable" stage was 0.16, 11.4 ± 1.8 °C, 8.5 °C, and 20.5 °C, respectively. The two stages of thermal experience (i.e., "consistent" and "variable") are interpreted separately in the discussion.

Diel patterns were also observed in individual hourly fish temperature plots, and there appeared to be opposite diel patterns between study years. In 2006, fish generally exhibited a diel pattern of experiencing warmer temperatures during the day and cooler

temperatures during the night as can be observed in individual hourly temperature plots for the fish represented by Tag IDs 150.46_99, 150.46_140, and 150.36_5 (Fig. 3.2). In contrast, fish tagged in 2010 generally exhibited a diel pattern of experiencing warmer temperatures during the night and cooler temperatures during the day, as can be observed in individual hourly temperature plots presented for all the fish in that year (Fig. 3.2). Diel patterns in thermal experience also varied between-individuals within a study year and within-individuals. For example, the fish represented by tag ID 150.32_160 from 2006 showed signs of both diel patterns described above. In contrast, the fish represented by tag ID 150.36_40 from the same study year showed a more consistent diel pattern of experiencing warmer temperatures during the day and cooler temperatures at night (Fig. 3.2).

During the "variable" stage of thermal experience, I observed some variability in thermal experience among stock complexes (Fig. 3.3). However, variability observed among stock complexes was largely attributed to differences among run-timing groups (Fig. 3.3). Among run-timing groups, summer-run fish experienced the warmest temperatures and "normal" late run fish experienced the lowest temperatures (Fig. 3.3). In addition, fish from the "early" late-run group experienced warmer temperatures than fish from the "normal" late-run group (Fig. 3.3) even though they are made up of the same stock complex and migrate through the SoG at similar times.

Descriptive statistics for oceanographic/meteorological variables used in my model measured over the study duration (Table 3.1) revealed that mean values for oceanographic/meteorological conditions were similar between the two years of study. However in 2010, surface waters reached higher maximum temperatures, and both

components of wind stress were higher for both minimum and maximum values. Mean temperature below the thermocline (> 20 m) in 2006 and 2010 was 9.4 ± 0.3 °C and 9.3 ± 0.3 °C, respectively. Vertical profiles of salinity and temperature structure of the water column in 2006 and 2010 show that surface waters reached warmer temperatures and lower salinities in 2010 (Fig. 3.4). Temperature and salinity below the thermocline were similar between the two years (Fig. 3.4).

3.3.2 Model results for fish thermal experience

Variables included in the models and the model selection procedures are presented in Table 3.2. The final "ocean" model included a significant effect for the interaction of study year with the periodic terms that represent diel patterns [sin(2π×hour/24):study year; df = 1146, $p<0.0004$; and cos(2π×hour/24):study year; df = 1146, $p<0.0001$]. Predictions of median hourly fish thermal experience over a day for each of the study years indicate opposite diel patterns in fish thermal experience between the study years (Fig. 3.5). In 2006, fish experienced peak temperatures around mid-day (12-2 pm PST), and the lowest temperatures during the night (12-2 am PST), whereas in 2010, fish experienced peak temperatures during the night (12-2 am PST), and the lowest temperatures around mid-day (12-2 pm PST; Fig. 3.5). In addition, fish in 2006 experienced higher and more variable median hourly temperatures than fish from 2010 (Fig. 3.5).

After incorporating the above significant variables from the "ocean" model into the "physio" model, the physiologic variables were not significant ($p > 0.01$) and were therefore removed prior to removal of any oceanographic variables during backward

selection (Table 3.2). For this reason I fell back to using the "ocean" model as it took advantage of the full data set rather than a subset of data for physiologically sampled fish.

The significant fixed effects alone (marginal R^2) in my final model explained only ~11% of the variability in the data. The fixed effects and random effects (conditional R^2) explained ~50.3%. Among random effects in my model, the amount of variability associated with individual, stock complex, and run-timing effects was ~ 30.7%, < 0.0001%, and ~8.5 %, respectively. The remaining variability (~49.7%) was due to within-individual variation. Model predictions for the expected median thermal experience for each run-timing group (i.e., early-summer, summer-run, "early" late-run, and "normal" late-run), were 11.9 °C, 12.4 °C, 11.9°C, and 11.2 °C, respectively. Model predictions show that summer-run fish experience the highest temperatures among run-timing groups (on average between 0.5 and 1.5 °C warmer), and "early" late-run fish experience temperatures that are on average 0.7 ° C warmer than "normal" late-run fish.

3.4 Discussion

The present study used small thermal data loggers to track individual homing sockeye salmon thermal experience in a coastal marine environment as they migrated to freshwater and related fish thermal experience to environmental conditions and physiological state of individual fish. By tracking individual fish thermal experience, I was able to observe two "stages' of fish thermal experience; the first stage being characterized by a period of "consistent" thermal experience followed by a " variable" stage with opposite diel patterns between the study years. Based on my models, the "variable" stage of thermal experience was not associated with environmental conditions

or fish physiological state, and the largest amount of variability in thermal experience in the data was attributed to within-individual variation.

The "consistent" stage of fish thermal experience directly following release ranged in duration between individuals in my data set and could be attributed to a number of potential factors. The first possible explanation is that there is an effect of capture/tagging on subsequent fish behaviour after release that influences their thermal experience either directly by fish seeking out cooler waters to facilitate physiological recovery from a stressor, or indirectly by fish exhibiting an escape response after release by diving down to cooler waters. In other studies using similar methods of tracking anadromous salmon behaviour in the ocean, authors have noted a "tagging" effect on fish behaviour immediately following release (Candy & Quinn, 1999; Quinn et al., 1989; Walker et al., 2000). Quinn et al. (1989), for example, noted that homing sockeye salmon that were manually tracked in a location near my release site initially dived to deep waters after release before ascending to surface waters, and in some cases this feature was distinguishable from subsequent behaviour.

Another possibility is that the thermal environment is homogeneous throughout the vertical water column in the location my fish were released; thus providing no scope for variation in thermal experience. Based on vertical profile data taken during tagging in 2010, temperature throughout the upper 50 m of the water column in Discovery Passage area is homogeneous at approximately 10.8 °C (S.M. Drenner, unpublished data). Furthermore, a study examining depth use of homing sockeye salmon that were tagged and released in the same location as my study found that sockeye salmon swam at an average depth of 26.2 m in an area directly downstream of the migration pathway of the

release site, but the same fish were observed swimming at more shallow depths (e.g., ~9.0 m) when they were in the SoG that could expose them to higher temperatures (Wilson et al., 2014a). I conclude that the observed "consistent" thermal experience immediately following release in my study could be associated with one or more factors such that the vertical environment is homogeneous at the release site and capture/handling may influence the duration of time a fish remains in this environment before migrating into the thermally stratified waters of the SoG. For these reasons, and because I was trying to relate thermal experience to surface conditions in the SoG, I did not include temperature recordings from this stage in my subsequent analysis. Combining depth sensors with thermal loggers in future studies would help resolve behavioural issues I observed in the "consistent" stage of thermal experience.

After the stage of "consistent" thermal experience, sockeye salmon exhibited a large amount of variability in thermal experience that persisted to freshwater entry. Variability in thermal experience is likely related to vertical movements in and out of the warmer surface layer as observed for sockeye salmon that were tagged with depth sensing tags (Quinn et al., 1989). Interestingly, sockeye salmon did experience temperatures that were well above what is considered metabolically optimal in freshwater [i.e., >18 °C; (Eliason et al., 2011)] and as high as those experienced after entering the Fraser River. However, out of a total of 12,232 hourly temperature readings in the "variable" thermal experience stage, only 69 (~0.6%) of these readings were greater than or equal to 18 °C providing evidence that sockeye salmon generally avoid warmer surface water. Overall, sockeye salmon showed a strong preference for cooler waters (mean temperature of 11.4 °C) at or near the thermocline during the "variable" stage of

thermal experience. I note that my finding of mean thermal experience of 11.4°C is below what is considered the thermal optimum for maintaining aerobic scope in freshwater environments by summer migrating sockeye salmon (Brett, 1971; Eliason et al., 2011). Moreover, metabolic costs are higher in seawater than in freshwater for sockeye salmon (Wagner et al., 2006), therefore the choice of occupying deeper and cooler water in the SoG could be a result of individuals attempting to minimize total metabolic costs in the marine environment at the expense for maximizing aerobic scope. Development of more robust models that predict metabolic rate from temperature for adult salmon in seawater would help resolve this trade-off.

During the "variable" stage of thermal experience, I observed diel patterns in individual fish temperature plots that were opposite between study years. This observation was supported by my models, which found a significant effect for the variables representing an interaction of diel patterns with study year. Diel patterns have been noted for anadromous salmon migrating in the open-ocean (Friedland et al., 2001; Hedger et al., 2009; Ishida et al., 2001; Ogura & Ishida, 1992; Ogura & Ishida, 1995; Reddin et al., 2011; Walker et al., 2000) and for coastally migrating Pacific salmon (Quinn et al., 1989; Walker et al., 2000). Among previous studies, the most common diel pattern was characterized by fish migrating in warmer, surface waters at night and cooler, deeper waters during the day. This pattern was consistent with diel patterns I observed in 2010. However, in 2006, diel patterns were opposite and were similar to those described in a study on post-spawning steelhead kelts in the ocean (Teo et al., 2013). Teo et al. (2013) noted that diving behaviour of steelhead kelts in the ocean appeared to be associated with the moon phase. During my study, the moon phase in 2006 at the first

date of tagging (August 11) was waning and changed to a waxing moon phase towards the end of the study. In contrast, the moon phase at the start of the study in 2010 (August 11) was a waxing moon and changed to a waning moon phase towards the end of the study. Therefore, the opposite diel patterns between our two study years could be associated with differences in moon phases. Other studies have suggested that diel patterns for anadromous salmon in the ocean could be associated with other factors including season, location, foraging, behavioural thermoregulation, predation and olfactory homing (Hinke et al., 2005; Ogura & Ishida, 1995; Quinn et al., 1989; Tanaka et al., 2000; Walker & Myers, 2009; Walker et al., 2000). During the period of migration examined in my study, sockeye salmon have ceased feeding (Hinch et al., 2006), ruling out foraging as a hypothesis for diel patterns. Oceanographic data I collected indicated that in 2010, surface waters reached higher temperatures and lower salinities than in 2006. Therefore in 2010, sockeye salmon could have been more likely to avoid these conditions (which would occur during mid-day) to optimize energy use (i.e., behavioural thermoregulation) or osmoregulatory function. Diel patterns could also be a result of reducing encounters with pinneped predators such as harbour seals (*Phoca vitulina*). Harbour seal population numbers have increased to potentially carrying capacity levels in the SoG since a ban on harvesting was implemented in 1970's (Olesiuk et al., 1990), and are known to utilize homing Pacific salmon as a food source in estuaries (Wright et al., 2007). Interestingly, Wright et al. (2007) speculated that harbor seal predation rates on salmon were higher during the night, and could be in response to the behaviour of their prey (i.e., salmon migrating in shallower waters at night), exemplifying the complexity of predator-prey behaviours. I conclude that diel patterns observed in my study could be

related to a number of factors (i.e, moon phase, reducing encounters with predators, olfactory/celestial navigation, and behavioural thermoregulation) that my study was not designed to test for. Overall, my results suggest that diel patterns of homing sockeye salmon in coastal marine environments is far more complex than previously thought, and multiple year studies are needed before generalizing behavioural patterns observed from single year studies.

The significant fixed variables together (e.g. variables representing an interaction between diel patterns and study year) in my final model explained a relatively low amount of the variability in thermal experience. In addition, none of the physiological variables included in my model were significant predictors of fish thermal experience, which was unlike previous studies with Fraser River sockeye salmon where physiological state of fish at the same capture sites as I used and with similar sampling approaches to ours have found strong relationships between reproductive hormone concentrations, osmoregulatory indices, and physiological stress metrics and subsequent migration rate and survival into freshwater environments (Cooke et al., 2008a; Cooke et al., 2006b; Crossin et al., 2009a; Crossin et al., 2007; Crossin et al., 2009b). The fact that I was not a able to clearly identify environmental and physiological correlates with thermal experience combined with the low amount of variability in thermal experience explained by the interaction of diel patterns and study year suggests that thermal habitat choice is a much more complex process than is involved with governing coastal migration rates and it likely involves multiple factors as I have alluded to above (e.g., minimizing metabolic costs, obtaining homing cues, avoiding predators).

The largest and second largest amount of variability in thermal experience in the data was attributed to within-individual variation and variation among individuals of a given stock complex, respectively. The SoG is a spatially and temporally complex environment; therefore individual fish may experience fine scale differences in the environment based on timing, location and chance that I was not able to account for. For example, although fish may share similar phenotypes, one fish may encounter more predators or experience different ocean conditions at a local scale. Furthermore, fine scale differences in environmental experience within-individuals could interact with the physiological state of the fish to produce variability in behaviour. Development of models that predict more fine-scale environmental conditions fish experience based on individual migration rates combined with tags that measure multiple environmental variables may aid in our ability to explain differences in thermal experience within-individuals.

I observed variability in thermal experience among stock complexes that I attributed to variability among run-timing groups. My model supported my observations from the data and indicated differences in thermal experience among run-timing groups, and a relatively low variability in the data explained by differences among stock complexes of a given run-timing group. As previously described, run-timing groups are aggregates of stock complexes based on the time they enter the Fraser River. Because of this, stock complexes within a run-timing group would be expected to encounter similar conditions because they migrate at similar times, and different run-timing groups would be expected to experience different conditions. Indeed, my model indicated that run-timing groups that migrate through the SoG at later dates generally experienced lower

temperatures, which I would explain based on surface water temperatures decreasing over the study duration. Interestingly, my model indicated differences within the late-run run-timing group. “Early” late-run fish experienced warmer temperatures than “normal” late-run fish. “Early” and “normal” late-run fish migrate at similar times through the SoG (the only difference is the date of river entry) and would be expected to experience similar environmental conditions. A possible explanation of the temperature differences between the two late-run groups could be that there are differences in behaviour. For example, “early” late-run fish may be more likely to exhibit vertical migrations than “normal” late-run fish. More frequent vertical migrations could be a reflection of a higher frequency of encountering predators or sampling natal olfactory cues in surface waters, which could increase the likelihood of “early” late-run fish deciding to leave the marine system and enter the river.

3.5 Conclusion

Changes in global temperatures could alter the physical structure of coastal marine environments (IPCC, 2007) and have profound effects on their biota (Pörtner & Farrell, 2008). Average annual sea surface temperatures in the SoG have already increased by 1 °C over the past century (Chittenden et al., 2009a), and are predicted to continue to rise based on changes in precipitation patterns and timing of freshet input from the Fraser River (Johannessen & Macdonald, 2009). For sockeye salmon that are already near the southern limit to their distribution (Welch et al., 1998), the ability to adapt to meet changing conditions is paramount. In order to understand how sockeye salmon will adapt to future conditions we need to understand current behaviours and the

factors associated with them. My research showed that sockeye salmon homing in the SoG experience diel patterns in thermal experience as well as variable temperatures. The variability in thermal experience was largely attributed to within-individual variation that was not explained by either physiological state of fish or environmental conditions suggesting how important fine-scale environmental conditions may be to homing sockeye salmon. Development of models that predict environmental conditions that fish experience based on individual migration rates combined with tags that measure multiple environmental parameters is needed to further our understanding of behaviour during this life-stage. Overall the factors governing sockeye salmon thermal experience during homing migration in coastal marine waters is complex and may vary between years. However, variability in thermal experience suggests that homing sockeye salmon may be able to adapt to changing environmental conditions in coastal marine waters.

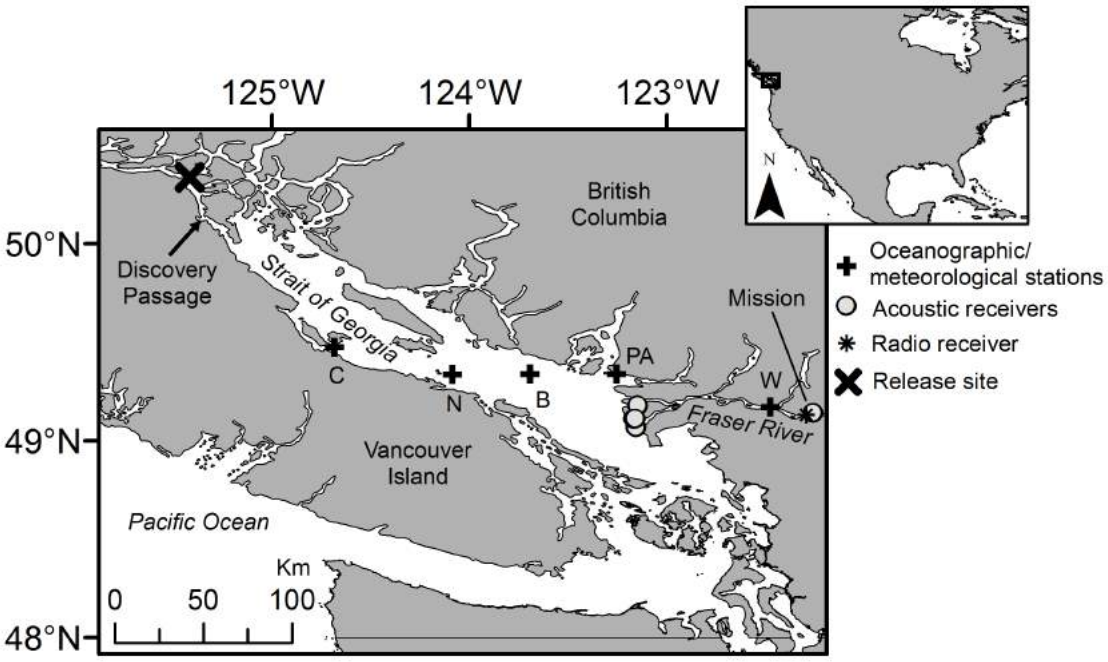

Figure 3.1 Map of study area. "C" = Chrome Island. "N" = Nanoose Bay. "B" = Buoy 46146. "PA" = Point Atkinson. "W" = Whonnock.

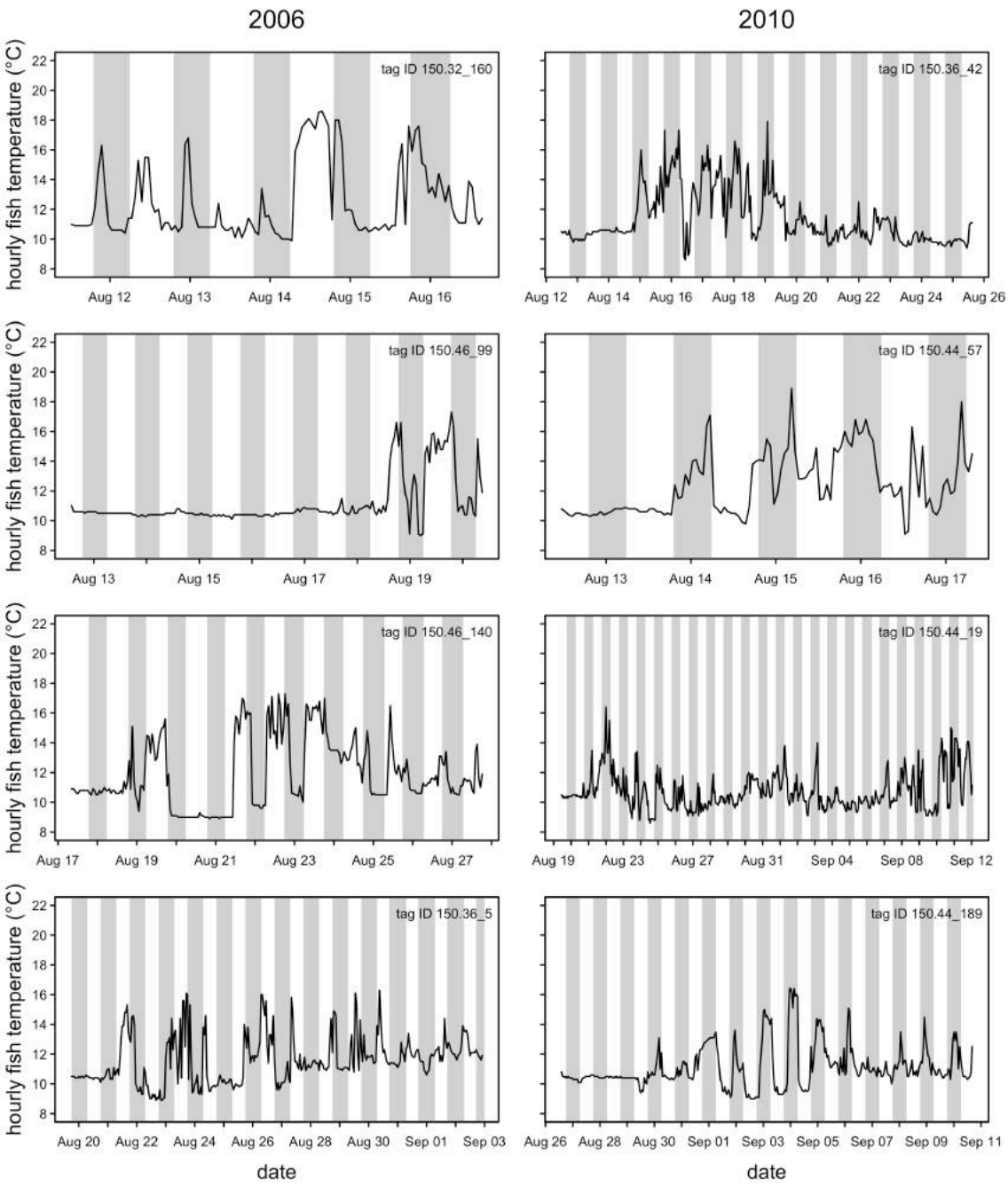

Figure 3.2 Hourly thermal (°C) experience recorded from eight individual sockeye salmon from time of release in Discovery Passage to Fraser River entry. Fish in the left column are from 2006, and fish in the right column are from 2010. Light grey shaded regions indicate "night". Individual fish were selected due to thermal profiles being representative of patterns described in text.

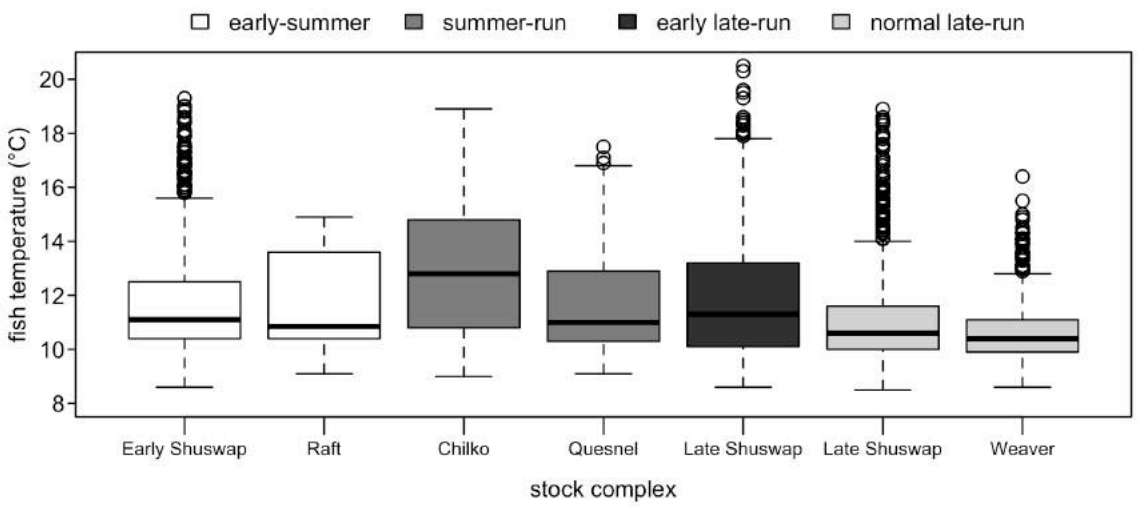

Figure 3.3 Boxplots of the thermal experience for various stock complexes represented in the data set. Only temperature data from the "variable" stage of thermal experience are shown (see text). The run-timing group that a stock complex belongs to is indicated in the figure legend. Run-timing groups are ordered from left to right by the date they enter into the Fraser River (i.e., earliest river entry date on the left and latest river entry date on the right). Within a run-timing group, stock complexes are not ordered in any particular manner. Solid bold horizontal lines within boxes represent the median, box limits represent the interquartile range (IQR), and whiskers represent 1.5x the IQR. Open circles represent outliers. Outliers were defined as outside 1.5x the IQR above the upper quartile and below the lower quartile.

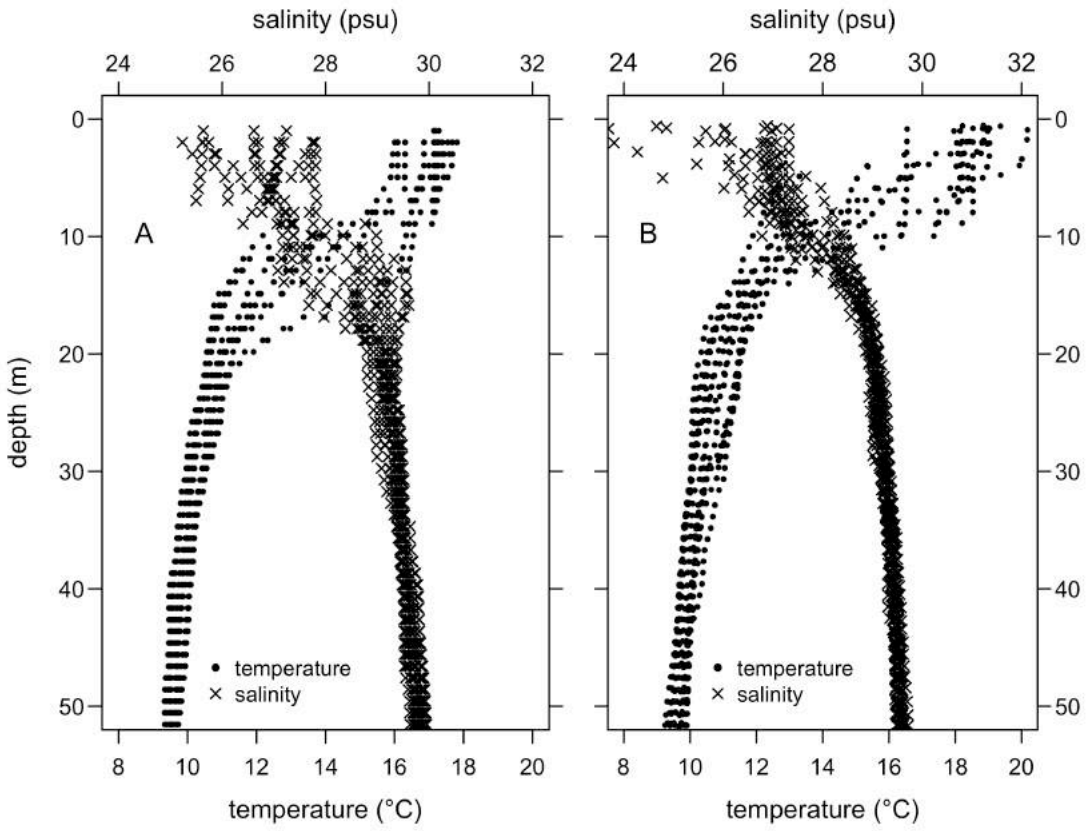

Figure 3.4 Vertical profile of the Strait of Georgia taken at Nanoose Bay in (A) 2006 and (B) 2010. All measurements taken over the month of August are shown.

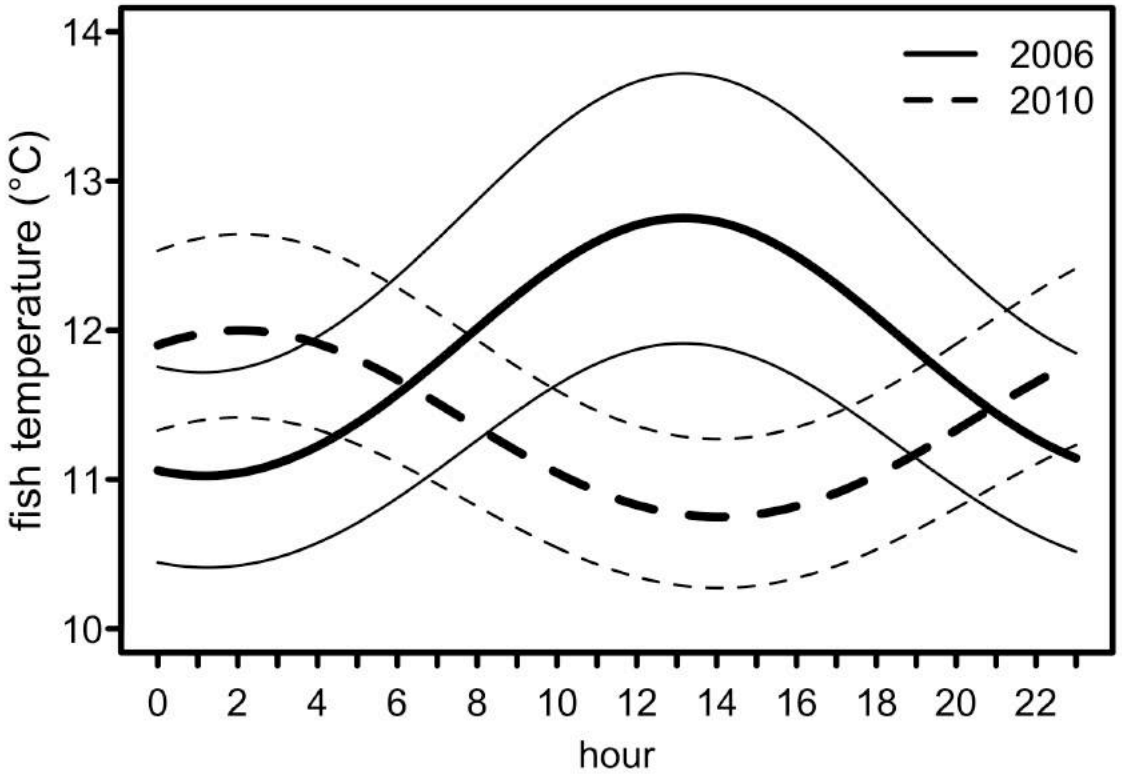

Figure 3.5 Model predictions for median hourly fish thermal experience over a 24-hour period for the two study years (2006 and 2010). Thin solid and dashed lines above and below bolded solid and dashed lines, respectively, indicate 95% confidence intervals.

Table 3.1 Descriptive statistics for oceanographic/meteorological variables used in my model (except tide) over the study duration (August 11 to September 6 in 2006 and August 11 to September 21 in 2010). Hourly measurements were taken at Buoy 46146 in the Strait of Georgia. Temperature is measured at 3 m depth. Along-shore wind stress is positive when directed to 290 degrees true compass bearing and cross-shore wind stress is positive when directed toward 20 degrees true compass bearing.

Variable	2006			2010		
	Mean (SD)	Min	Max	Mean (SD)	Min	Max
Sea surface temperature (°C)	16.0 (± 2.3)	11.3	21.3	16.4 (± 2.4)	11.4	23.6
Along-shore wind stress (N/m^2)	-0.03 (± 0.04)	-0.20	0.09	0.00 (± 0.07)	-0.31	0.37
Cross-shore wind stress (N/m^2)	0.00 (± 0.01)	-0.03	0.04	0.00 (± 0.02)	-0.11	0.13

Table 3.2 Table of fixed effects and model selection procedure for the "ocean" and "physio" models. For variables in the "ocean" model, "wi-" and "bi-" before variables indicate a variable representing a within-individual and a between-individual effect respectively (see text). Variables are reported in the order they were removed from the model during backwards selection. P-values are reported for each variable after removal of previous non-significant variables. In the "step removed" column, a (-) indicates the variable was retained in the final model.

"ocean" model			"physio" model		
step removed	variable	p-value	step removed	variable	p-value
1	wi-ST3m	0.95	1	Cl	0.44
2	bi-CrSh	0.87	2	Sodium	0.53
3	sampled	0.74	3	Sex:Testosterone	0.09
4	bi-AlSh	0.51	4	Testosterone	0.52
5	wi-CrSh	0.50	5	Sex	0.42
6	wi-AlSh	0.13	6	Lactate	0.03
7	tide	0.05	-	study year	0.07
8	bi-ST3m	0.02	-	sin(2π×hour/24)	0.11
-	study year	0.15	-	cos(2π×hour/24)	< 0.0001
-	sin(2π×hour/24)	0.09	-	sin(2π×hour/24):study year	0.0008
-	cos(2π×hour/24)	< 0.0001	-	cos(2π×hour/24):study year	< 0.0001
-	sin(2π×hour/24):study year	0.0004			

"ocean" model			"physio" model		
step removed	variable	p-value	step removed	variable	p-value
-	cos(2π×hour/24):study year	< 0.0001			

CHAPTER 4: ENVIRONMENTAL CONDITIONS AND PHYSIOLOGICAL STATE INFLUENCE ESTUARINE MOVEMENTS OF HOMING SOCKEYE SALMON

4.1 Introduction

Anadromous salmon migrate from feeding grounds in the ocean to natal freshwater sites to spawn. During this reproductive migration, salmon face changing environmental and physiological conditions that can influence migratory behaviour and survival (Healey, 2000; Hinch et al., 2006). Among the various environmental stages of the reproductive migration (i.e., open ocean, coastal, estuarine, riverine), the estuarine stage is perhaps the most challenging due to a high density of predators, variable thermal and osmotic conditions, and anthropogenic factors such as commercial fisheries and pollutants. In addition, homing salmon must gain cues from the environment for navigation (Dittman & Quinn, 1996; Keefer & Caudill, 2014), undergo physiological changes necessary for freshwater entry, and manage their energy reserves, which are needed to fuel migration, maturation and spawning (Hinch et al., 2006; Høgåsen, 1998; Thorstad et al., 2010).

Despite these diverse and variable year-to-year challenges, the timing of salmon migrations through estuaries and into rivers is remarkably predictable among populations (Groot & Margolis, 1991). This predictability of timing is generally thought to be associated with population-specific adaptations to historic conditions experienced during the freshwater stage of migration such as temperature, flow and other abiotic factors (Hansen & Jonsson, 1991; Hodgson et al., 2006; Jonsson & Jonsson, 2009; Taylor,

1991). All the same, modest variability in timing is observed among individuals within a given population (Cooke et al., 2004a; Jonsson et al., 2007; Stasko, 1975), in that a given population may enter the river over a period of several weeks, which is known to influence migration success (Hinch et al., 2012). Individual variability in behaviours, such as migration rate and river entry timing, are likely individual responses to different environmental conditions (Hodgson et al., 2006; Jonsson et al., 2007), an individual's energetic state and the time required for individuals to make appropriate physiological adjustments prior to entering the river (Hinch et al., 2006; Høgåsen, 1998). Indeed, studies combining physiological biopsy and telemetry revealed that coastally migrating sockeye salmon that migrated faster into the river were more reproductively mature, had lower somatic energy reserves and were more osmotically prepared for freshwater (Cooke et al., 2008a; Crossin et al., 2009a; Crossin et al., 2007).

Environmental conditions in estuarine regions can also influence homing salmon behaviour. Olfactory homing is thought to allow salmon to locate freshwater entry points from estuarine waters (Døving et al.; Hasler & Scholz, 1983a; Keefer & Caudill, 2014; Quinn & Dittman, 1990), presumably by salmon gathering cues in surface waters that are influenced by freshwater discharge (Døving & Stabell, 2003) and potentially other environmental conditions such as wind patterns that influence the mixing and transport of these surface waters. For example, wind-driven spread of olfactory cues was related to migratory route for homing Atlantic salmon in a Norwegian fjord (Davidsen et al., 2013). Likewise, salinity in estuaries may also play an important role in river entry timing. In one study, lower estuarine salinities encountered by returning sockeye salmon were associated with earlier river entry (Thomson & Hourston, 2011). Furthermore, diel

(Quinn et al., 1989; Smith & Smith, 1997) and tidal (Aprahamian et al., 1998; Levy & Cadenhead, 1995; Stasko, 1975) patterns in behaviour have been documented for salmon migrating in estuaries, which are likely related to avoiding predators, locating navigational cues, making osmoregulatory adjustments, and conserving energy.

Very few studies have related both physiology and environment to marine migration behaviour of anadromous salmon within a single study (Drenner et al., 2012) despite the potential for gaining an understanding of interactions between the environment, physiology, and behaviour. Thus, the aim of my study was to build on previous research by relating both environmental conditions and physiological state to migratory behaviour of homing salmon in estuarine waters and at subsequent river entry. My specific objectives were to: a) relate environmental conditions (e.g. wind patterns, discharge, salinity) and individual fish physiological (e.g. reproductive maturity, ionoregulatory state and stress levels as revealed by blood biopsies) and energetic state to migration rate in the estuarine region; b) relate migration pathway used by sockeye salmon in estuarine waters to environmental conditions; and c) test for diel patterns and tidal influences on arrival timing in the estuary and at subsequent river entry. This was achieved by taking blood biopsies and implanting acoustic transmitters into homing Fraser River sockeye salmon in marine waters of BC, Canada to follow passage through strategically located acoustic listening arrays. Fraser River sockeye salmon are comprised of hundreds of genetically distinct populations with different migratory behaviours (Groot & Margolis, 1991), allowing additional examination of population-specific behaviour within each study objective. Furthermore, Fraser River sockeye salmon are ecologically, economically and culturally valuable species that have experienced variable

returns in recent years (Cohen, 2012), and thus a better biological understanding is needed to aid in their management, in particular during their estuarine migration where the majority of commercial fisheries occur.

4.2 Methods

4.2.1 Study site

The SoG is a deep inland basin located between mainland BC and Vancouver Island (Fig. 4.1) that is approximately 200 km long, 40 km wide, and has an average and maximum water depth of 155 and 400 m, respectively. The SoG is strongly influenced by freshwater discharge from the Fraser River. In the summer this warmer, less dense water entering the SoG dilutes the surface layer, causing a strongly stratified interface between the shallow (<10 m) brackish surface layer and deeper (>10 m) more saline bottom layer (Thomson, 1981). Tidal currents and wind-generated circulation also strongly influence the environment within the SoG (Thomson, 1981). Currents in the northern SoG maintain a slow (< 0.5 m s^{-1}) counterclockwise rotation driven mainly by tidal currents and Coriolis effects on the northward-flowing component of the estuarine circulation. Currents in the southern part of the SoG are driven by a combination of winds and river discharge with currents circulating in a clockwise direction. These slower currents (~ 0.1 to 0.2 m s^{-1}) are also affected by the mixed, mainly semidiurnal tidal currents, which are stronger in the south (~ 1 m s^{-1}) and decrease in strength to the north. Rising tides result in flood currents entering the strait from the south and north (the tides meet in the northern sector of the SoG), while falling tides produce ebb currents that exit the SoG to the south and north.

Hourly water height (m) and mean daily salinity data were compiled from DFO coastal monitoring sites at Point Atkinson and Chrome Island, respectively (Fig. 4.1). I assigned tidal stage to each hourly water height measurement over the study duration as either flood (the water height at a given hour was greater than the prior hour but less than the following hour), ebb (the water height at a given hour was less than the prior hour but greater than the following hour), low (the water height at a given hour was less than the prior and following hour), or high (the water height at a given hour is greater than the prior and following hour). Hourly near-surface water temperature (°C) and wind data [AlSh (N/m^2), CrSh (N/m^2)] were compiled from the Environment Canada Meteorological Buoy 46146 (Fig. 4.1). Buoy 46146 measured wind variables at 10 m above the sea surface and water temperature at 3 m depth. Along-shore wind stress ranged from positive to negative values indicating southeasterly and northwesterly winds, respectively (i.e., parallel to the sockeye salmon migration route) (Fig 4.2.). Cross-shore wind stress ranged from positive to negative values indicating southwesterly and northeasterly winds, respectively (i.e., perpendicular to the sockeye salmon migration route) (Fig. 4.2). Fraser River hourly discharge (m^3/s) measured at Hope was collected from Water Survey of Canada, Environment Canada (Fig. 4.1).

4.2.2 Fish capture and tagging procedure

All tagging and sampling was conducted with the approval of the Animal Care Committee of the University of British Columbia, in accordance with the Canadian Council on Animal Care. Adult sockeye salmon (n = 365) returning to the Fraser River were captured by commercial troll (n = 340) or commercial purse seine (n = 25) fisheries

in northern Discovery Passage ~ 215 km north of the Fraser River mouth (Fig. 4.1) from August 5 - 18, 2010. Individual fish were brought on board the vessel and transferred to a holding tank that was continuously flushed with ambient seawater for <15 min (troll) or <120 min (purse seine) prior to fish undergoing either biopsy or control treatments. The biopsy procedure followed that established for the non-lethal, unanaesthetized handling and sampling of sockeye salmon (Cooke et al., 2005) where individual fish were moved from the holding tank to a foam-padded, v-shaped trough with a constant supply of ambient seawater. A 3-ml blood sample was taken from the caudal vasculature and stored on ice for up to 8hrs (Clark *et al.*, 2011). A small (< 4 mm, 0.03g) gill tissue sample was taken from the gill filament tips (data not reported herein), and an adipose fin punch (0.05 g) was taken for DNA stock identification and stored in 95% ethanol prior to analysis. Fork length was measured to the nearest cm and GSE was determined with a hand-held microwave radio emitter (Distell Fish FatMeter FM 692, Distell Inc., West Lothian, Scotland, UK; Crossin & Hinch 2005). Individually coded acoustic transmitters (Vemco V16-3x, 16 mm diameter and <70 mm length) were inserted through the mouth into the stomach with a plastic applicator, and a spaghetti tag (Floy Tag & Mfg. Inc., Seattle, WA, USA) was applied anterior to the dorsal fin through the dorsal musculature to allow visual identification of tagged fish in the case that fish were recaptured by fisheries, or for visual identification of fish on spawning areas. A control treatment, which was performed on 80 (~ 22%) fish, involved only the insertion of an acoustic transmitter, which reduced the extent and time of fish handling (< 3 min versus < 5 min). At least one control fish was tagged following the tagging of 3-8 biopsy fish. Fish were released directly overboard immediately following either treatment. At the end of the workday blood

samples were centrifuged, and the blood plasma was transferred into liquid N_2 in preparation for subsequent analysis of plasma concentrations of metabolites (glucose, cortisol, lactate) and sex hormones (testosterone, 17β-oestradiol), as well as osmolality and ion concentrations (Na^+, Cl^-).

4.2.3 Characterization of sockeye salmon behaviour

Individual fish movements were monitored after release using fixed acoustic telemetry arrays [see (Heupel et al., 2006) for details on the technology] that recorded and stored date, time and the individual tag ID when a tagged fish was in close proximity to the array. The first point of detection was ~84 km south of the release site on the NSOG array, which was a former POST array that is currently an OTN array. The NSOG array consisted of 27 receivers spanning west-to-east across the SoG, from Vancouver Island to BC mainland, crossing the northern tip of Texada Island (Fig. 4.1). This curtain of receivers allowed us to assess migratory pathway of individual fish (i.e., west to east position of where individual fish arrived at the NSOG array), and to test for associations of migratory pathway with measured environmental and stock-specific variables. The next point of detection was ~131 km to the southeast of the NSOG array on a series of acoustic receivers that were strategically located in the arms of the Fraser River (Fig. 4.1), and were used to determine date and time of river entry for individual fish.

Individual fish migration rate was estimated in kilometers per day using the shortest distance between locations. Migration rates were calculated for two sections of migration: a) from the release site to the NSOG array (i.e., northern SoG region), and b) from the NSOG array to Fraser River entry (i.e., central SoG region). I divided sockeye

salmon migration through the SoG into two sections because this allowed us to test whether associations between environmental or physiological factors and migration rate changed as migration progressed towards river entry. In addition, I expected that any behavioural effects due to capture, handling and tissue biopsy to be short-lived (Donaldson *et al.*, 2012), and therefore only detected in the northern SoG given that this section of the migration took on average ~2 days based on prior estimates (Crossin et al., 2009a).

4.2.4 Laboratory assays and physiological data

DNA analysis on adipose fin clips was used to determine individual fish stock origin [mean percentage error < 1% (Beacham et al., 2004)]. Plasma osmolality, ions (Na^+, Cl^-), glucose and lactate were measured using procedures outlined in Farrell et al. (2001) to provide information on osmotic and stress status. Plasma cortisol (for stress), testosterone and 17β-oestradiol (for reproductive status) were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Neogen). Testosterone and 17β-oestradiol samples were extracted in ethyl ether in accordance with the manufacturer's protocols. Cortisol, testosterone and 17β-oestradiol samples were all run in duplicate at appropriate dilutions. Additional details on assays are provided in Farrell et al. (2001).

Visual inspection of scatterplots and VIF analysis (Zuur et al., 2010) revealed collinearity among many physiological variables. Therefore, principal component analysis [PCA; following protocols presented in Field et al. (2012)] was performed on all physiological variables (i.e., cortisol, glucose, lactate, testosterone, 17β-oestradiol , Na^+,

Cl^- , osmolality) to create synthetic variables that represented two or more physiological variables, thereby reducing the number of variables included in my analysis. All physiological variables had a Kaiser-Mayer-Olkin (KMO) score > 0.5. Based on PCA results, scores were assigned to individual fish for the first and second principle components (PC1 and PC2), which explained 40% and 23% of the variation in the data, respectively (Table 4.1). PC1 represented overall ionic, osmotic and stress condition of fish because lactate, cortisol, osmolality, Na^+, and Cl^- loaded heavily on PC1 (Table 4.1). PC2 represented reproductive maturity because testosterone and 17β-oestradiol loaded heavily on PC2 (Table 4.1). Glucose was the only variable that loaded heavily on the third PC axis (PC3) (Table 4.1), and therefore was included in models as a separate variable rather than including PC3 scores.

4.2.5 Linking environmental data with biotelemetry data

Environmental variables (temperature, salinity, discharge, AlSh, CrSh) were paired with migration rate estimates for individual sockeye salmon within each migration section. In the northern SoG region, the mean of each variable was calculated over 1-day prior to reaching the NSOG array. I selected a time course of 1-day because it represented the approximate minimum number of days it took for sockeye salmon to travel from the release site to the NSOG array, and due to uncertainty of when fish enter the SoG from Discovery Passage, thereby reducing the chance of relating environmental conditions in the SoG to fish that are in Discovery Passage. In addition, 98% of fish took > 1 day to reach the NSOG array. Therefore, I felt that a 1-day interval represented the environmental conditions experienced by the greatest number of fish possible. Fish that

took < 1 d (n = 4) were not excluded from analyses. In the central SoG region, the mean of each variable was calculated from last detection on the NSOG array to first detection on receivers at river entry.

Visual inspection of scatterplots and VIF analysis revealed collinearity among environmental variables in both migration sections. Specifically, salinity was strongly related to temperature and CrSh in the northern and central SoG regions, respectively, and river discharge was strongly related to day-of-release (DOR) and day of last NSOG detection (NSOG date) in the northern and central SoG regions, respectively. However, PCA on environmental variables resulted in poor separation of variables among PC axes. Therefore, rather than using PCA as was used on physiological variables, I selected among collinear environmental variables by choosing variables that I believed would have the greatest influence on migration rate based on previous studies. I selected date variables (DOR and NSOG date) in place of discharge for the northern and central SoG regions, respectively, because date could broadly represent environmental conditions and/or physiological state of fish (Crossin *et al.*, 2009). In addition, salinity was selected in place of temperature and CrSh for the northern and central SoG regions, respectively, because salinity was associated with sockeye salmon migration timing in a previous study, and therefore was of particular interest (Thomson & Hourston, 2011).

4.2.6 Data Analysis

Analysis was performed only on sockeye salmon stocks (i.e., aggregates of populations in a spawning region) with large enough sample sizes in the data set to perform rigorous statistical testing [Chilko (n = 46), Early Shuswap (n = 53), Late

Shuswap (n = 130)]. These sockeye salmon stocks have overlapping migration timing in the estuarine region, but river entry timing can differ among and within stocks (Crossin et al., 2007; Hinch et al., 2012). In particular, since 1995, some portion the Late Shuswap stock began entering the river earlier than historic norms, resulting in increased *en route* mortality for these "early" migrants (Hinch et al., 2012). To further examine this early river entry behavioural phenomenon, I calculated the first and third quartiles of river entry dates for Late Shuswap sockeye salmon, and categorized individuals as 'early-timed' (n = 37) or 'normal-timed' (n = 27) Late Shuswap fish if they entered the river before the first quartile or after the third quartile, respectively. While this selective approach excluded 66 individuals, it ensured that the fish assigned to either group were representative of the two river entry behaviours.

Relationships between individual fish migratory pathway at the NSOG array (i.e., NSOG receivers divided into 4 sections grouped from west to east) and predictor variables (AlSh, CrSh, DOR, stock-group) were evaluated using multinomial modeling and model selection via Akaike's information criterion corrected for small sample sizes (AIC_c) (Burnham & Anderson, 2002). Multinomial modeling and model comparison based on ΔAIC_c and AIC_c weights (w_i) was performed using R packages (R Core Development Team, 2012) 'nnet' (Venables & Ripley, 2002) and 'MuMIn' (Bartoń, 2013), respectively. Model averaging was applied to a 95% confidence set of models (all models with a cumulative summed weights ≥ 0.95) to incorporate model selection uncertainty (Burnham & Anderson, 2002). Models were fit with standardized continuous predictor variables (Gelman, 2008), which allowed us to estimate and directly compare effect sizes among predictor variables (Schielzeth, 2010). Variables were selected as

being associated with the response variable when the effect size was statistically different from zero (i.e., when the 95% confidence intervals for estimates of effect size did not intersect zero).

Differences in migratory pathway used by sockeye salmon along 4 grouped sections of the NSOG array (e.g. NSOG pathway) were tested with a χ^2 test weighted for the number of receivers included in each of 4 possible NSOG pathways. In addition, χ^2 tests were used to test whether sockeye salmon arrived at the NSOG array or entered the Fraser River at day or at night (based on civil twilight) weighted for the number of hours in each diel state, and at different tidal stages (e.g. flood, ebb, low, high) weighted for the number of hours in each tidal stage over the study. If significant differences were found among NSOG pathways or tidal stages, *post hoc* weighted χ^2 tests were made for all pair-wise comparisons, and Bonferroni corrections were used to adjust p-values to account for multiple tests.

Relationships between migration rate and predictor variables were examined using linear regression, AIC_c model selection, and effect size as described above for NSOG migratory pathway analysis. Migration rate data were analyzed in two models sets run separately for each section of migration (i.e., northern and central SoG regions). The first set of models (termed 'environmental') took advantage of the full data set (n = 163 and n = 147 for the northern and central SoG regions, respectively), the primary focus being to assess whether environmental variables influenced migration rate. Additional predictor variables [treatment (biopsy or control), capture method (purse seine or troll), FL, and date variables (DOR or NSOG date)] were also included in the 'environmental' model because these variables could potentially influence migration rate, and therefore I

wanted to account for them to properly estimate the effects of environmental variables. Specific predictor variables included in the 'environmental' models examining migration rate in the northern SoG region were salinity, AlSh, CrSh, treatment (biopsy or control), capture method (purse seine or troll), FL, and DOR. The same predictor variables were included in the 'environmental' model examining migration rate in the central SoG region with the exception of temperature in place of CrSh and NSOG date in place of DOR.

To assess whether physiological variables influenced migration rate, a second set of models (termed 'physiological') was fit to data from a subset of fish (n = 99 and n = 90 for the northern and central SoG regions, respectively) for which physiological data was available (i.e., biopsied fish). Predictor variables included in the 'physiological' models were physiological condition (PC1 scores), reproductive maturity (PC2 scores), glucose, GSE, and sex [assigned based on reproductive hormone concentrations (Cooke et al., 2006a)]. In addition to physiological variables, any variables selected based on effect size from the 'environmental' models were also included in the 'physiological' model because the previous model indicated they were important in explaining migration rate.

I also included a variable representing stock-group (i.e., Chilko, Early Shuswap, 'early-timed' Late Shuswap, 'normal-timed' Late Shuswap) and interactions between stock-group and all other predictor variables in both 'environmental' and 'physiological' models. Stock-group was included in all models for the central SoG region, and was therefore not subject to model selection, because of prior known differences in migration behaviour among stock-groups in the estuary (Cooke et al., 2004a; Groot & Margolis, 1991).

Diagnostics for heteroscedasticity, normality and independence of residuals were visually inspected, and revealed violation of heteroscedasticity in migration rate models for the central SoG region. Therefore, I used generalized least squares (GLS) modeling in the central SoG region, and variance structure selection was applied to residuals of the stock-group that was identified as contributing to heteroscedasticity (Zuur et al., 2009). Model predictions for a range of values of a given variable present in the 95% confidence set of models was done while using the median value for the other continuous variables and selecting a level of a categorical variable that I would expect to better represent a freely migrating fish (e.g. vigorous fish rather than lethargic or moderately impaired fish).

4.3 Results

4.3.1 Northern SoG region

Among sockeye salmon from the targeted stock-groups, 46 Chilko, 53 Early Shuswap, 37 'early-timed' and 27 'normal-timed' Late Shuswap fish were detected on the NSOG array. Compared with 'normal-timed' Late Shuswap fish, Chilko, Early Shuswap and 'early-timed' Late Shuswap fish were tagged/released and reached the NSOG array at earlier dates (Fig. 4.3). Migration rate in the northern SoG region was similar among stock-groups (Fig. 4.4). The mean, minimum and maximum migration rate for all stocks combined in the northern SoG region was 42.5, 10.1, and 93.0 km/day, respectively.

Sockeye salmon displayed preferences for migratory pathways and near-shore migration upon arrival at the NSOG array (grouped into 4 segments) (χ^2 test = 29.09, df =

3, $p < 0.001$) (Fig. 4.5, Table 4.2). Most sockeye salmon migrated down the west side of the SoG along Vancouver Island (NSOG pathway 1, receiver numbers 1-6) and the east side of the SoG on the west side of Texada Island (NSOG pathway 3, receiver numbers 15-20) (Fig. 4.5, Table 4.2) with no significant difference between these two pathways (χ^2 test 0.77 df = 1, $p = 0.38$). Relatively fewer sockeye salmon used the strait between Texada Island and British Columbia mainland (NSOG pathway 4, receiver numbers 21-27) compared with NSOG pathway 1 (χ^2 test = 22.75, df = 1, $p < 0.001$) and NSOG pathway 3 (χ^2 test = 15.22, df = 1, $p < 0.001$) (Fig. 4.5). In addition, sockeye salmon used NSOG pathway 1 more than the middle of the NSOG (NSOG pathway 2, receiver numbers 7-14) (χ^2 test =12.14, df = 1, $p = 0.003$) (Fig. 4.5). No other significant differences were found among NSOG migratory pathways. In addition, none of the predictor variables (i.e., stock-group, DOR, CrSh, AlSh) were associated with NSOG pathway used by sockeye salmon and therefore, no further information is presented for this model.

Significantly more sockeye salmon reached the NSOG array during the day than at night (χ^2 test = 26.73, df = 1, $p < 0.001$) and at different tide stages (χ^2 test = 13.50, df = 3, $p = 0.004$) (Table 4.3). *Post hoc* comparisons showed that sockeye salmon reached the NSOG array more frequently on ebb tides compared to flood (χ^2 test = 13.46, df = 1, $p = 0.001$), but there were no other significant differences among tide stages.

Migration rate in the northern SoG region was associated with AlSh, treatment, salinity, stock, and the interaction of stock and salinity based on effect size for the 'environmental' model (Fig 4.6). Predictions for the relationship between AlSh and migration rate showed a positive relationship, indicating that under positive values of

AlSh (i.e., stronger southeasterly winds) sockeye salmon migrated faster to the NSOG array (Fig. 4.7, upper left panel). Model predictions also indicated that sockeye salmon that experienced higher salinity migrated faster than sockeye salmon that experienced lower salinity, but this relationship was not as strong for 'normal-timed' Late Shuswap fish (Fig. 4.7, upper middle panel), which were the latest migrating of the fish groups tested. Lastly, sockeye salmon that were biopsied for blood and gill tissue migrated ~ 6.5 km/day slower than sockeye salmon from the 'control' group (i.e., about a 6 h delay). The top-ranked 'environmental' model for the northern SoG region explained a low amount of the variability in the data (adjusted-R^2= 0.20) (Table 4.4).

The 'physiological' model for the northern SoG region indicated an effect of AlSh and the interaction between glucose and stock on migration rate (Fig. 4.6). Model predictions indicated that Chilko and 'early-timed' Late Shuswap fish with higher plasma glucose concentrations migrated faster to the NSOG array, while Early Shuswap and 'normal-timed' Late Shuswap fish with higher levels of glucose migrated slower to the NSOG array (Fig. 4.7, upper right panel). However, glucose, stock, or the interaction of glucose and stock were not present in all top-ranked models (Table 4.4), which suggests uncertainty in regards to its overall importance as a predictor of migration rate. In addition, salinity was no longer present in any top-ranked models once it was incorporated into 'physiological' models that had reduced samples sizes (Table 4.4). The top ranked 'physiological' model for the northern SoG region explained a similar amount of the variability in the data (adjusted-R^2= 0.21) as the top ranked 'environmental' model, and both top ranked models contained the variables AlSh and stock, indicating these variables account for the largest amount of the variability in the data (Table 4.4).

4.3.2 Central SoG region

Of the 36 Chilko, 47 Early Shuswap, 37 'early-timed' and 27 'normal-timed' Late Shuswap fish detected on receivers in the Fraser River, the 'normal-timed' Late Shuswap fish migrated slower in the central SoG region (Fig. 4.4). Additionally, migration rates for 'normal-timed' Late Shuswap fish were slower in the central SoG region compared to the northern SoG region (Fig. 4.4).

River entry occurred more during the day than at night (χ^2 test = 19.90, df = 1, $p < 0.001$), and there was a significant difference in river entry among tide stages (χ^2 test = 24.60, df = 3, $p < 0.001$) (Table 4.3). *Post hoc* comparisons revealed that sockeye salmon entered the river less during low tide compared to ebb tide (χ^2 test = 10.71, df = 1, $p = 0.006$) or flood (χ^2 test = 18.41, df = 1, $p < 0.001$), but there were no other differences between tidal stages during river entry.

Migration rate in the central SoG region was associated with AlSh, stock and the interaction between stock and NSOG last detection date based on 'environmental' model selection (Fig. 4.8). Predictions for the relationship between AlSh and migration rate in the central SoG region were opposite from predictions in the northern SoG region and showed a negative relationship. This indicates that under negative values of AlSh (i.e., stronger northwesterly winds) sockeye salmon migrated faster from the NSOG array to the Fraser River (Fig. 4.7, lower left panel). Model predictions also indicated that Chilko and Early Shuswap fish that reached the NSOG array at earlier dates migrated faster into the Fraser River (Fig. 4.7, lower right panel). In contrast, migration rates for both 'early-timed' and 'normal-timed' Late Shuswap fish were not strongly related to the date they

were last detected on the NSOG array, and 'normal-timed' Late Shuswap fish migrated much slower compared to all other stocks (Fig. 4.7).

Once variables selected from the 'environmental' model were incorporated into the 'physiological' model for the central SoG region, none of the physiological variables were selected based on effect size (Fig. 4.8), even though PC2 was present in all top-ranked 'physiological' models (Table 4.4). The top-ranked model that included both AlSh and the interaction of stock with NSOG date explained an exceptionally high amount of the variation in the data (adjusted-$R^2 = 0.91$) (Table 4.4) that could not be solely attributed to differences among stock-groups because the adjusted-R^2 value decreased to only 0.74 when stock-group was removed from the model.

4.4 Discussion

The present study combined telemetry, tissue biopsy and environmental monitoring to examine environmental and physiological influences on homing sockeye salmon behaviour in coastal and estuarine waters. My estimates for migration rate in the SoG (mean = 37 km/day, SD ± 22 km/day) were within ranges reported in previous studies on homing sockeye salmon in estuaries (Crossin et al., 2007; Madison et al., 1972; Quinn, 1988; Quinn et al., 1989; Stasko et al., 1976). As anticipated, there was variability in migration timing and rate among stock-groups in my study that were mostly attributed to differences between the 'normal-timed' Late Shuswap stock-group and the other stocks. 'Normal-timed' Late Shuswap fish had much slower migration rates in the central SoG region compared to all other stock-groups, which reflects the previously known tendency of this particular stock to mill in the estuary prior to entering the river

(Hinch et al., 2012). It was first believed that the key trait separating 'early-timed' and 'normal-timed' Late Shuswap fish was that the 'early-timed' Late Shuswap fish did not mill in the estuary resulting in earlier river entry for this segment of the Late Shuswap stock. Thus, river entry date has been the metric used to categorize Late Shuswap fish as either 'early-timed' or 'normal-timed' (Cooke et al., 2004) as was done in the present study. However, of particular note, the 'early-timed' Late Shuswap fish were consistently earlier migrants than the 'normal-timed' Late Shuswap fish throughout the area examined in my study. This has been noted by other studies (Hinch et al., 2012) and indicates the 'early entry' behavioural phenomema of a segment of the Late Shuswap stock has already commenced by the time these fish arrive in the inner coastal and estuarine areas.

While migrating through estuaries, homing salmon are thought to rely on a number of cues to aid in orientation and navigation (Keefer & Caudill, 2014). For example, both homing Atlantic and Pacific salmon have been observed following coastlines during their estuarine migration (Davidsen et al., 2013; Quinn et al., 1989). In my study, sockeye salmon tended to follow close to the coastlines against Vancouver Island and against the west side of Texada Island. Currents in the northern SoG maintain a slow counterclockwise rotation during the time of year sockeye salmon are migrating in the estuarine region (Thomson, 1981), and some sockeye salmon may use these currents that flow southward along Vancouver Island to swim in an energetically efficient manner. Alternatively, following coastlines (or using shallower depths associated with coastlines) may assist in navigation through complex coastal systems. Interestingly, outmigrating sockeye salmon smolts tend to use the strait on the east side of the SoG between Texada Island and the BC mainland (Welch et al., 2009), which may suggest juveniles did not

imprint on particular parts of the coastline during outmigration and provides further evidence of the importance of coastlines in Pacific salmon migration across multiple life stage.

Many studies on homing salmon have examined the use of tides and diel patterns [reviewed in (Drenner et al., 2012)] without reaching a consensus on the direction of relationships and patterns (Smith & Smith, 1997). My study indicated that sockeye salmon in the estuary and at river entry moved more frequently during the day, which is similar to other studies on sockeye salmon that noted faster swimming in estuaries during the day (Madison et al., 1972; Quinn, 1988; Wilson et al., 2014a). All fish in my study were tagged and released during the day, which could influence arrival timing on the NSOG array that was a mean of ~2.4 days travel time from the release site. Although there was a large amount in variability among fish in the number of days it took to reach the NSOG line (i.e., minimum= 0.7 days and maximum = 8.6 days), ~ 40% of fish reached the NSOG array between 1 and 2 days after release, and therefore, release timing may have affected diurnal patterns on the NSOG line. Diurnal movements could be related to gathering visual cues during the daytime, such as polarized light, that supplements other navigation or orientation cues.

Sockeye salmon in my study reached the NSOG array less frequently during flood tides, compared to ebb, and entered the Fraser River less frequently during low tides compared to flood or ebb. Variable use of tide by sockeye salmon in the SoG has been noted in earlier studies (Wilson et al., 2014a). Interestingly, Levy and Cadenhead (1995) found that sockeye salmon migrate more frequently into the Fraser River on flood tides, potentially as a strategy for energy conservation. Although my study did not detect a

similar tidal pattern associated with river entry, the sockeye salmon stock from Levy and Cadenhead (1995) have a much longer in-river migration distance to spawning grounds compared to the sockeye salmon from my study, and therefore, energy conservation may be more important for that particular stock. Overall, the factors influencing diel patterns and tidal use of homing salmon in estuaries are complex and may be species, stock, and location specific.

One of the predominant mechanisms salmon are thought to use in estuaries for orientation and navigation is olfactory homing (Hasler & Scholz, 1983a; Keefer & Caudill, 2014; Quinn & Dittman, 1990), where fish gather olfactory cues that are emitted into estuaries from freshwater entry points. The presence, concentration and spatial distribution of olfactory cues in estuaries are dependent on environmental forces such as river discharge, tides and wind-induced currents. In the SoG, oceanography is heavily influenced by wind-induced currents (Thomson, 1981), and my study found that stronger southeasterly winds were associated with faster migration rates for sockeye salmon migrating in the northern SoG region (i.e., from the release site to the NSOG array). Stronger southeasterly winds over the SoG would result in stronger estuarine outflow in the northern sector of the strait towards the ocean. This outflow would push freshwater exiting the Fraser River northward along the SoG in the upper 50-70 m of the water column (Thomson, 1994), which corresponds to the depths homing sockeye salmon utilize in estuarine waters (Quinn et al., 1989; Wilson et al., 2014a). Therefore, under stronger southeasterly winds, sockeye salmon entering the northern SoG from Discovery Passage would encounter a stronger Fraser River signal, which could result in increased migration rates under a few possible scenarios described below.

One possibility is that stronger southeasterly winds established a gradient of olfactory cues that sockeye salmon followed, thereby increasing their homing precision and thus rate of migration. Consistent with this explanation, the horizontal position of homing Atlantic salmon in a Norwegian fjord was related to wind direction, presumably because the fish followed brackish water that contained olfactory cues that were spread across the fjord by wind (Davidsen et al., 2013). A stronger olfactory cue may also accelerate reproductive maturation by triggering an increase in circulating reproductive hormones through a neuroendocrine pathway as evidenced by elevated levels of gonadotropin-releasing hormone (sGnRH) in the olfactory bulb of homing chum salmon (*Oncorhynchus keta*) in an estuary (Ueda, 2011), which can lead to increases in steroid hormones that fuel gamete development (Ueda & Yamauchi, 1995). Interestingly, more reproductively mature sockeye salmon (as evidenced from higher concentrations of circulating reproductive hormones) migrated faster from coastal waters into the Fraser River than less reproductively mature individuals (Cooke et al., 2008a; Crossin et al., 2009a; Crossin et al., 2007). In my study, PC2, which represented reproductive maturity, was present in all top-ranked 'physiological' models for the central SoG region, suggesting it may be an important variable. Model estimates for PC2 indicated that more reproductively mature fish migrated faster into the river, which is consistent with findings from previous studies. However, I cannot rule out the possibility of no effect of PC2 on migration rate based on coverage of the confidence intervals for this variable. Additional field studies that examine endocrine and neural responses along varying migration distances (or olfactory gradients) [see examples from (Ueda, 2011)] are needed to gain a better understanding of how homing salmon respond to olfactory cues in estuarine

environments. Despite these additional studies, the challenge remains for linking neuroendocrine responses to behaviour of wild migrating fish.

Conversely to the northern SoG region, stronger northwesterly winds were associated with faster migration rates for sockeye salmon migrating in the central SoG region (i.e., from the NSOG array to the Fraser River). I offer two possible explanations for this association. First, once sockeye salmon have adequately located the Fraser River plume, energy conservation may become more important before embarking on the energetically demanding up-river migration in warmer waters (Farrell et al., 2008; Patterson et al., 2007). Therefore, they take advantage of wind-generated currents in the direction of the Fraser River to aid in movements as an energy-saving strategy. An alternative explanation is that stronger northwesterly winds could have forced higher salinity surface waters exiting Discovery Passage southeastward along the SoG exposing sockeye salmon to higher salinities. Since sockeye salmon are progressively becoming more acclimated for freshwater while migrating through the SoG (Shrimpton et al., 2005), they may have attempted to avoid higher salinity water by migrating faster towards freshwater. Supporting my latter explanation, homing sockeye salmon that were captured in marine waters and were experimentally acclimated to freshwater migrated faster into the Fraser River after release into the estuary compared to fish held in either seawater or iso-osmotic water (Hinch *et al.*, 2008). Avoidance of higher salinity water through faster migration by sockeye salmon that are becoming freshwater acclimated could also aid in explaining my finding that higher salinity in the northern SoG region was associated with faster migration rates.

Interestingly, an earlier study found that sockeye salmon experiencing lower salinity in coastal waters along the southwest side of Vancouver Island (e.g. Juan de Fuca Strait) migrated faster into the Fraser River (Thomson & Hourston, 2011). Sockeye salmon physiological state (including osmoregulatory state) has been shown to differ between fish sampled while using either Juan de Fuca Strait or Discovery Passage as migratory routes, which was attributed to previous environmental experience (Evans et al., 2011). Therefore, I attribute the opposite responses to salinity between Thomson and Hourston (2011), in which stocks had entered the SoG via Juan de Fuca Strait, and my study, in which stocks had entered the SoG via Discovery Passage, to differences in prior environmental experience and different physiological states of sockeye salmon from the two distinct migratory routes. Further studies that experimentally manipulate salinity levels and test for physiological and behavioural responses [as in Cooperman et al. (2010)] are needed to gain a better understanding of how osmoregulatory state plays a role in migratory behaviour of homing anadromous salmon.

My results indicated that the association of glucose and migration rate varied by stock in the northern SoG region. Glucose is mobilized in response to exercise and physiological stress, and a previous study on homing sockeye salmon sampled in coastal marine waters found glucose was related to river entry timing (Crossin et al., 2007). My finding indicates there were stock-specific responses to exercise or physiological stress induced by the capture and tagging event. However, glucose, stock and the interaction between glucose and stock were not present in all top-ranked models, which suggests uncertainty as to their overall importance to migration rate in the northern SoG region.

Tissue biopsy is a technique commonly used to examine the physiological mechanisms underlying migration of Pacific salmon (Cooke et al., 2008b) and has been previously validated in the field (Cooke et al., 2005). However, results from my study indicated that sockeye salmon from the 'biopsy' treatment migrated slower than the 'control' group in the northern SoG region, but there was no effect of treatment on migration rate in the central SoG region. Physiological recovery from a stressor has been shown to occur over a 24-48 hour period in homing anadromous salmon (Donaldson et al., 2010), which corresponds to the approximate mean amount of travel time from the release site to the NSOG array (~2.4 days) and suggests that sockeye salmon had recovered from the 'biopsy' treatment by the time they were migrating in the central SoG region. Overall, the effect size of tissue biopsy on migration rate in my study was very small as 'biopsied' fish traveled ~ 6.5 km/day slower than 'control' fish, which represents ~16% of the average migration rate in the northern SoG region. Furthermore, the effects of tissue biopsy are likely insignificant compared to the larger effects associated with the capture event. More importantly, my finding that tissue biopsy (or the additional handling time associated with tissue sampling) can have an effect on migration rate highlights the importance of incorporating control treatments into studies using tissue biopsy, especially when examining behaviour immediately after release. Researchers could also consider incorporating variable handling times into study designs to specifically test for an effect of handling time.

This study presented details on migration routes, rate and timing of homing sockeye salmon in the SoG, and further provided empirical evidence of how environmental characteristics are associated with migration timing and rate. These results

are of particular importance for Fraser River sockeye salmon, which are in need of a better biological understanding due to recent variability in returning numbers of spawners (Cohen, 2012). Specifically, information on movement patterns (and the variables influencing them) is useful for forming management strategies of commercial fisheries, which target Fraser River sockeye salmon during this phase of the homing migration. In addition, my results showing an effect of wind and a short-term effect of tissue biopsy on estuarine migration rate are broadly applicable to those studying biological processes influencing anadromous salmon during their marine homing migration.

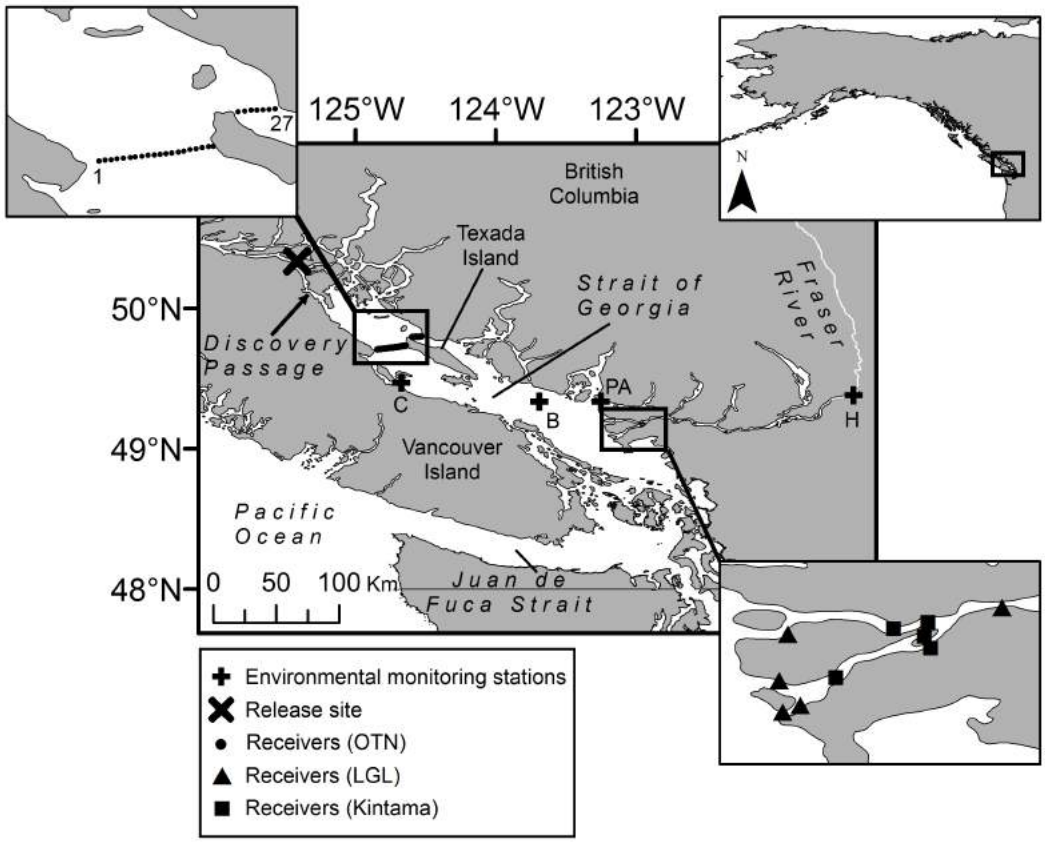

Figure 4.1 Map of study area. 'C' = Chrome Island, 'B' = Buoy 46146, 'PA' = Point Atkinson, 'H' = Hope. Kintama receivers were paired at each location in the lower Fraser River (n = 10), whereas all other receiver points represent a single receiver at each location (i.e., 27 OTN receivers, 5 LGL receivers).

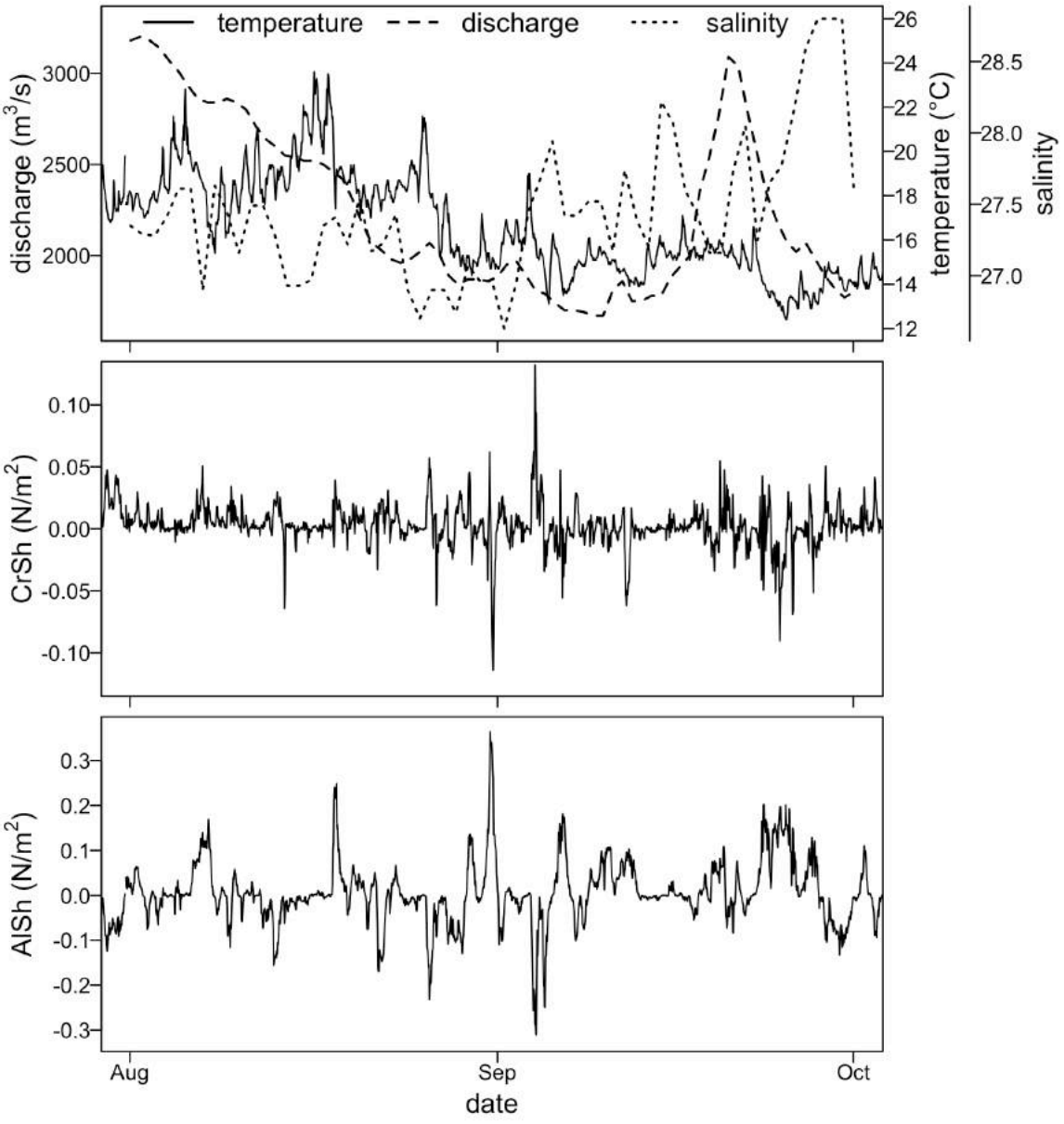

Figure 4.2 Environmental variables collected from various monitoring sites from August 1, 2010 to October 1, 2010.

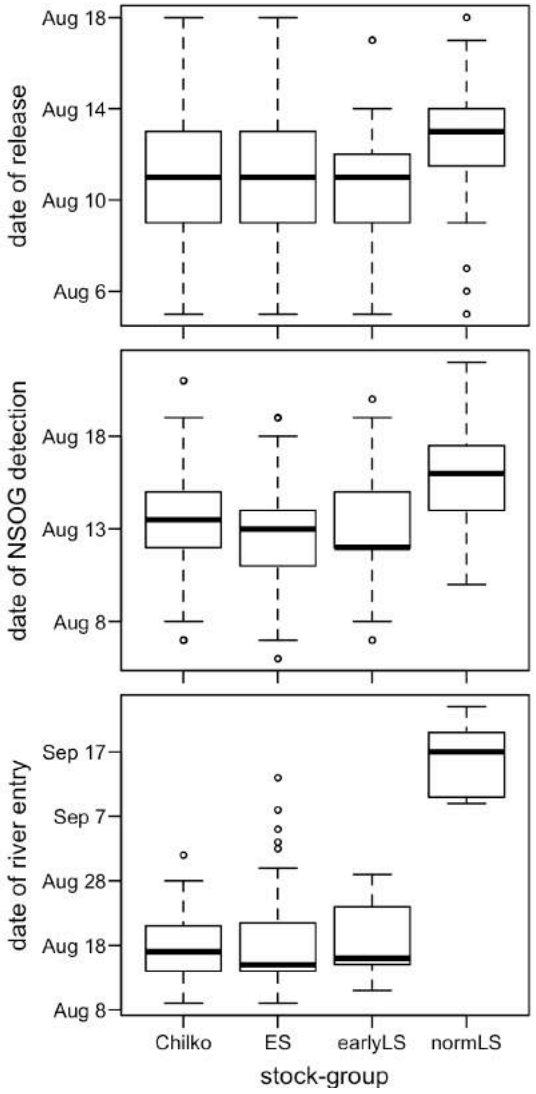

Figure 4.3 Boxplots of dates when sockeye salmon stocks were captured and released (upper), detected on the NSOG detection array (middle), and entered the river (lower). Data are presented for Chilko, Early Shuswap (ES), 'early-timed' Late Shuswap (earlyLS), and 'normal-timed' Late Shuswap (normLS) stock-groups. Solid bold horizontal lines within boxes represent the median, box limits represent the interquartile range (IQR), and whiskers represent 1.5x the IQR. Open circles represent outliers. Outliers were defined as outside 1.5x the IQR above the upper quartile and below the lower quartile.

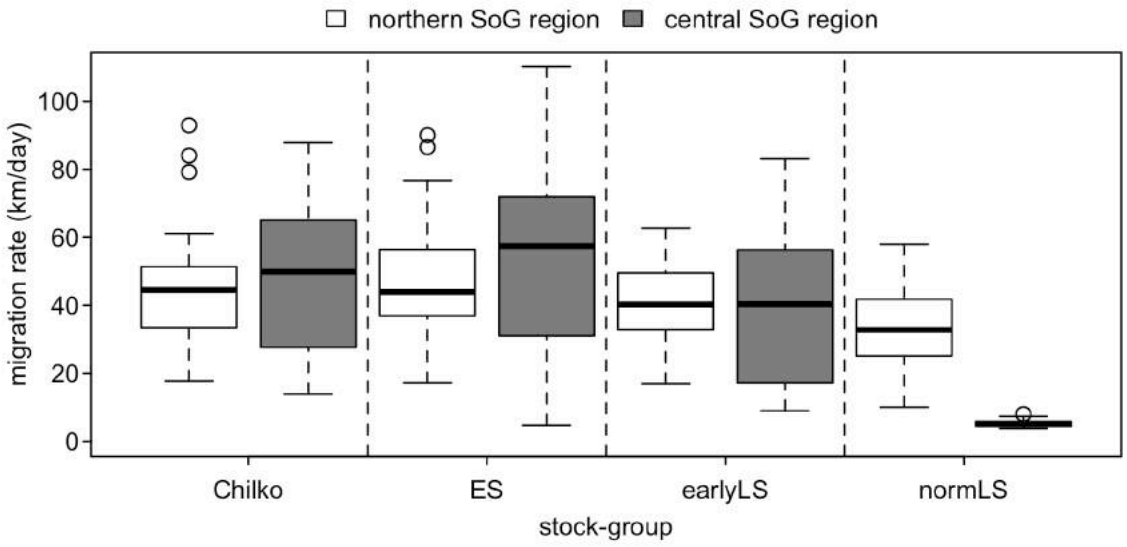

Figure 4.4 Boxplots of migration rates for Chilko, Early Shuswap (ES), 'early-timed' Late Shuswap (earlyLS), and 'normal-timed' Late Shuswap (normLS) stock-groups. Migration rates are shown for each stock-group in the northern (white) and central (grey) SoG regions. Solid bold horizontal lines within boxes represent the median, box limits represent the interquartile range (IQR), and whiskers represent 1.5x the IQR. Open circles represent outliers. Outliers were defined as outside 1.5x the IQR above the upper quartile and below the lower quartile.

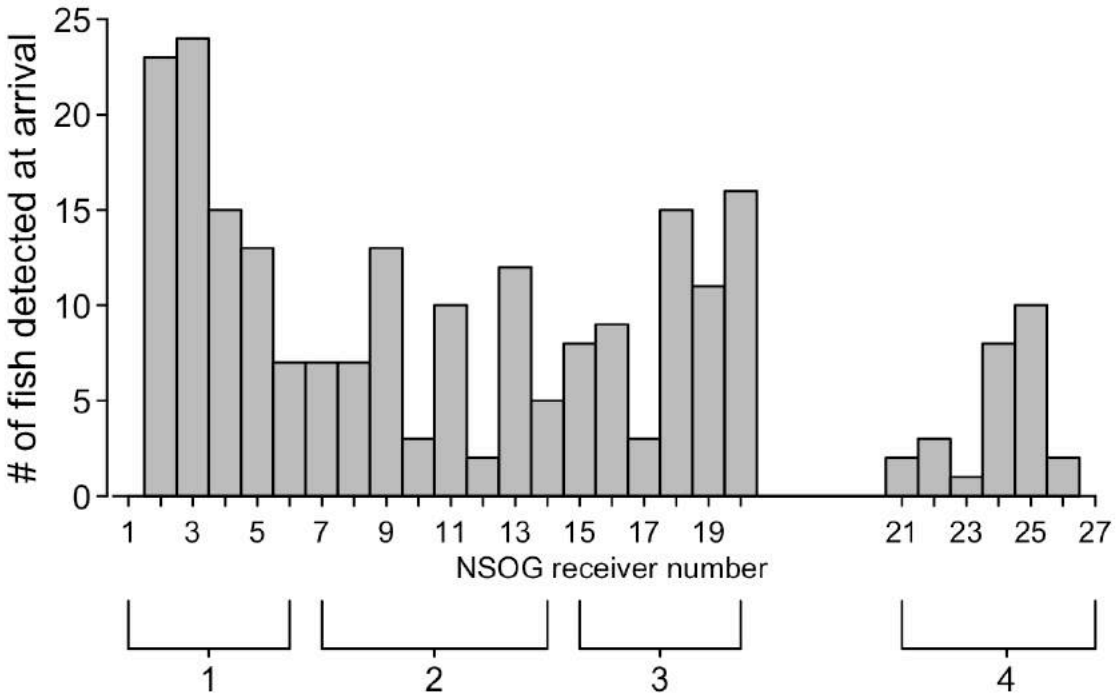

Figure 4.5 Number of individual sockeye salmon detected at arrival on receivers along the NSOG detection array. NSOG receiver numbers range from 1 to 27 going from west to east across the SoG, and the space between receiver numbers 20 and 21 represents where the receiver line intersects Texada Island (see Fig. 1). The numbers (1-4) below brackets at the bottom of the figure indicate the NSOG pathway groupings of receivers used in analyses. All Chilko, Early Shuswap and Late Shuswap fish (n = 229) are represented in the figure.

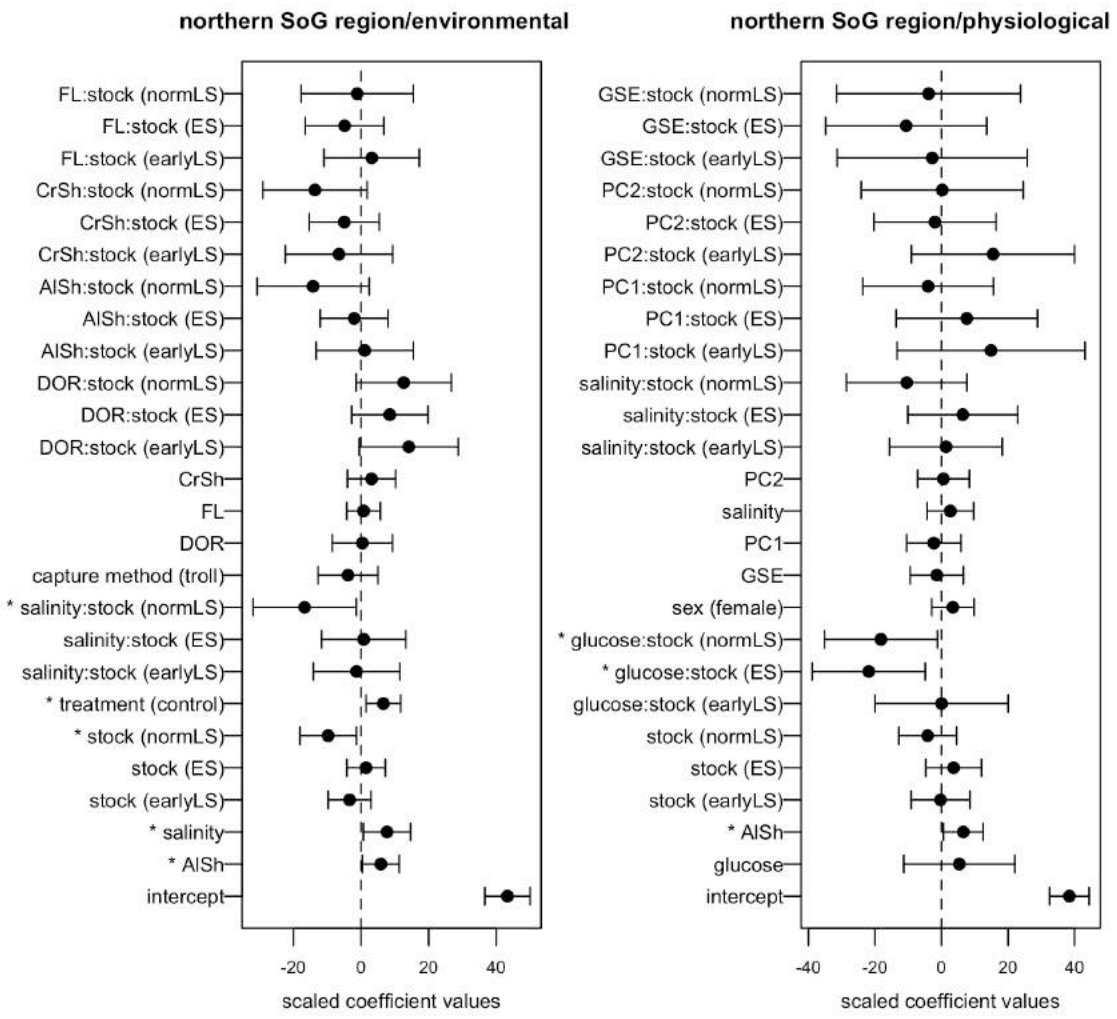

Figure 4.6 Model-averaged scaled parameter estimates (circles) with 95% confidence intervals (lines) for migration rate in the northern SoG region from two models. The 'environmental' model (left) used the full data set (n = 163) with the primary goal of assessing whether environmental variables influenced migration rate, but also included additional variables [i.e., FL (fork length), DOR (day-of-release), capture method (troll or purse seine) and treatment (biopsy or control)] that had data available for the full data set. The 'physiological' model (right) used data from a subset of fish (n = 99) for which physiological data was available to assess whether physiological variables influenced migration rate, but also included any variables selected based on effect size from the

'environmental' model. An asterisk preceding the variable names signifies that the 95% confidence intervals for the scaled parameter estimate did not intersect zero. Additional abbreviations are presented as ES (Early Shuswap), earlyLS ('early-timed' Late Shuswap), normLS ('normal-timed' Late Shuswap), CrSh (cross-shore wind stress), AlSh (along-shore wind stress), GSE (gross somatic energy), PC1 (first principle component; represented overall fish condition), and PC2 (second principle component; represented reproductive maturity).

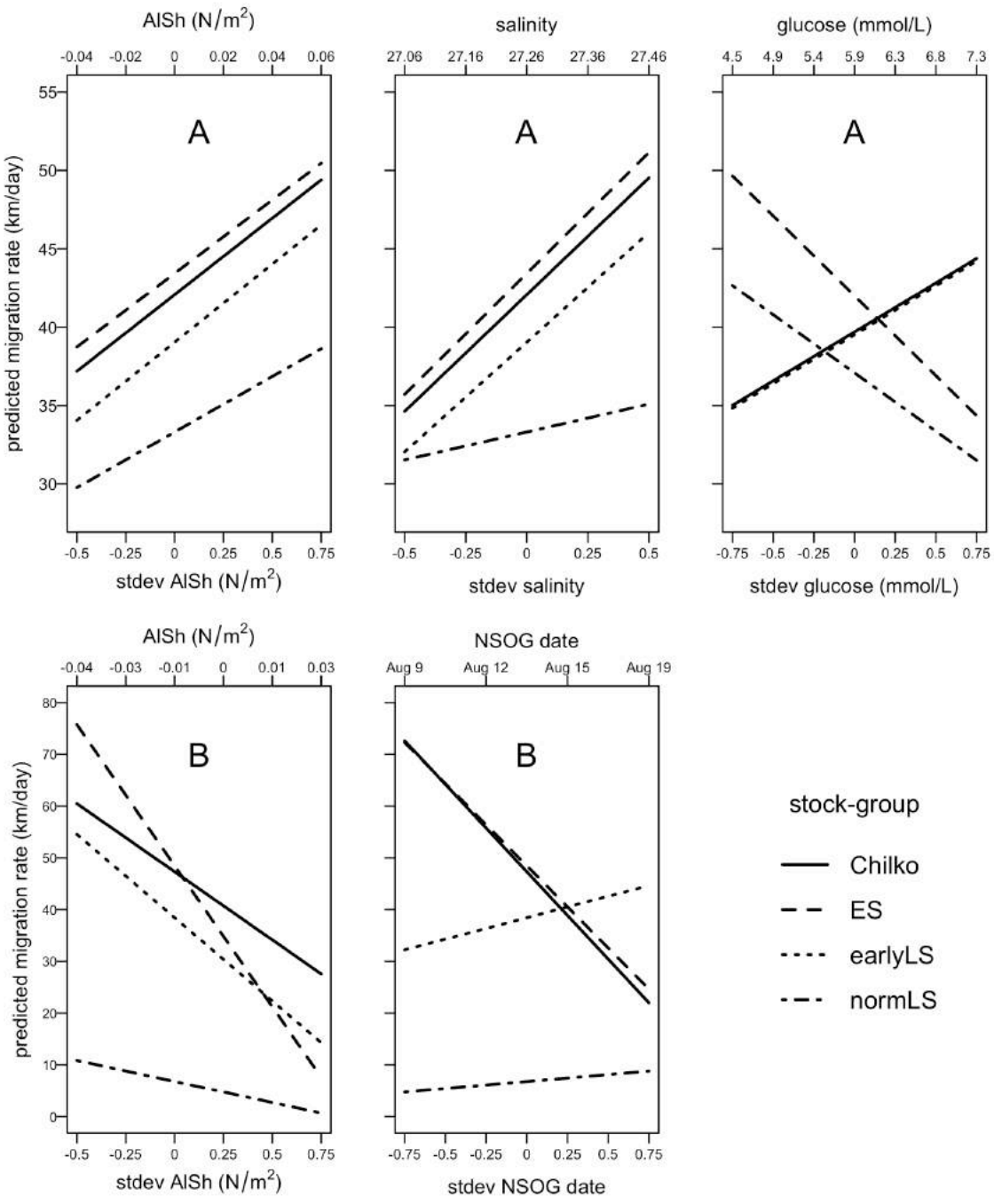

Figure 4.7 Model averaged predictions for migration rate based on variables selected as being associated with migration rate in the northern (A) and central (B) SoG regions. The upper x-axis is the actual value of the variable, and the lower x-axis is the standardized variable (in SD units). Model predictions are presented for the Chilko, Early Shuswap

(ES), ‘early-timed’ Late Shuswap (earlyLS), and ‘normal-timed’ Late Shuswap (normLS) stock-groups. Abbreviations are presented as AlSh (along-shore wind stress) and NSOG date (day of last NSOG detection).

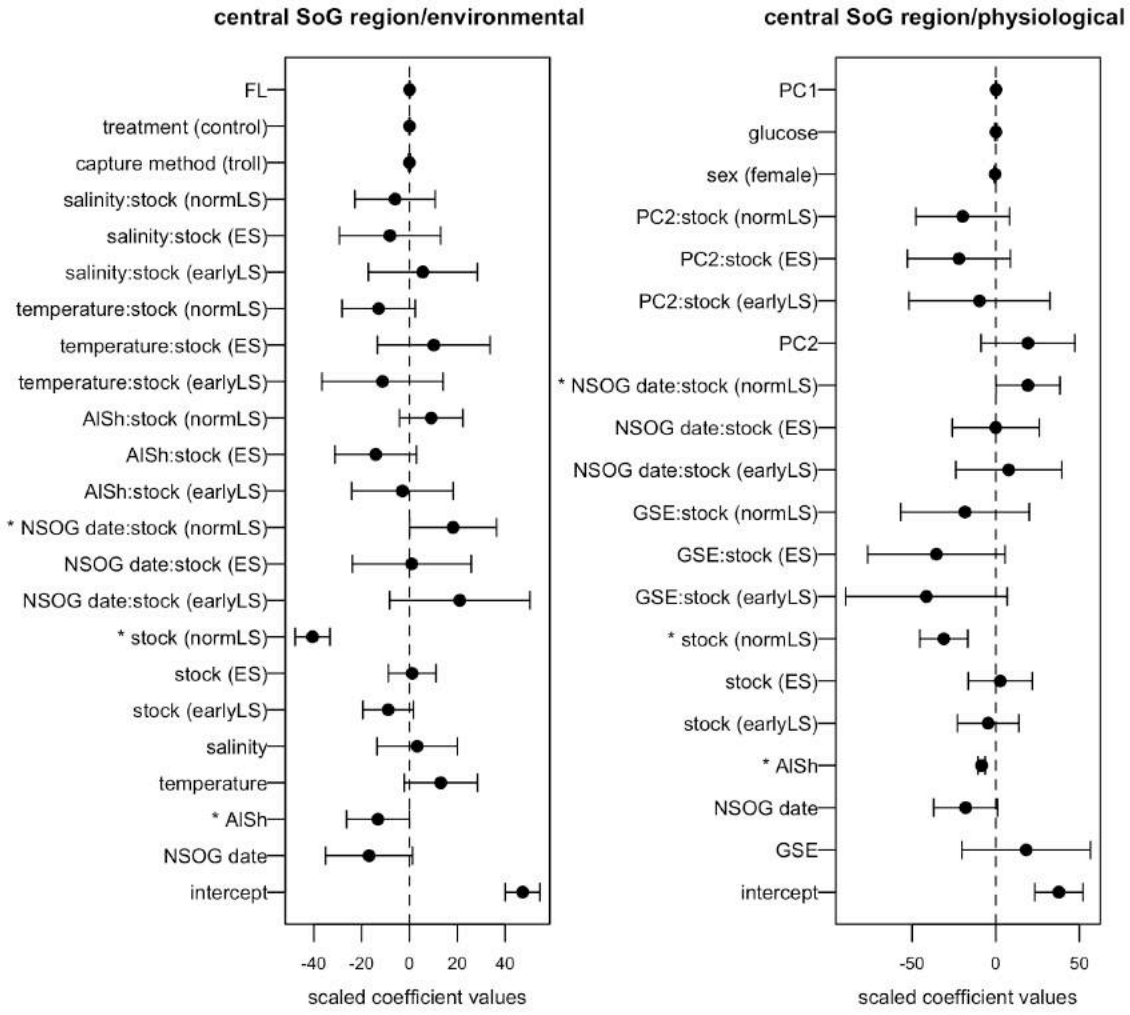

Figure 4.8 Model averaged scaled parameter estimates (circles) with 95% confidence intervals (lines) for migration rate in the central SoG region from two models. The 'environmental' model (left) used the full data set (n = 147) with the primary goal of assessing whether environmental variables influenced migration rate, but also included additional variables [i.e., FL (fork length), NSOG date (day of last NSOG detection), capture method (troll or purse seine) and treatment (biopsy or control)] that had data available for the full data set. The 'physiological' model (right) used data from a subset of fish (n = 90) for which physiological data was available to assess whether

physiological variables influenced migration rate, but also included any variables selected based on effect size from the 'environmental' model. An asterisk preceding a variable name signifies that the 95% confidence intervals for the scaled parameter estimate did not intersect zero. Additional abbreviations are presented as ES (Early Shuswap), earlyLS ('early-timed' Late Shuswap), normLS ('normal-timed' Late Shuswap), CrSh (cross-shore wind stress), AlSh (along-shore wind stress), GSE (gross somatic energy), PC1 (first principle component; represented overall fish condition), and PC2 (second principle component; represented reproductive maturity).

Table 4.1 Output of the principle component analysis (PCA) including all 8 blood variables. The PCA was successively run with stepwise elimination of variables based on plasma variables having either a) a low KMO statistic, or b) not having a loading > ±0.6 (shown in bold) for any factor which also had other > ±0.6 loadings (i.e., not agreeing strongly with other variables within a factor).

Plasma variable	PC1 loading	PC2 loading	PC3 loading	H2
Eigenvalues	3.20	1.82	1.43	
% variance explained	40	23	18	
Cortisol	**0.68**	0.38	-0.23	0.90
Estradiol	-0.13	**0.78**	0.48	0.85
Testosterone	-0.20	**0.79**	0.42	0.87
Glucose	0.44	0.38	**-0.66**	0.88
Lactate	**0.72**	0.36	-0.33	0.76
Chloride	**0.72**	-0.38	0.51	0.94
Omolality	**0.90**	-0.13	0.34	0.94
Sodium	**0.80**	-0.05	0.19	0.81

Table 4.2 Comparisons of the number of sockeye salmon detected upon arrival at each NSOG position. Numbers in parentheses indicate percentages.

NSOG position	NSOG receiver numbers	Number of sockeye salmon detected at arrival
1	1-6	57 (35)
2	7-14	37 (23)
3	15-20	48 (30)
4	21-27	21 (13)

Table 4.3 Comparisons of the number of sockeye salmon reaching the NSOG detection array or entering the Fraser River during day or night and at different tidal stages. Numbers in parentheses indicate percentages.

Location	Condition	Number
NSOG array	day	132 (81)
	night	31 (19)
	flood	48 (29)
	high	15 (9)
	ebb	89 (55)
	low	11 (7)
Fraser River entry	day	116 (79)
	night	31 (21)
	flood	51 (35)
	high	4 (3)
	ebb	68 (46)
	low	24 (16)

Table 4.4 Model selection results based on AIC_c showing top models ($\Delta AIC_c < 2$), to a maximum of 5 models, from a 95% confidence set of models. Models results are shown for 'environmental' and 'physiological' models in the northern and central SoG regions. The 'environmental' model used the full data set (n = 163 and n = 147 for the northern and central SoG regions, respectively) with the primary goal of assessing whether environmental variables influenced migration rate, but also included additional variables [i.e., FL (fork length), DOR (day-of-release), NSOG date (day of last NSOG detection), capture method (troll or purse seine), treatment (biopsy or control), and stock] that had data available for the full data set. The 'physiological' model used data from a subset of fish (n = 99 and n = 90 for the northern and central SoG regions, respectively) for which physiological data was available to assess whether physiological variables influenced migration rate, but also included any variables selected based on effect size from the 'environmental' model. Stock was included in all models for the central SoG region due to prior known differences, and therefore was not subject to model selection. Additional abbreviations are presented as AlSh (along-shore wind stress) and PC2 (second principle component; represented reproductive maturity)

Migration section/model step	Model	K	ΔAIC_c	W_i	Adjusted-R^2
Northern SoG Region					
environmental	AlSh, salinity, treatment, stock	4	0.00	0.06	0.20
	AlSh, treatment, stock × salinity	5	0.81	0.04	
	AlSh, salinity, treatment, stock, capture method	5	0.92	0.04	
	AlSh, salinity, treatment, stock, release date	5	1.58	0.03	
	AlSh, treatment, stock × salinity, release date	6	1.66	0.03	
physiological	AlSh, stock × glucose	4	0.00	0.04	0.21
	AlSh, stock	2	0.38	0.03	
	AlSh, stock × glucose, sex	5	0.56	0.03	
	AlSh	1	0.76	0.02	
	AlSh, glucose	2	1.09	0.02	

Migration section/model step	Model	K	ΔAICc	Wi	Adjusted-R2
Central SoG Region					
environmental	stock × NSOG date, stock × temperature, salinity, stock × AlSh	8	0.00	0.15	0.91
	NSOG date, temperature, salinity, stock × AlSh	6	0.17	0.13	
physiological	stock × NSOG date, PC2, AlSh, sex	6	0.00	0.11	0.91
	stock × NSOG date, PC2, AlSh	5	0.01	0.11	
	stock, NSOG date, PC2, AlSh, sex	5	0.46	0.08	
	stock, NSOG date, PC2, AlSh	4	0.67	0.08	
	stock × NSOG date, PC2, AlSh, glucose	6	1.33	0.06	

CHAPTER 5: TRANSCRIPTOME PATTERNS AND BLOOD PHYSIOLOGY ASSOCIATED WITH HOMING SUCCESS OF SOCKEYE SALMON DURING THEIR FINAL STAGE OF MARINE MIGRATION

5.1 Introduction

Animal migrations are challenging life history events whereby fitness advantages are gained by moving between habitats (Dingle, 1996). To reproduce, often only once, all anadromous salmon migrate from ocean feeding grounds to freshwater spawning areas. This reproductive migration represents one of the most demanding phases of the entire salmon life cycle because within a matter of a few months leading up to spawning, migrants undergo extensive physiological shifts associated with reproductive maturation and freshwater acclimation, as well as cope with complex environmental challenges (Groot et al., 1995; Healey, 2000; Hinch et al., 2006). Mortality prior to reaching spawning areas should not be unexpected, and can act as a powerful selection mechanism for semelparous species such as Pacific salmon that only have one opportunity to reproduce in a lifetime [with the exception of cutthroat trout and steelhead (Groot & Margolis, 1991)]. Of great concern, however, is that in recent years, adult mortality during homing for some salmon populations has reduced the population size to below replacement rates, warranting conservation and management concerns for these ecologically, economically and culturally valuable species (Boisclair, 2004; Bradford, 1995; Nehlsen et al., 1991; Parrish et al., 1998). This concern has resulted in biotelemetry studies that, to date, have largely focused on factors associated with pre-spawn mortality in-river; which has been linked with water temperature (Martins et al., 2012b; Thorstad et

al., 2008), stress (Cooke et al., 2006a, Thorstad et al., 2008), energetic state (Crossin et al., 2009a) and disease (Miller et al., 2011) to name just some. In contrast, fewer studies have investigated factors that influence marine survival, in part because of the greater logistical and financial challenges of collecting and tracking the fate of adult salmon in a marine environment (Drenner et al., 2012). However, with the advent of new acoustic arrays in the SoG, as well as in the Fraser River estuary it is now possible to follow the fate of Fraser River adult sockeye salmon during the final stage of their marine migration.

By combining individual biotelemetry with physiologic biopsy of blood and gill tissues, previous studies (Cooke et al., 2008a; Cooke et al., 2006a; Cooke et al., 2006b; Crossin et al., 2009a; Crossin et al., 2007; Crossin et al., 2009b) specifically focused on homing Fraser River sockeye salmon discovered that the physiological state of fish sampled in coastal waters was associated with marine and freshwater survival in the SoG and the Fraser River watershed, respectively. Physiological variables found to be associated with survival included GSE, as well as a number of variables related to stress, reproductive status and osmoregulation (e.g. cortisol, reproductive hormones, ions and osmolality) in addition to gill tissue variables involved in osmoregulation (NKA enzyme activity). These results seem particularly important during the challenging final marine phase of the sockeye salmon migration because the fish are simultaneously preparing their osmoregulatory system for a seawater to freshwater transition [as evidenced from plasma ions and NKA activity; (Flores et al., 2012; Shrimpton et al., 2005)], undergoing reproductive maturation and developing secondary sexual characteristics (Hinch et al., 2006; Høgåsen, 1998). In addition to any pathogens (e.g., parasites, bacteria, viruses, fungi) acquired during previous life history phases, they may also encounter novel

freshwater pathogens in the river system. Lastly, entrance into freshwater and encountering higher water temperatures in river/lakes compared with seawater (sockeye salmon migrate in the summer) may increase the pathogenicity of some pathogens (Miller et al., 2014). As a result, sockeye salmon migrating in estuaries characteristically increase levels of circulating stress hormones and metabolites (cortisol, lactate, glucose) (Cooke et al., 2006a; Crossin et al., 2007) that are closely tied with reproductive maturation, energy use, immune function, olfactory navigation, and ionoregulation (Barton, 2002; Carruth et al., 2002). Thus any disruption to these on-going processes minimally impacts energy management, which is also crucial because homing salmon nearing freshwater cease to feed and must conserve energy for intense swimming activity to migrate upriver and spawn (Brett, 1995).

Application of functional genomic techniques, such as microarray analysis and qRT-PCR, to ecological studies has enabled a much finer and detailed analysis of physiological mechanisms (microarrays can measure the expression of tens of thousands of genes from one tissue sample). Indeed, genomics techniques have already provided valuable insights into the physiologic mechanisms in homing sockeye salmon (Evans et al., 2011; Flores et al., 2012; Jeffries et al., 2012; Jeffries et al., 2014; Miller et al., 2011; Miller et al., 2009; Shrimpton et al., 2005). Of particular note was the use of a 16k gene salmonid cDNA microarray that identified a mortality-related signature (MRS) in homing sockeye salmon sampled in seawater and freshwater (Miller et al., 2011). Fish with the MRS had a 13.5-fold greater chance of dying prematurely in freshwater, and showed signs of infection, stress and osmoregulatory perturbations (Miller et al., 2011).

In the present study, an improved 4x44k gene salmonid oligonucleotide microarray (Jantzen et al., 2011) and qRT-PCR was added to the arsenal of physiological analyses of blood and gill biopsies and biotelemetry to investigate the physiological mechanisms associated with successful homing of sockeye salmon from the marine environment into the Fraser River. The primary objective was to test whether gene expression is related to marine migration success to freshwater entry for homing sockeye salmon using the 44k gene salmonid oligonucleotide microarray, as done previously with a smaller microarray to investigate freshwater survival (Miller et al. 2011). The prediction was that gene expression patterns of unsuccessful migrants would show signs of an elevated stress and immune response. The second objective was to relate physiological parameters including sex measured from blood biopsy, and other factors such as energetic state, fish size, fish stock (e.g. population), fisheries capture method, and biopsy treatment to marine migration success, and to compare these results with those from microarray analysis and from previous studies. A final objective was to assess whether factors other than survival were associated with the gene expression profiles such as energetic state, fish size, fish stock (e.g. population), fisheries capture method, and physiological parameters measured from blood biopsy. This study represents the first attempt to relate gene expression patterns of wild-migrating homing salmon to fate in the marine environment.

5.2 Materials and methods

All experiments were conducted with the approval of the Animal Care Committee of the University of British Columbia, in accordance with the Canadian Council on Animal Care.

5.2.1 Fish capture, biopsy and telemetry

Adult sockeye salmon (n = 400) were captured in August, 2010 by either troll (n = 375) or purse seine (n = 25) commercial vessels in northern Discovery Passage, ~ 215 km north of the Fraser River during their normal migration (Fig. 5.1). These fish were the same individuals used in chapter 4 of this thesis, with the exception of the addition of 35 fish to this study for a holding experiment (see below). After capture, individual fish were brought on board the vessel and transferred to a holding tank that was flushed with free-flowing ambient seawater. Fish were held for <15 min on troll vessels and <120 min purse vessels before biopsy or implanting a biotelemeter. There were two treatment groups. The biopsy treatment (n = 285) (~ 78% of fish tagged and released) followed established procedures handling and biopsying of unanaesthetized sockeye salmon in a non-lethal manner (Cooke et al., 2005). Each individual fish was moved from the holding tank to a foam-padded, v-shaped trough with the gills irrigated with cold seawater. A 3 ml blood sample was quickly taken from the caudal vein and stored on ice until processing at the end of the workday, when blood was centrifuged, and plasma was transferred into liquid N_2 (Farrell et al., 2001). The plasma was used to assess concentrations for stress parameters (glucose, cortisol, lactate), sex hormones (testosterone, 17β-oestradiol) and osmoregulatory state (concentrations of Na^+ and Cl^-, as well as plasma osmolality) in the laboratory at a later date. Next, a small (< 4 mm) gill biopsy was taken from gill filament tips for gene expression analysis. Gill filament tips were separated into two vials containing RNAlater that were refrigerated for 24 h before being transferred to liquid N_2. An adipose fin punch (0.5 g) was stored in 95% ethanol prior to analysis for DNA stock identification [mean percentage error < 1% (Beacham et

al., 2004)]. Fork length was measured to the nearest cm and GSE was determined with a hand-held microwave radio emitter [Distell Fish FatMeter FM 692, Distell Inc., West Lothian, Scotland, UK; (Crossin & Hinch, 2005)]. Finally, an individually coded acoustic transmitter (Vemco V16-3x, 16 mm diameter and <70 mm length) was gastrically inserted with a plastic applicator and a spaghetti tag (Floy Tag & Mfg. Inc., Seattle, WA, USA) was applied anterior to the dorsal fin through the dorsal musculature to assist visual identification after release. The non-biopsy treatment group (n = 80) (~ 22% of fish released) experienced reduced handling and was used to test for tissue biopsy effects on subsequent migration success by limiting handling to the implantation of the acoustic transmitter (i.e., no blood biopsy, gill biopsy, GSE sample and spaghetti tag) and taking a adipose fin punch. Total handling time was < 3 min for the non-biopsy group and < 5 min for biopsy group.

All fish were immediately released overboard following either treatment. The release site was deliberately at the southern boundary for commercial fishery to minimize possible recapture of tagged fish in legal fisheries between Southern Discovery passage and first acoustic receivers in the lower Fraser River. Nonetheless, two tags were recaptured by commercial fishing vessels close to the release site and were excluded from the analysis. However, other unaccounted fishery captures of tagged fish could have biased the results.

For a short-term holding study, fish (n = 35) were captured at the same location, biopsied, but not released and instead were transferred to a metal holding cage fixed to the side of a dock to assess rates of tag retention and short-term mortality. Additional to the 400 fish described above, control fish (n = 30) that had not been treated after capture

were also placed into the cage to compare survival between tagged and untagged fish. Fish were held in the cage for approximately 32 h, which approximated the time for a fish to swim to the first telemetry array based on previous studies (Crossin et al., 2009a). Any behavioural effects due to capture, handling and tissue biopsy were expected to be short-lived (Donaldson *et al.*, 2012), and therefore would be detected during this initial period of migration (i.e., first 32 h). All surviving fish from the short-term holding study were released, but were not included in survival estimates or analyses described below.

Individual fish were detected at fixed acoustic telemetry arrays along the migration route [see Heupel et al. (2006) for details on the technology], from which a survival estimate was determined. Each fish detection was registered and stored an individual tag ID and an associated timestamp. These data were downloaded from receivers at a later date. The NSOG acoustic telemetry array (Fig. 5.1) provided the first point of detection, ~ 84 km southward from the release site [a former POST array that is currently maintained by OTN]. The NSOG array consisted of 27 receivers spanning ~25 km across the SoG, from Vancouver Island to the BC mainland, crossing the northern tip of Texada Island (Fig. 5.1). Successful migration to the NSOG array was compared with survival from the 32-h holding experiment to assess short-term impacts of tagging on survival. A series of acoustic receivers (maintained by Kintama Research, Nanaimo, BC, Canada or LGL Ltd., Victoria, BC, Canada) within the estuarine arms of the Fraser River was the next point of detection, ~ 131 km southeast of the NSOG array (Fig. 5.1) and extended ~ 85 km upriver of the river mouth (Fig. 5.1). A successful river entry represented a single detection on any of these lower Fraser River receivers. The major challenges with biotelemetry data are tag signal collisions that prevent fish from being

detected or imperfect detection efficiencies of an array. Previous estimates of detection efficiency for the NSOG array and the Kintama-maintained lower Fraser River receivers are 100% for acoustic-tagged adult salmon with the same tag specifications as used here [see Crossin et al. (2009a)]. In fact, all fish not detected on the NSOG array were not detected on any of the Fraser River receivers. Furthermore, it can be reasonably assumed that detection efficiency in the lower Fraser River was as good as Crossin et al. (2009a) because of the added LGL acoustic receivers in the present study. False detections were removed from the data set and were defined as any individual tag that was detected only once within 24 h across either the NSOG array or any Fraser River receiver.

5.2.2 RNA and microarray preparation

5.2.2.1 Total RNA isolation

Total RNA was purified from gill tissue using Magmax™-96 for Microarrays Kits (Ambion Inc, Austin, TX, USA) with a Biomek NXP (Beckman-Coulter, Mississauga, ON, Canada) automated liquid-handling instrument. Approximately 2 gill filaments stored in RNAlater were homogenized with stainless steel beads in TRI-reagent (Ambion Inc, Austin, TX, USA) on a MM301 mixer mill (Retsch Inc., Newtown, PA, USA). One hundred µl aliquots of the aqueous layer of the homogenate were pipetted into 96-well plates and extractions were carried out according to the manufacturer's instructions using the "No-Spin Procedure" for tissues, on the Biomek NXP. RNA yield was determined by measuring the A_{260} of the eluate. Purity was assessed by measuring the A_{260}/A_{280} ratio of the eluate. Solutions of RNA were stored at -80 ºC.

5.2.2.2 cRNA Labeling

Amplification and labeling steps were performed on 96-well plates all at once and with the same kit batches to minimize technical artifacts. 1.0 µg of total RNA was amplified using the Amino Allyl MessageAmp^{TM}II-96 kit (Ambion, TX, USA) according to manufacturer's instructions. A dye coupling reaction was performed using 5 µg of amino-allyl aaRNA (cRNA) and Alexa dyes (Invitrogen, Carlsbad, CA). During dye labeling, samples were processed individually by adding DMSO to the Alexa dye tube and coupling buffer to the appropriate cRNA. The dye mix was then combined with the sample mix and incubated for 1h at room temperature. For microarray experiments, a pooled tissue reference comprised of the RNA from all of the fish used in the experiment was used against each individual sample. All individual (experimental) samples were fluorescently tagged with Alexa 555 (Invitrogen, Carlsbad, CA) and the reference tagged with Alexa 647 (Invitrogen, Carlsbad, CA). Samples and references were purified using the Amino Allyl MessageAmp^{TM}II-96 kit (Ambion, TX, USA) according to manufacturer's instructions and eluted in 35 µl of elution buffer. A fragmentation mix containing 825 ng of Alexa-555 labeled cRNA sample, 825 ng of the Alexa-647 labeled reference cRNA pool, 10X blocking agent (Agilent Technologies) and 25X fragmentation buffer (Agilent Technologies) was made and incubated at 60 ºC for 30 min. Equal volumes of the fragmented cRNA were added to GEx Hybridization buffer HI-RPM (Agilent Technologies) and spun down at room temperature for 1 min to reduce bubbles. These were immediately frozen and stored in the dark at -20 ºC until use.

5.2.2.3 Salmon arrays

The cGRASP (http://web.uvic.ca/grasp/) 44K Salmonid Oligo Array (Agilent Technologies) was used in this study. The array is comprised of approximately 22,000 60-mer oligos that are 95% conserved between rainbow trout and Atlantic salmon, plus an additional 14,866 Atlantic salmon and 5,661 rainbow trout contig sequences, resulting in a microarray with large transcript representation and very low redundancy. At the time of use, 84% of the features were well annotated with stringent hits (e-value cutoff: 1e-10) to public databases (December 17, 2009).

In each of the technical steps leading to slide hybridization, samples were either randomized (RNA extraction, hybridization) or ran all at once (amplification, labeling); as a result, extensive biological replication was chosen over technical replication, as my approach ensured that technical variation would not be confounded by biological variables of interest. Hybridizations were performed in batches of up to 48 samples and conducted by a single technician over a 10-day period to minimize technical variance. 55 µl of sample was loaded onto 4x44k Agilent slides using the quad hybridization chambers in the Tecan-HS4800 Pro Hybridization Station (Tecan Trading AG, Switzerland). Slide processing steps within the Tecan HS4800 were conducted as follows: one 1 min slide wash step (aCGH prehybridization buffer) at 65 ^{o}C, sample injection at 63 ^{o}C with agitation, hybridization for 17 h at 63 ^{o}C with high viscosity mode agitation, two 1 min washes (GE wash 1 with 0.005% Triton-X102) at 23 ^{o}C with a 1 min soak time, two 1 min washes (GE wash 2 with 0.005% Triton-X102 and 0.01% surfactant) at 37 ^{o}C with a 1 min soak time, followed by slide drying at 30 ^{o}C for 2 min.

Slides were scanned using the Tecan LS Reloaded scanner (TecanTrading AG, Switzerland) and the Array-Pro Analyzer software according to manufacturer's instructions. Images were quantified using Imagene (BioDiscovery, El Segundo, CA, www.biodiscovery.com) and spots with poor quality or no signal (<2 standard deviations from background) at both wavelengths were flagged. Raw microarray intensity data were normalized in GeneSight (version 4.1, BioDiscovery, Inc. El Segundo, CA, www.biodiscovery.com) using the local intensity-dependent loess normalization in order to remove intensity-dependent dye bias (Yang et al., 2002). All data were $\log_2$ transformed and an intensity ratio was computed by taking the differences in log transformed intensities between the sample and reference control. These log-transformed intensity ratios were used in all further analyses.

Features with observations missing for greater than 50% of the samples were eliminated from the analysis, leaving ~ 26 k features upon which analyses were based. For hierarchical clustering and principle component analysis (PCA), the remaining features with missing values had their intensity ratios imputed using the K-nearest neighbour method, but were unmodified for ANOVAs.

Microarray data were deposited in the NCBI Feature Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/).

5.2.3 Quantitative Reverse-Transcriptase PCR (qRT-PCR)

MRS biomarker TaqMan assays were developed based on the MRS signature as defined on the GRASP 16K salmon microarray (from the Miller et al., 2011 study). Because the mapping of genes across the 16K microarray and the cGRASP 44K Agilent

array is very poor, to compare results to the earlier study (i.e., Miller et al., 2011), the MRS biomarkers were applied that had previously been validated to predict the MRS microarray signature (K.M. Miller, unpublished data). QRT-PCR of biomarker genes in gill tissue was conducted using TaqMan assays run on the Fluidigm BioMark^{TM} platform (Fluidigm Corp. San Francisco, CA). Technical details on the Fluidigm BioMark^{TM} can be found in Miller et al. (2014).

5.2.4 Blood plasma laboratory analysis

Plasma osmolality, ions (Na^+, Cl^-), glucose and lactate were measured using procedures outlined in Farrell et al. (2001). Plasma cortisol, testosterone and 17β-oestradiol were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Neogen). Testosterone and 17β-oestradiol samples were extracted in ethyl ether in accordance with the manufacturer's protocols. Cortisol, testosterone and 17β-oestradiol samples were all run in duplicate at appropriate dilutions. Additional details on assays are provided in Farrell et al. (2001).

5.2.5 Data analysis

DNA analysis revealed that four fish stocks (Chilko, Early Shuswap, Harrison, Late Shuswap) accounted for the majority of the fish, which then allowed for a focused analysis of these stocks and a comparison with previous work on the marine survival of these stocks (Cooke et al., 2006b; Crossin et al., 2009a). Differences in survival among stocks to the NSOG array and the lower Fraser River receivers were examined with a test of equal proportions. Variables most associated with marine survival were identified with

generalized linear models (GLMs; family=binomial, link=logit), using marine survival (i.e., a successful river entry) as the response variable. Sample size was maximized by separately running three sub-models, each with a set of related explanatory variables, which allows for comparison among models and for complementary rather than competing models (Planque et al., 2011) while preventing over-fitting and reducing co-linearity.

The primary study objective was addressed by the survival ~ transcriptome sub-model, which tested the gene expression features that were related to marine survival (n = 145 fish for which microarrays were run). Principle component analysis was used to construct PC axes that captured variability in gene expression for all genes that had complete expression profiles across all fish (i.e., ~26 k genes were successfully run for all fish). The PC scores for each of the first five PC axes of each fish were used as explanatory variables. To further investigate the broad classes of genes related to marine survival, a functional analysis was performed on genes from any PC axis from microarray results found to be significantly associated with marine survival. Using PathWay Studio^{TM} (Nikitin et al., 2003), gene-set enrichment analysis was performed to identify processes that are over-represented in the gene expression profiles. Redundancy reduction was performed using REVIGO (Supek et al., 2011) on significant ($p < 0.01$) gene ontology terms resulting from the functional analysis. T-tests were used to relate MRS biomarker gene expression load to any PC axes that were significantly related to marine survival to test for similarities between the present study and Miller et al. (2011). I choose to take an unsupervised approach using PCA to summarize gene expression and contrasting survivorship along PC axes rather than a supervised approach comparing

survivors and non-survivors because there are numerous potential factors related to survival that may not be represented in the gene expression patterns.

The second study objective was addressed by the survival ~ blood physiology sub-model, which identified blood plasma variables related to marine survival (n = 250 biopsied fish). Explanatory variables included: cortisol, lactate, glucose, sodium, chloride, testosterone, osmolality, sex and GSE. MANOVA comparisons were used to compare plasma variables and GSE among stocks (Cooke et al., 2006a; Crossin et al., 2009a). Variables with significant differences among stocks based on MANOVA results were added as interactions with stock in the model. In addition, data exploration revealed potential differences in plasma variables between troll and purse seine capture methods, and therefore, MANOVA comparisons were used to compare plasma variables between capture methods. Visual inspection of multi-panel scatterplots indicated possible co-linearity between some plasma variable pairs. Based on a Pearson correlation coefficient >0.8 and VIF >3 (Zuur et al., 2010), osmolality, which was identified as a co-linear with lactate, cortisol, Na^+ and Cl^-, was excluded from subsequent analyses.

The second study objective was also addressed by the survival ~ non-physiology sub-model, which pooled biopsied and non-biopsied fish (n = 313) to identify factors beyond blood plasma and genomics that were related to marine survival. Explanatory variables entered in this model included: capture method (troll versus purse seine), biopsy treatment, stock, DOR and FL. This model also assessed the null hypothesis that neither tissue biopsy nor capture method had an effect on marine survival.

Within each sub-model, all continuous explanatory variables were standardized (i.e., subtracting global mean from each value and dividing by two times the standard

deviation) (Gelman, 2008), which allowed for the estimation and comparison effect sizes among predictor variables. To identify potentially important variables within each sub-model, an all-subsets regression was conducted, and models were ranked based on AIC corrected for small sample sizes (AIC_c) (Burnham & Anderson, 2002), ΔAIC_c and AIC_c weights (w_i) using the 'MuMIn' package (Bartoń, 2013) in R (R Core Development Team, 2012). Model averaging was applied to a 95% confidence set of models (all models with a cumulative summed $w_i \geq 0.95$) to incorporate model uncertainty (Burnham & Anderson, 2002). Model fit was assessed using adjusted-R^2. The relative support for individual predictor variables in the models was evaluated based on whether the 95% confidence intervals for estimates of effect size intersected zero (i.e., if the 95% confidence intervals did not intersect zero there is greater support for the variable being associated with marine survival). Diagnostics for heteroscedasticity, normality and independence of residuals were visually inspected in all models. Model predictions were made based on all variables present in the 95% confidence set of models, and using the median value for continuous variables and selecting a level of a categorical variable that was predicted to have the least effect on survival. All data analyses were performed using R v. 2.15.2 (R Core Development Team, 2012).

To better understand factors influencing gene expression and to compare results between microarray and blood plasma data, thereby addressing the third study objective, further GLMs were constructed for any PC axis from microarray data significantly related to marine survival. For these GLMs, the PC scores of the PC axis was the response, and predictor variables included fish stock, capture method, sex, blood plasma variables, GSE and FL. Otherwise, model diagnostics, model construction, selection, and

assessment followed the procedure described above (i.e., all subsets regression, ranking via AIC_c, model averaging, and investigation of effect sizes).

5.3 Results

All biopsied (n = 35) and control (n = 30) salmon survived the 32-h holding study in the metal net pen, and there was no tag regurgitation. Of the 313 salmon tagged and released, 71 – 80 % survived to the first detection point (e.g., the NSOG array) and 53 – 66% to the lower Fraser River array depending on salmon stock (Table 5.1). However, no significant differences in survival existed among sockeye salmon stocks to either the NSOG array (df = 3; P = 0.76) or the lower Fraser River array (df = 3; P = 0.52).

PC axis 1 (PC1) of the PCA on gene expression from the microarray captured 19% of the variability, and cumulatively the first five PC axes contained 41% of the total variability (Table 5.2). Of these five PC axes, the survival ~ transcriptome sub-model selected only PC1 as being related to marine survival (Fig. 5.2a), such that fish with more positive PC1 scores had a lower probability of survival to river entry (Fig. 5.3A). The top survival ~ transcriptome sub-model that contained only PC1 explained a small proportion of the data variability (adjusted-R^2 = 0.10) (Table 5.3).

The biological processes associated with PC1 of microarray data, and therefore marine survival, was explored further in a functional analysis. Survival of fish in the 25% and 15% ends of PC1 were contrasted to identify groups of fish with the strongest differences in survival. Fish in the 15% most PC1-positive grouping had a proportional marine survival of 68.2% (15 out of 22 fish) whereas the 15% most PC1-negative grouping had a proportional marine survival of 95.5% (21 out of 22 fish). The

proportional survival was significantly different between the two PC1 groupings [two-sided Fisher exact test; $P = 0.046$, and an Odds Ratio of 9.35 (95% confidence interval = 1.03 - 461.0)]. Therefore, to identify the strongest genomic signal for use in a functional analysis, the PC scores of individual fish (n = 145) were ranked along the first PC axis, and fish in the 15% most PC1-positive and 15% most PC1-negative groupings were selected (n = 44). Fold-change was then calculated by averaging intensity ratios across individuals within a given PC1 grouping, then calculating the difference in averaged ratios between PC1 groupings as [mean(PC1-negative 15%) – mean(PC1-positive 15%)]. The resulting value (x) was converted to fold-change [if $x > 1$, then fold-change = $2^{(x)}$; if $x \leq 1$, then fold-change = $(-1)2^{(x)}$]. Thus, a positive fold-change value reflects up-regulation of a gene in the 15% most PC1-negative group of fish that had significantly higher probability of survival. The 50 most up-regulated and 50 most down-regulated genes (based on fold-change values) included genes involved in metabolic processes, cell proliferation, apoptosis, protein synthesis, stress, immune response, protein binding, structural proteins and osmoregulation (Appendix C). In agreement, the functional analysis indicated protein biosynthesis, cellular metabolism, apoptosis, stress and immune defense, protein binding, oxidative phosphorylation, and structural processes were biological processes significantly overrepresented among genes most differentially regulated between the PC1 groupings (Table 5.4).

Based on t-tests relating MRS biomarker load to PC scores of fish in the 15% most PC1-positive and the 15% most PC1-negative groupings, 40 out of 58 (70%) biomarker genes were significant at $p < 0.05$, 30 out of 58 (52%) biomarker genes were significant at $p < 0.01$, and 22 out of 58 (38%) biomarker genes were significant at $p <$

0.001 (Table 5.5). Comparisons of biomarker load between the PC1 groupings indicated the majority (33 out of 40; ~ 83%) of significant biomarker genes were up-regulated in the 15% most PC1-negative group, which had a higher probability of marine survival (Table 5.5). Notably, significant immune-, stress-, and osmoregulatory-related MRS biomarkers that were up-regulated in the 15% PC1-negative group included genes involved in antigen presentation via the proteasome (PSMB4), proteolysis or inflammation (MMP25), apoptosis (PRF1, RALB), osmotic stress (CIRP), interferon immune response (IFNA2, IRF1, MX_ONTS, RIG-1, MCSF), immune response through activation of the complement cascade (C4B), viral release (SGTA), osmoregulation (NKAA1B, NKAA3), and other immune related responses (CD4, NKA_B1, ZAP7) (Table 5.5). In contrast, significant immune-, stress-, and osmoregulatory-related MRS biomarkers that were down-regulated in the 15% PC1-negative group included genes involved in interferon immune response (STAT1), oxidative stress (WDR16, SHOP21), and osmoregulation (NKAA1C) (Table 5.5).

MANOVA comparisons among stocks indicated significant differences among stocks for plasma testosterone ($df = 3$; $P < 0.001$), 17β-oestradiol ($df = 3$; $P < 0.003$) and glucose ($df = 3$; $P = 0.03$), as well as GSE ($df = 3$; $P < 0.001$) (Table 5.6). Therefore, a stock interaction term was included in the survival ~ blood physiology sub-model, and the Harrison stock were excluded because of a low sample size (n = 4). In addition, MANOVA comparisons between capture methods indicated significantly higher plasma cortisol ($df = 1$, $P < 0.001$), lactate ($df = 1$, $P < 0.001$), glucose ($df = 1$, $P < 0.001$), osmolality ($df = 1$, $P < 0.001$), sodium ($df = 1$, $P = 0.02$) and chloride ($df = 1$, $P = 0.003$) between the purse seine and troll capture methods (Fig. 5.4); therefore only troll-captured

fish (n = 225 versus n = 25 for seine caught fish) were used in the survival ~ blood physiology sub-model. The survival ~ blood physiology sub-model selected lactate and interactions between glucose and the Early Shuswap and Late Shuswap stocks as variables associated with marine survival (Fig. 5.2b). The survival ~ blood physiology sub-model predicted a higher probability of marine survival for fish with lower plasma lactate (Fig. 5.3B; upper right), and the probability of marine survival was negatively related to plasma glucose for Early Shuswap fish but positively related for Late Shuswap fish (Fig. 5.3B; lower left). The top survival ~ blood physiology sub-model explained a quarter of the data variability (adjusted-R^2 = 0.25; Table 5.3).

The survival ~ non-physiology sub-model selected only FL as being associated with marine survival, with no support for an effect of capture method, biopsy treatment or stock (Fig. 5.2c). The survival ~ non-physiology sub-model predicted a higher probability of survival to river entry for fish with a greater FL (Fig. 5.3C). The top survival ~ non-physiology sub-model explained a small proportion of the data variability (adjusted-R^2 = 0.06) (Table 5.3).

Beyond the relationship to marine survival, PC1 from microarray results was negatively correlated with DOR, FL and plasma glucose, but not related to stock, sex, cortisol, lactate, Cl^-, Na^+, testosterone, GSE or capture method (Fig. 5.2d). Model-averaged and scaled parameter estimates indicated that fish that had more negative PC1 scores were released on a later date, had a higher plasma glucose concentration, and had a greater FL (Fig. 5.2d). The top model predicting PC1, which included DOR, FL and glucose, explained over one third of the data variability (adjusted-R^2 = 0.39) (Table 5.3).

5.4 Discussion

This study, which combined physiological sampling of blood tissue and genomic analysis of gill tissue with biotelemetry, is the first to successfully link gene expression to survival of homing sockeye salmon during final stages of marine migration prior to river entry. Migration typically took 40 h to the first telemetry line (e.g., the NSOG array), during which 20 - 29% of the tagged fish died, unlike the full survival of cage-held fish for approximately the same period. A further 11 - 25 % died during the next period of migration to river entry. Moreover, clear genomic expression differences were identified between those fish that showed the best survival (nearly 100%) and those than had just over even odds of survival. Marine survival was specifically related to multiple physiological processes including stress, immune response, metabolic processes and osmoregulation.

Survival estimates to river entry from the present study fall within the ranges reported previously for homing Fraser River sockeye salmon sampled north of the SoG [i.e., 52 – 75%; (Cooke et al., 2006a; Cooke et al., 2006b; Crossin et al., 2009a)]. All such telemetry studies assume that survival estimates are not biased due to imperfect receiver detection efficiencies, tag collisions, tagging/handing induced mortality, tag regurgitation and unreported fish capture. Nevertheless, detection efficiencies of acoustic telemetry arrays were previously estimated as 100% (Crossin et al., 2009a) and additional receivers were used in the lower Fraser River bolster this detection efficiency. Tag collisions were reduced by releasing fish individually over a two-week period, and the acoustic tags were programmed to emit a random signal every 40 - 120 s. No tag regurgitation was observed during the holding study and fish were released at the

southern boundary for commercial fishery to reduce tag recapture, with commercial fisheries aware of the tagging activities. Finally, a large sample size of fish (n = 400) was released to increase the confidence in the assumption that tagged fish generated reliable survival estimates that are applicable to the larger population.

The genomic signature identified here and related to survival involved many of the multiple biological processes (responses to infection and stress, protein biosynthesis, metabolism, and osmoregulation) previously identified as a MRS genomic signature by Miller et al. (2011) even though they were run on two different microarray platforms (i.e., 16K microarray versus 44K oligonucleotide microarray). Additionally, among the 43 biomarkers specifically identified from the MRS in Miller et al. (2011), 67% were also significantly related to PC1 from the microarray results in this study, which was related to marine survival. Furthermore, biomarkers related to an osmoregulatory signal (i.e., CIRP, SHOP21, NKAA1B, NKAA1C, NKAA3), a key biological process identified in the Miller et al. (2011) MRS genomic signature, were among the biomarkers significantly related to PC1 from microarray results in the present study.

Despite similar genomic signatures (hereafter collectively referred to as the MRS genomic signature) detected between studies assessing marine (herein) versus freshwater (Miller et al., 2011) survival, the survival outcome was reversed. Fish that had the MRS genomic signature in the present study experienced higher marine survival, whereas fish with the similar genomic signature in Miller et al. (2011) experienced lower freshwater survival. These differences are clearly shown in the relationships of MRS biomarker genes between the PC1 groupings of fish in the present study, where the majority of significant MRS biomarker genes were up-regulated in the PC1 grouping that had a

higher probability of marine survival (33 out of 40 (~ 83%) significant biomarker genes up-regulated in the 15% most PC1-negative group), whereas, these same genes were generally up-regulated in fish with poor freshwater survival in the Miller et al. (2011) study. Prior to providing interpretations in regards to study differences, it is important to first consider some of the more salient findings from Miller et al. (2011).

In addition to finding the MRS was related to freshwater survival, Miller et al. (2011) found that, upon entry into freshwater, the intensity of the MRS was considerably strengthened from that observed in the marine environment, with significantly higher expression of immune and anti-viral related genes, a pattern that continued to intensify at the spawning grounds. Moreover, in freshwater, over 65% of the MRS affected biological processes were consistent with a response to viral infection, lending support to the hypothesis that the MRS is a viral-mediated disease signature that emanates from the marine environment but further expressed in freshwater. Furthermore, in the marine environment, all three of the measured NKA isoforms (NKAA1B and NKAA3, NKAA1A) were up-regulated in MRS fish similar to levels of fish sampled in freshwater, coincident with elevated chloride and osmolarity in blood plasma. Based on these findings, Miller et al. (2011) hypothesized that pre-mature shifts in seawater preparedness could result in osmotic dysfunction and enhance motivation of MRS fish to move into freshwater, which was supported by a relatively faster migration rate of MRS-classified fish both in marine and freshwater environments (Miller et al., 2011). While faster migration of potentially compromised individuals seems counterintuitive, given that anadromous salmon have evolved to contend with simultaneous onset of reproductive development and senescence, it is possible that signals associated with advanced

senescence, like enhanced stress and immunity, may accelerate their drive to move towards spawning grounds before they die.

Given the discordant results on the relationship between the MRS and survival between the present study and Miller et al. (2011), a potential explanation is that survival of MRS-affected fish in the marine environment may not yet be ill effected by enhanced stress and immuno-compromise. Conversely, osmotic discomfort may have pushed fish harder in their migration to freshwater, and as a result, these fish may have showed increased survival in the marine environment (observed herein) due to reduced chance of predation by pinneped predators if predation risk is assumed to be time-dependent. However, once in freshwater, survival of MRS-affected fish may diminish as they become more compromised due to advancement of a disease-state (as observed in Miller et al., 2011), which could result from exposure to elevated temperatures (Jeffries et al., 2012; Wedemeyer, 1996) or stressful flow conditions in river. While this hypothesis is certainly plausible, unlike in Miller et al. (2011), the present study found no evidence that marine migration rates were enhanced in fish containing the MRS (the 'high survival' group herein), although there was some evidence of osmotic dysfunction in MRS fish from the present study.

MRS fish in this study had elevated expression of NKAA1b, a gill NKA isoform associated with seawater acclimation (Richards et al., 2003; Shrimpton et al., 2005). This osmoregulatory isoform was also up-regulated in MRS fish sampled in the marine environment in Miller et al. (2011) and continued to increase upon entering freshwater, diminishing near spawning grounds (Miller et al., 2011). Another gill NKA isoform, NKAA3, which was previously shown to be up-regulated in marine migrating sockeye

salmon (Evans et al., 2011) was also up-regulated in MRS fish in both the present study and Miller et al. (2011). Other proteins (CIRP and SHOP21) associated with hyperosmotic stress during adaptation of salmon smolts to seawater (Pan et al., 2002; Pan et al., 2004) provide somewhat conflicting results between studies. For example, SHOP21, part of the ubiquitin-ligase complex inducible upon exposure to osmotic stress (Pan et al., 2002), was down-regulated in MRS fish in the present study, whereas it was up-regulated in MRS fish in Miller et al. (2011) as well as in sockeye salmon with higher freshwater survival in Evans et al. (2011). In addition, CIRP, which increases in juvenile salmon during the transition from fresh to seawater (Pan et al., 2004) and decreases upon transition of adult sockeye salmon from seawater to freshwater (Evans et al., 2011), was up-regulated in MRS fish in the present study, whereas it was down-regulated in MRS fish in Miller et al. (2011). While these results are difficult to interpret, it indicates some potentially different osmoregulatory signals between studies. However, it is important to note differences among studies examining gene expression in homing sockeye salmon, including study year, and hence environmental conditions, as well as the environment where survival was examined [i.e., marine survival in the present study and freshwater survival in both Miller et al. (2011) and Evans et al. (2011)].

Environmental experience of sockeye salmon likely influences all aspects of an individual's physiological state. Evans et al. (2011) sampled homeward migrating sockeye salmon in two sites in the marine environment and one in freshwater, finding gene expression profiles of sockeye salmon differed the most between marine sampling sites, even more so than between marine and freshwater sampling sites. These findings led Evans et al. (2011) to hypothesize that the differences in gene profiles between

marine sampling sites was due to different environmental experiences of fish using distinct migratory routes in the ocean. Along these same lines, sockeye salmon that used similar migratory routes [as in both the present study and Miller et al. (2011)] in different years would have experienced different environmental conditions (and potentially different pathogens) that could influence physiological state and survival. Moreover, since the present study did not examine freshwater survival, direct comparisons cannot be made between studies in regards to how the MRS relates to survival. Nevertheless, results from the present study do support the notion that an infection-related signal emanates from the marine environment, although this signal is associated with increased survival in the marine environment. Molecular level processes can be further explored through relationships with other factors such as blood physiology that were also related to PC1 from microarray results and to survival itself.

Two variables (plasma glucose, FL) were related to both PC1 from microarray results and to marine survival itself. The relationships of these variables with survival were in similar directions between models (they influenced survival in the same direction between analyses), providing additional support for interpretations. Moreover, consistencies in findings between multiple approaches demonstrated that genomic techniques captured many of the same physiological processes as traditional blood biopsy, in addition to identifying fine-scale molecular responses in great detail. Not only that, but by pairing genomic techniques with more traditional analyses, direct comparisons can be made to previous studies conducted on the homing migration of sockeye salmon in the marine environment.

The date a fish was sampled (often referred to as Julian date of sampling) has been related to physiological conditions that vary along temporal trajectories during migration such as reproductive maturation and osmoregulatory preparations for freshwater (Crossin et al., 2009a). In this study, the date a fish was captured and released (e.g., DOR) was associated with PC1 from microarray results; the relationship indicating fish with a higher probability of marine survival (i.e., the MRS-affected fish) arrived later at the tagging site. This finding was somewhat surprising given sampling took place over a two-week period, and DOR was not related to survival itself in the model with the largest sample sizes (e.g., the 'survival ~ fish-related' sub-model). Ultimately, fish that arrived later at the tagging site were more likely to have the MRS; suggesting the intracellular infection response signature was stronger in these individuals. Sockeye salmon stocks can differ in their migration timing through the SoG, although migration timing does overlap (Crossin et al., 2007; Groot & Margolis, 1991), and stock-specific responses to the MRS were detected in Miller et al. (2011). Stock was not related to PC1 from microarray results and the data suggests that the three stocks were equally distributed throughout the relatively narrow period of sampling. In the future, larger sample sizes would be needed to adequately investigate stock-specific relationships with genomic signatures in the marine environment.

Among variables related to PC1 from microarray results, FL was also significantly related to survival itself. Size-selective survival in the ocean is common in salmon with larger fish typically having higher survival (Ewing & Ewing, 2002; Healy, 1982; Saloniemi et al., 2004). Both models in this study indicated that fish with larger FL had higher marine survival. However, in contrast to these results, a previous study found

sockeye salmon with smaller FL had higher marine survival (Cooke et al., 2006a), which the authors attributed to smaller fish potentially avoiding gill net fisheries in the lower Fraser River located downstream of their detection sites. During the present study, there were no in-river commercial fisheries that could have captured fish prior to reaching detection sites in the lower river. Therefore, once the potential for a fishery-imposed size bias was removed, larger fish may have had a selective advantage over smaller fish given the assumption that fish size is related to overall condition (Duffy & Beauchamp, 2011; Tomaro et al., 2012) and thus the ability to fight infection (Arkoosh et al., 2006) and evade predators (Mesa et al., 1994). Interestingly, the relationship between FL and PC1 from microarray results also suggests that larger fish were more apt to carry the MRS, which could negatively influence survival once fish enter the river (Miller et al., 2011).

Elevated stress in migrating fish can have a number of secondary effects such as immune suppression (Barton, 2002), increased energy use (Barton, 2002), depressed reproductive development (Carruth et al., 2002), osmoregulatory imbalances (Mommsen et al., 1999), and behavioural impairments (Wingfield et al., 1998) that result in an inability to escape predators, fight off infection, and therefore can have fitness consequences (Wood et al., 1983). Glucose and lactate are metabolites mobilized in response to stress and exercise in fish (Farrell et al., 2001; Pagnotta & Milligan, 1991; Suski et al., 2006; Wood et al., 1983) and both were associated with marine survival herein. Plasma glucose was inversely related to PC1 from microarray results, meaning it was associated with fish that showed signs of an elevated stress response, but could also be related metabolic processes that were also associated with PC1 from microarray results. Additionally, since plasma glucose was found to significantly interact with stock

in this study, it may indicate stock-specific responses to stressors, a finding that has been previously observed (Cooke et al., 2006a). Based on the relationships of glucose with PC1 from microarray results and survival, fish with elevated levels of plasma glucose had a higher probability of survival. This would coincide with biomarker gene results, which also indicated fish with an elevated stress response had a higher probability of survival.

In contrast to the relationship between glucose and marine survival, fish with elevated levels of plasma lactate had lower marine survival. Similar relationships between plasma lactate and marine survival have also been found in previous studies on homing sockeye salmon (Cooke et al., 2006a; Cooke et al., 2006b; Crossin et al., 2009a), and plasma lactate emerges as perhaps the most consistent blood plasma variable related to marine survival among studies. Interestingly, plasma lactate was not related to PC1 from microarray results suggesting that this acute stress response was either not captured in at least the first PC axis from microarray results or statistical power was enhanced for the analysis using blood plasma data due to larger sample sizes. As noted by others, elevated plasma lactate could be a result of exhaustive exercise due to fisheries capture.

Indeed, fisheries capture and fisheries gear type can influence stress, behaviour and survival (Chopin et al., 1996; Donaldson et al., 2010; Milligan, 1996; Skomal, 2007; Wilson et al., 2014b). In this study, evidence of a fisheries capture effect was shown by elevated concentrations of plasma stress indices (plasma cortisol, glucose, lactate and osmolality) in purse seine captured fish compared to troll captured fish. Plasma stress levels from purse seine captured fish in this study were consistent with those previously reported for purse seine captured sockeye salmon in the marine environment (Cooke et al., 2006a). In the present study, 33% of purse seine captured fish died prior to reaching

the Fraser River and had a mean and maximum plasma lactate concentration of 12.1 and 23.1 mm L^{-1}, respectively. In contrast, purse seine captured fish that survived to the Fraser River had mean and maximum plasma lactate concentrations of 11.6 and 24.1 mmol L^{-1}, respectively. In both cases, mean plasma lactate concentrations approached the threshold above which salmon have difficulty recovering from anaerobic stress (Jain & Farrell, 2003). Notably, maximum plasma lactate concentrations for both purse seine captured fish that died and those that survived to river entry were well beyond the threshold not to be exceeded for successful river entry in sockeye salmon (Crossin et al., 2009a).

Physiological indices of stress such as lactate, cortisol, and glucose are released after contact with a perceived stressor and peak after 1-2 hours (Farrell et al., 2001; Milligan, 1996; Wood et al., 1983; Wydoski et al., 1976). In this study, troll captured fish were sampled < 15 minutes after fish initially encountered fishing gear, whereas purse seine captured fish were sampled no less than 30 minutes to over an hour and a half after they initially encountered fishing gear. *Post hoc* examination showed a significant (df = 22; $P = 0.01$) relationship between plasma lactate and holding duration prior to sampling for purse seine captured fish. Therefore, it is proposed that different stress levels observed between capture methods in this study were attributed to the differences in the amount of time between initial gear contact and tissue sampling, rather than different magnitudes of stress induced between the two capture methods. Furthermore, although it is difficult to establish baseline stress values because samples cannot be obtained from fish in the wild without capture, it is justifiable that blood samples that were taken relatively quickly from troll captured fish would be more representative of pre-capture

stress levels compared to blood samples taken from purse seine captured fish. Future studies that desire to examine the effects of gear type on stress physiology should ensure similar holding times of fish across treatments prior to physiological sampling.

Tissue biopsy is a technique commonly used to examine the physiological mechanisms underlying Pacific salmon migration (Cooke et al., 2008b). Results from this study indicated that tissue biopsy likely had little effect, as there were no differences in short-term survival after release for fish exposed to tissue biopsies compared to control fish. Additionally, during the 32-hour holding experiment, survival was 100% for biopsied fish, corresponding to results of Cooke et al. (2005), which originally validated the sampling technique. Overall, tagging and tissue biopsy likely represents a negligible influence over survival compared to the capture event, but it remains important to incorporate controls for tissue sampling in study designs when possible.

In conclusion, genomic patterns of homing sockeye salmon sampled in the marine environment were related to their survival to river entry, and revealed a similar signature to that previously identified in a separate study (i.e., Miller et al. (2011)). However, this genomic signature was associated with enhanced survival in the marine environment herein, and to reduced survival in freshwater previously (Miller et al., 2011). While there are a few potential explanations for this variance, clearly further study will be required to fully understand these results. Nevertheless, a consistent genomic signature related to infection, stress, osmoregulation, and metabolism highlight the overwhelming importance of these biological processes during the homing stage of salmon migrations. Furthermore, the parallel use of microarrays and blood biopsies in this study demonstrated that gene expression captured much of the same physiological processes as more traditional blood

plasma data, as well as providing further resolution of physiological and molecular mechanisms responsible for migration success.

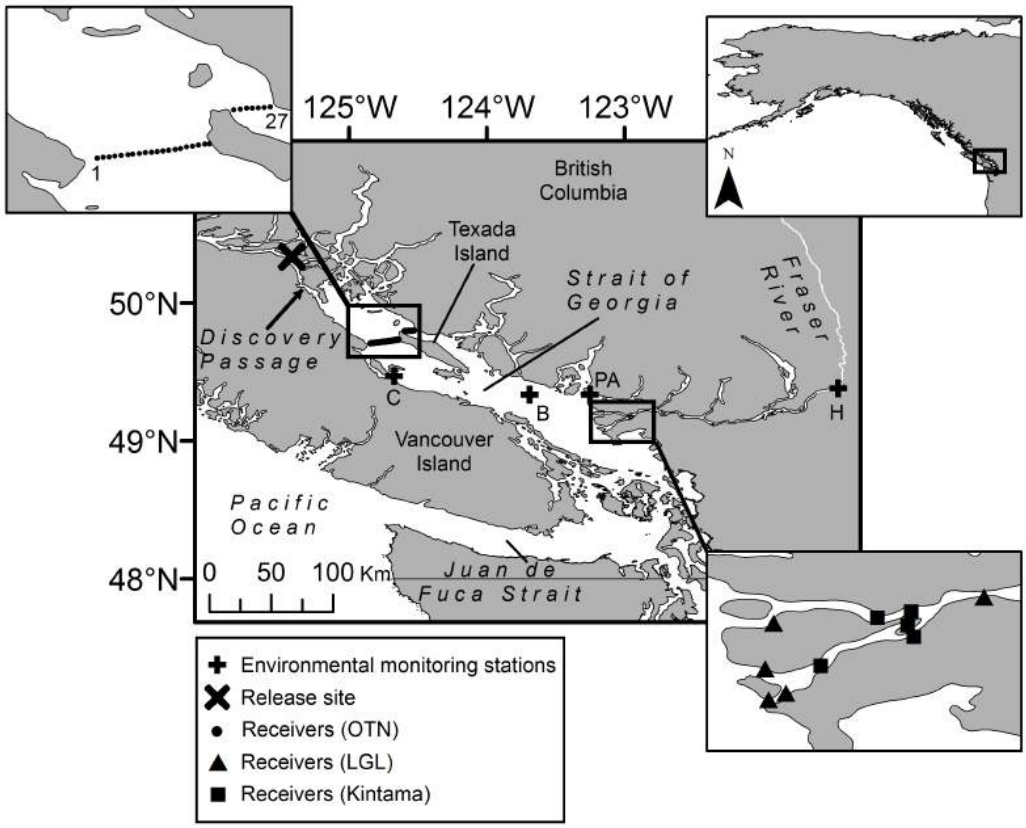

Figure 5.1 Map of study area. 'C' = Chrome Island, 'B' = Buoy 46146, 'PA' = Point Atkinson, 'H' = Hope. Kintama receivers were paired at each location in the lower Fraser River (n = 10), whereas all other receiver points represent a single receiver at each location (i.e., 27 OTN receivers, 5 LGL receivers).

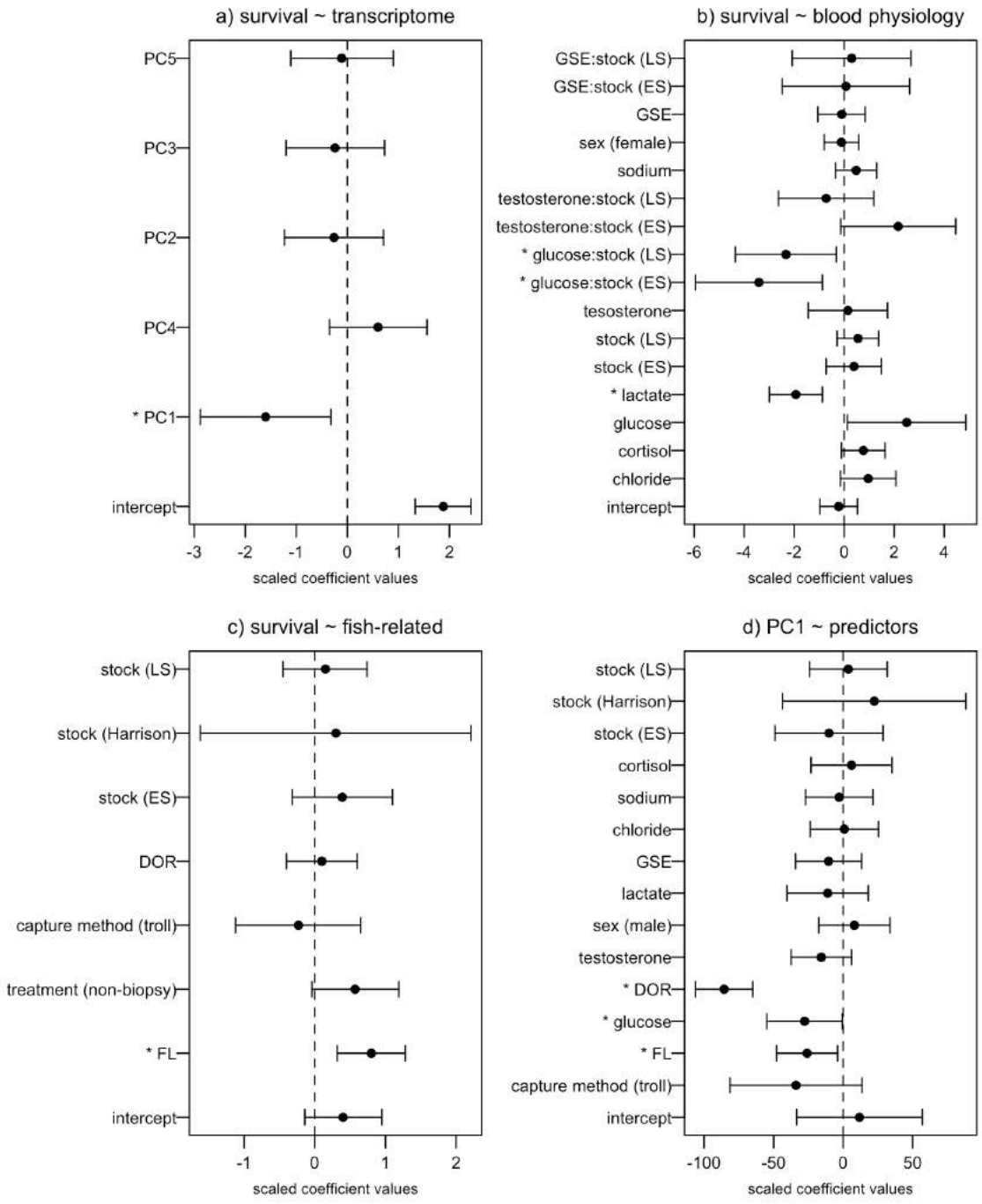

Figure 5.2 Model averaged scaled parameter estimates (circles) with 95% confidence intervals (lines) for three models examining the factors related to marine survival: a) survival ~ transcriptome; b) survival~ blood physiology; c) survival ~ fish-related, and one model examining the factors related to PC1 of gene expression from microarray data;

d) PC1 ~ predictors. An asterisk preceding the variable names signifies that the 95% confidence intervals for the scaled parameter estimate did not intersect zero. Abbreviations are given for Early Shuswap (ES), Late Shuswap (LS), gross somatic energy (GSE), day-of-release (DOR) and fork length (FL).

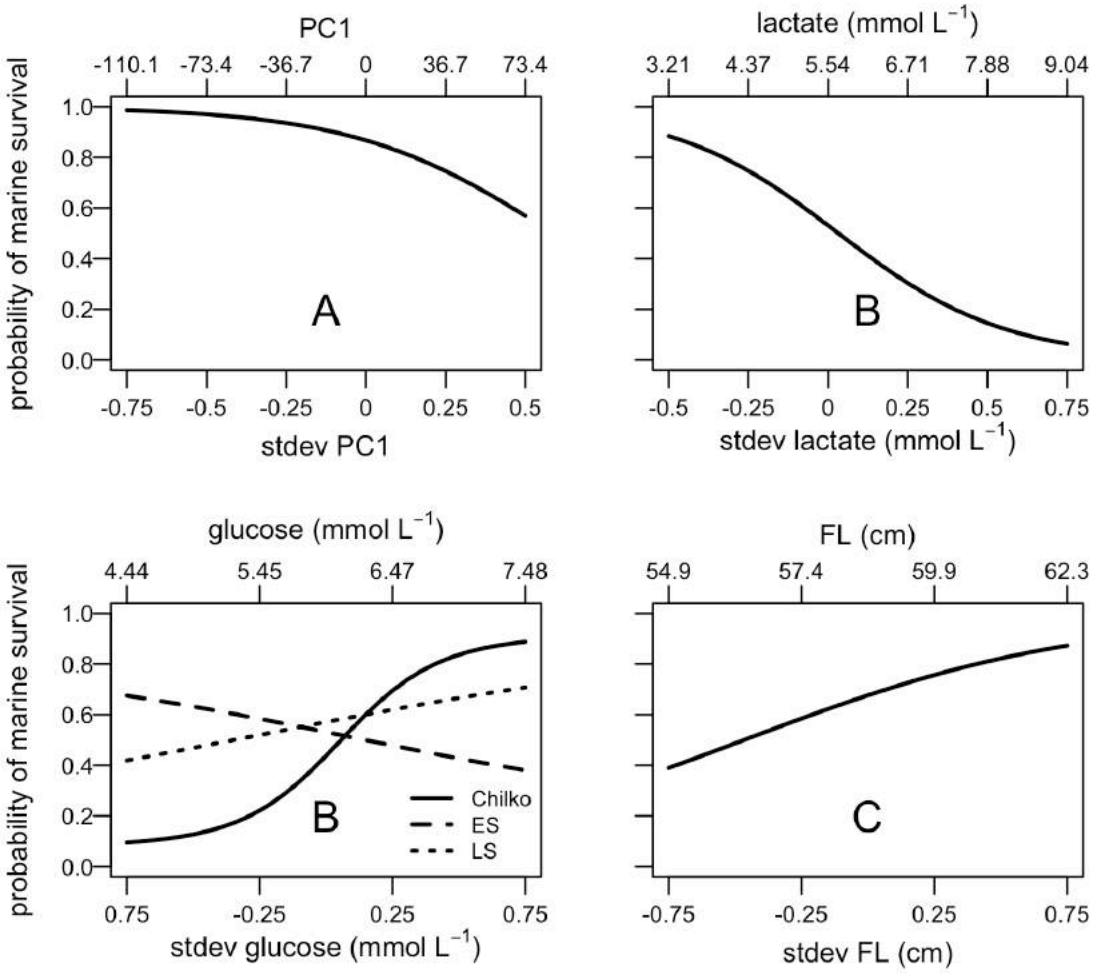

Figure 5.3 Model averaged predictions for the probability of marine survival based on variables selected as being associated with marine survival in three sub-models: survival ~ transcriptome (A); survival~ blood physiology (B); survival ~ fish-related (C). The upper x-axis is the actual value of the variable, and the lower x-axis is the standardized variable (in SD units). Abbreviations are given for fork length (FL), Early Shuswap (ES) and Late Shuswap (LS).

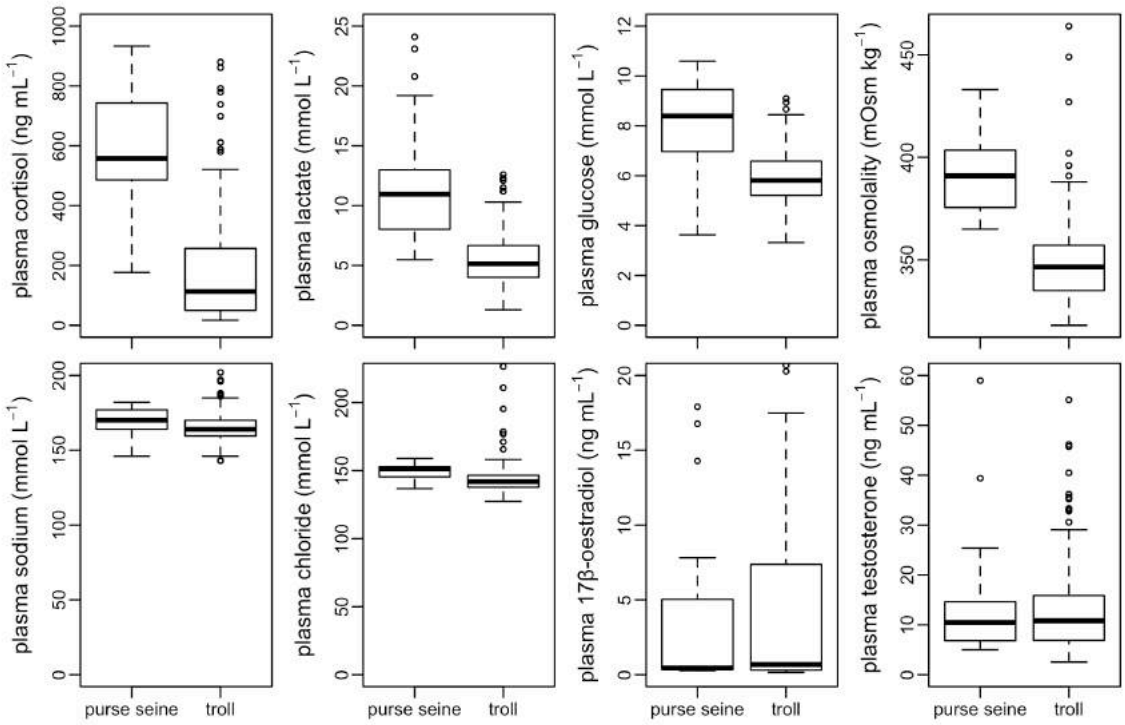

Figure 5.4 Comparison of blood plasma variables between purse seine and troll capture methods. Solid bold horizontal lines represent median values, box limits represent the interquartile rage (IQR), and whiskers represent 1.5x the IQR. Open circles represent outliers. Significant differences were found for cortisol (df = 1, $P < 0.001$), lactate (df = 1, $P < 0.001$), glucose (df = 1, $P < 0.001$), osmolality (df = 1, $P < 0.001$), sodium (df = 1, $P = 0.02$) and chloride (df = 1, $P = 0.003$) between capture methods.

Table 5.1 Numbers of sockeye salmon tagged and released, and detected at the Northern Strait of Georgia (NSOG) and lower Fraser River acoustic receiver locations by fish stock. Numbers in parenthesis indicate the cumulative percent success to the respective receiver location.

stock	number released	number detected at NSOG	number detected at Fraser River
Chilko	68	48 (71)	36 (53)
Early Shuswap	71	53 (75)	47 (66)
Harrison	5	4 (80)	3 (60)
Late Shuswap	169	130 (77)	102 (60)

Table 5.2 Summary of the first five principle components (PCs) generated from PCA analysis of microarray data.

principle components	stdev	proportion of variance	cumulative proportion
PC1	73.39	0.19	0.19
PC2	55.54	0.11	0.30
PC3	36.08	0.05	0.34
PC4	33.34	0.04	0.38
PC5	29.18	0.03	0.41

Table 5.3 Model selection results based on AICc showing top models (ΔAIC_c <2), to a maximum of 5 models, from a 95% confidence set of models. Model results are shown for 'gene expression', 'non-physiological' and 'blood physiology' models. Abbreviations are given for day-of-release (DOR), fork length (FL), glucose (gluc), lactate (lact), chloride (Cl^-), sodium (Na^+), testosterone (T), and cortisol (cort).

model type	model	K	ΔAIC_c	W_i	adjusted-R^2
survival~transcriptome	PC1	1	0.00	0.20	0.10
	PC1, PC4	2	0.46	0.16	
	PC1, PC2	2	1.76	0.08	
	PC1, PC3	2	1.86	0.08	
survival~ blood physiology	lact, gluc, stock X gluc, stock X T, Cl^-, cort	10	0.00	0.10	0.25
	lact, gluc, stock X gluc, stock X T, Cl^-	9	0.30	0.08	
	lact, gluc, stock X gluc, stock X T, cort, Na^+	10	1.51	0.05	
	lact, gluc, stock X gluc, stock X T, Cl^-, cort, Na^+	11	1.67	0.04	
	lact, gluc, stock X gluc, stock X T, Cl^-, Na^+	10	1.79	0.04	
survival ~ fish-related	FL, treatment	2	0.00	0.31	0.06
	FL,	1	1.38	0.15	
	FL, treatment, capture method	3	1.69	0.13	
	FL, treatment, DOR	3	1.76	0.13	
PC1 ~ predictors	DOR, FL, gluc capture method, T	5	0.00	0.02	0.39
	DOR, FL, gluc, capture method	4	0.22	0.02	
	DOR, FL, gluc, T	4	0.38	0.02	
	DOR, FL, gluc	3	0.79	0.02	
	DOR, FL, gluc, capture method, sex	5	1.28	0.01	

Table 5.4 Functional groupings of genes from gene set enrichment analysis based on fold change values calculated between fish from the 15% most PC1-positive and 15% most PC1-negative groups of fish. Redundancy reduction was performed on significant ($p<0.01$) functional groupings.

Name/GO ID	Name	# of genes	Median Fold Change	P-value
Apoptosis				
GO:0043066	negative regulation of apoptosis	177	1.059	< 0.001
Cell Proliferation				
GO:0007090	S phase of mitotic cell cycle	106	1.030	< 0.001
GO:0000278	mitotic cell cycle	248	-1.024	< 0.001
GO:0001938	positive regulation of endothelial cell proliferation	29	-1.085	< 0.001
GO:0015074	DNA integration	5	1.288	0.001
GO:0008283	cell proliferation	231	1.044	0.004
GO:0007568	aging	115	-1.008	< 0.001
GO:0031018	endocrine pancreas development	90	1.116	< 0.001
Immune Response				
GO:0019083	viral transcription	75	1.133	< 0.001

Name/GO ID	Name	# of genes	Median Fold Change	P-value
GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	6	1.279	0.002
GO:0044130	negative regulation of growth of symbiont in host	8	1.159	0.004
GO:0051918	negative regulation of fibrinolysis	9	1.167	0.002
Metabolic Processes				
GO:0006200	ATP catabolic process	195	-1.036	< 0.001
GO:0006006	glucose metabolic process	87	-1.039	< 0.001
GO:0015991	ATP hydrolysis coupled proton transport	22	-1.003	< 0.001
GO:0045471	response to ethanol	79	1.043	0.004
GO:0006600	creatine metabolic process	7	-1.175	0.005
GO:0010888	negative regulation of lipid storage	7	1.066	0.008
GO:0006521	regulation of cellular amino acid metabolic process	44	1.062	< 0.001
GO:0046688	response to copper ion	23	1.060	0.003
Oxidative Phosphorylation				
GO:0022904	respiratory electron transport chain	85	1.104	< 0.001
GO:0051881	regulation of mitochondrial membrane potential	10	1.116	0.006
Oxidative Stress				

Name/GO ID	Name	# of genes	Median Fold Change	P-value
GO:0050665	hydrogen peroxide biosynthetic process	5	-1.207	0.002
Protein Binding and Metabolism				
GO:0051437	positive regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	70	1.080	< 0.001
GO:0031145	anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	78	1.072	< 0.001
GO:0050821	protein stabilization	40	1.001	0.008
GO:0042026	protein refolding	7	-1.077	0.006
GO:0006898	receptor-mediated endocytosis	38	-1.060	0.007
Protein Synthesis				
GO:0010467	gene expression	348	1.026	< 0.001
GO:0008380	RNA splicing	251	1.064	< 0.001
GO:0006415	translational termination	80	1.133	< 0.001
GO:0006412	translation	308	1.054	< 0.001
GO:0016070	RNA metabolic process	141	1.049	< 0.001
GO:0016071	mRNA metabolic process	103	1.035	< 0.001
GO:0006413	translational initiation	63	1.078	0.001
GO:0051028	mRNA transport	59	1.027	0.002

Name/GO ID	Name	# of genes	Median Fold Change	P-value
GO:0006396	RNA processing	83	1.090	0.003
GO:0006366	transcription from RNA polymerase II promoter	213	1.040	0.004
GO:0046939	nucleotide phosphorylation	9	1.281	0.006
Structural Component				
GO:0030198	extracellular matrix organization	65	-1.069	0.001
GO:0097435	fibril organization	7	1.213	0.002
GO:0034501	protein localization to kinetochore	6	1.344	0.002
GO:0006928	cellular component movement	77	1.034	0.004
GO:0007155	cell adhesion	325	-1.044	0.005
GO:0031529	ruffle organization	12	1.153	0.002

Table 5.5 MRS biomarker genes selected for qRT-PCR analysis, and resulting p-values from t-tests relating biomarker load to PC1 scores of fish from the 15%-PC1 negative and 15% most PC1-positive groupings. Positive and negative fold change values indicate genes were up- and down-regulated, respectively in the 15%-PC1 negative grouping that had a higher probability of marine survival relative to the 15% most PC1-positive grouping that had a lower probability of marine survival. When possible, individual biomarker general functions were inferred using RefSeq (http://www.ncbi.nlm.nih.gov/refseq/) and Ingenuity Target Explorer (https://targetexplorer.ingenuity.com/index.htm).

Gene name	Assay_name	p-value	Fold change	General function	Accession number(s)	Primers sequences F	Category
Complement C4-B precursor	C4B	0.014 *	0.983	complement cascade; Immune	CB518123	F-TCCAACCACATCGCATTATCC R-ATCTCTGACACCACTGACCACAA	MRS-multi-tissue
Complement component C7 precursor	C7	0.459	0.036	Complement component C7 precursor	CA052045	F-ACCTCTGTCCAGCTCTGTGTC R-GATGCTGACCACATCAAACTGC	MRS-multi-tissue
C-X-C chemokine receptor type 4	CXC4	0.444	0.200		CA054133	F-GGAGATCACATTGAGCAACATCA R-GCTGCTGGCTGCCATACTG	MRS-multi-tissue
Elongation factor 1-alpha, oocyte form	EEF1AO	0.029 *	-0.384		CA053679,CB497678	F-CGGAACGACGGTCGATCT R-GCTCACATCGCCTGCAAGT	MRS-multi-tissue
BTB/POZ domain-containing protein KCTD10	KCTD1	0.003 **	-1.327	Potassium ion transport,immune response	CA062065	F-TGTTTGTTAAAAGGGGACACAGTG R-GTGAAGTGTTATCTGGGCTGAAAG	MRS-multi-tissue
Krueppel-like factor 2	KIF2	0.081	0.613	positive regulation of transcription	CA058059	F-GCAGCCTTCCCAATTGCA R-AAGGCTCACATGCGAACACA	MRS-multi-tissue
Oncorhynchus mykiss mRNA for macrophage colony-stimulating factor (csf1 gene)	MCSF	0.006 **	0.783	Macrophage colony stimulating factor	CA061415	F-GCTCTCTCAATCCTTGGCTTTAC R-ACCAGCATAATTGAAAACCAGAGG	MRS-multi-tissue
Matrix metalloproteinase-25 precursor	MMP25	< 0.001 ***	1.132	proteolysis, inflammatory response	CB516773	F-TGCAGTCTTTTCCCCTTGGAT R-TCCACATGTACCCACACCTACAC	MRS-multi-tissue
Oncorhynchus mykiss G-protein (P-ras) mRNA, complete cds	PRAS	< 0.001 ***	1.917		CA059617	F-GCAGGATGAGCAGAGGAAGAA R-GGCCTGGGCAATGTAACACT	MRS-multi-tissue

Gene name	Assay_name	p-value	Fold change	General function	Accession number(s)	Primers sequences F	Category
Ras-related protein Ral-B precursor	RALB	0.002 **	0.482	Apoptosis	CA054290	F-TGTGCCTGTCCATTTTGTTCAA R-GTGTCTGTGTGAACGAACTTTGG	MRS-multi-tissue
SUMO-activating enzyme subunit 2	SAE2	0.486	0.077	protein modification, ubiquitin cycle	CA055135	F-TTGGCGCTTGGCTGAAG R-CCACAAGAGGAAGCTCCATGA	MRS-multi-tissue
secretogranin II [Ctenopharyngodon idella]	SCG	< 0.001 ***	1.109		CA053613	F-GGATGTGAAGAATCCAACACTGAT R-ACACCACTTCAAACTAGCCATACATT	MRS-multi-tissue
Small glutamine-rich tetratricopeptide repeat-containing protein A	SGTA	< 0.001 ***	0.508	viral release	CB517023	F-GGAGATTCAGTTCCGACAACAGTT R-TGTAGCTCTTGGTTTAGCTGTTTAATG	MRS-multi-tissue
Signal transducer and activator of transcription 1-alpha/beta	STAT1	0.023 *	-0.760	Transcription activation, viral response	CB517962	F-TGTCACCGTCTCAGACAGATCTG R-TGTTGGTCTCTGTAAGGCAACGT	MRS-multi-tissue
Transmembrane protein 18	TMEM	0.074	-0.706		CB513847	F-TCACATGGCAAACATCTGCAT R-AAAAAGTTTAGGCAGTCGAAGCA	MRS-multi-tissue
UNKNOWN	UK8	0.120	0.293		CA059261	F-ACAGACAAGCATCTCTTATTGGAC R-AAAACCACAATGCTGGGTCTTG	MRS-multi-tissue
UNKNOWN	UKBC15	0.113	0.240		CB515132	F-GTGCATTGTTTTTCCAACTTTCC R-CATAGCGGGCCCATGTCT	MRS-multi-tissue
WD repeat protein 16	WDR16	< 0.001 ***	-2.990	cell proliferation, tumor, oxidative stress	CB514231	F-GGAGGAGAGGGACAGGTACGT R-GTCTCAATGAGCCGGTGACA	MRS-multi-tissue
Tyrosine-protein kinase ZAP-70	ZAP7	0.039 *	0.590	immune, T-cell	CA052716	F-TCACCTCCGGACCTTTCATT R-CCATGTGGGAAGCCTTTTCTT	MRS-multi-tissue
Actin, alpha skeletal muscle	ACA	0.050 *	0.773	Structural protein	CB494344, CB498135	F-TGGACAGGGAAGCCAGGAT R-CAGCACCATGAAGATCAAGATCA	MRS-gill_fate
ADP-ribosylation factor 6	ARF6	0.059	0.650		CA064167	F-GCTGTACAACTGATGTGAATGGATT R-GGGCCCAAGTAAGCAAGTGTT	MRS-gill_fate
ADP-ribosylation factor-like protein 8B	ARL8B	< 0.001 ***	0.516	cell proliferation, metabolism	CA050777	F-CATGTTCTCCAGACAGTCCTGTATG R-AAACATTTGGATTTGGCAATAGC	MRS-gill_fate
UNKNOWN	CA054698	0.020 *	0.600		CA054698	F-CAGTAGCGTTTATCTGTCTGCATTAGT R-GCATGGTTATTCAGCGGTTTACA	MRS-gill_fate
UNKNOWN	CA055640	< 0.001 ***	1.030		CA055640	F-GCACCTGCGATAGAAGAGCAT R-GAGATGGAATCCGCAGAAGCT	MRS-gill_fate

Gene name	Assay_name	p-value	Fold change	General function	Accession number(s)	Primers sequences F	Category
UNKNOWN	CA063814	0.011 *	0.537		CA063814	F-AACCTTTGAGTATGAGCAGTTCCA R-GGACGCTTTGCTATTGTTTCAAC	MRS-gill_fate
Tropomyosin-1 alpha chain	CB486176	0.019 *	0.638		CB486176	F-ACGTTGTACTTTCCGACCACTATTT R-GCTACAGCTGTCAGTATATGCTCATTG	MRS-gill_fate
86791 pfam05110, AF-4, AF-4 proto-oncoprotein	CB511853	0.001 **	1.059		CB511853	F-TTTCTTCTCCTACTGCTTGGTGATC R-AGGCGACCTGCACCTTCTC	MRS-gill_fate
UNKNOWN	CB512538	0.812	0.136		CB512538	F-TCCTTAAAGCAGGCGGCATA R-AATGCTCCTGTCACTTTCTTAAATAGG	MRS-gill_fate
C-type lectin domain family 4 member M	CLC	0.356	-0.157		CA056108	F-GGCCGAGCAGCAGAGACA R-CCGGGCCTCTTTTTCCA	MRS-gill_fate
COMM domain-containing protein 7	COMMD7	< 0.001 ***	0.616	Protein synthesis	CB512167	F-CAAAGCCAGTATGGACTGTTTCAG R-TTGTTTTCTGCTGCCCCTCTA	MRS-gill_fate
FYN-binding protein	FYB	< 0.001 ***	0.980	T-cell signal cascade	CA053392	F-TGCAGATGAGCTTGTTGTCTACAG R-GCAGTAAAGATCTGCCGTTGAGA	MRS-gill_fate
Heterogeneous nuclear ribonucleoprotein A1	HNR1	0.002 **	0.532		CA057305	F-ACTACATAAAACCTCATTGGAATGCTT R-GCTACAAGAAGAGATGTGTACAATAGAGAA	MRS-gill_fate
Histone acetyltransferase HTATIP	HTA	< 0.001 ***	0.843		CA062248	F-CTTGTAACAGTTCGACATGGCTTATT R-TGGTGAAGCATTTCTGTATGTCAA	MRS-gill_fate
Keratin, type II cytoskeletal 8	KRT8	< 0.001 ***	-0.880	Cell structure	CA770356,CB492778	F-CGATTGAGCGGCTGGATAA R-GCATTGTTTACCTTTGACTTGAATTG	MRS-gill_fate
Platelet-activating factor acetylhydrolase precursor	PLA	0.008 **	0.487		CA054819	F-AGTTTCCTCATGCGACTGATGTT R-GGGTAAAGCAGCCGATCTTCT	MRS-gill_fate
Peptidyl-prolyl cis-trans isomerase A	PPIA	0.095	0.283	pathogen virulence factor	CB499629,CB511232	F-CCAATGGATCCCAGTTCTTCA R-TGCCGAACACAACGTGCTT	MRS-gill_fate
Perforin-1 precursor	PRF1	0.016 *	0.778	apoptosis, lysis of viral infected cells	DY711337	F-AAGGCCGCAACAACACATG R-TCACAAAGGTGAAGCAGAGAGAAA	MRS-gill_fate

Gene name	Assay_name	p-value	Fold change	General function	Accession number(s)	Primers sequences F	Category
Proteasome subunit beta type 4 precursor	PSMB4	< 0.001 ***	0.435	gamma interferon-inducible proteasomal genes with roles in antigen presentation	CA055298	F-CGATTATGACGCCACCTGTAAA R-CACACGCTAAACCCCATGGT	MRS-gill_fate
60S ribosomal protein L6	RPL6	0.004 **	1.282		CB495313	F-CGCCACCACAACCAAGGT R-TCCTCAGCCTCTTCTTCTTGAAG	MRS-gill_fate
SAM domain-containing protein SAMSN-1	SAMSN	0.848	0.096		CB511635	F-ACAGTCTCAATAGTGGACAAAGC R-ACAGAACTGGCCTGTGTAGG	MRS-gill_fate
ADP/ATP translocase 2	SLC1	< 0.001 ***	0.468	Metabolism	CK990577,CA057185,CB493265,CK991225,CA058445,CA042906,CK990722,CA047726,CA057203,CB510912,CB497820	F-CAACCGCCGTTTTGGAGAT R-AATGAATGAGACCGCAGTTTCA	MRS-gill_fate
Transcription initiation factor TFIID subunit 11	TAF11	0.275	-0.267	Protein synthesis	CA051151	F-GGAAGTACTAGAAGTGACCAGTTTTGG R-AATGACCACAGCATCAATGTTTG	MRS-gill_fate
Tropomyosin-1 alpha chain	TPM1	0.760	-0.200		CB517835,CA064436	F-AGACTTGCCCTAAGTGCCAAAA R-ACTAATGATGGTGAAAACTTGAAGACA	MRS-gill_fate
complement factor C3	C3	0.507	-0.357	complement factor C3; indiction complement system	-	F-ATTGGCCTGTCCAAAACACA R-AGCTTCAGATCAAGGAAGAAGTTC	Immune
CD4	CD4_ONMY	< 0.001 ***	0.836	T-cell Activity/B-cell activity	-	F-CATTAGCCTGGGTGGTCAAT R-CCCTTTCTTTGACAGGGAGA	Immune
IFN-alpha	IFNA2	< 0.001 ***	1.215	Interferon responses typical of anti-viral activity	-	F-CGTCATCTGCAAAGATTGGA R-GGGCGTAGCTTCTGAAATGA	Immune
interferon regulatory factor 1	IRF1	< 0.001 ***	1.524	anti-viral response; interferon activity	CB511515	F-CAAACCGCAAGAGTTCCTCATT R-AGTTTGGTTGTGTTTTTGCATGTAG	Immune
MHC I	MHC1	0.407	-0.815	Cellular immune responses typical of responses to intracellular pathogens	-	F-GCGACAGGTTTCTACCCCAGT R-TGTCAGGTGGGAGCTTTTCTG	Immune
Mx	MX_ONTS	< 0.001 ***	1.415	Interferon responses typical of anti-viral activity	-	F-AGATGATGCTGCACCTCAAGTC R-CTGCAGCTGGGAAGCAAAC	Immune
NKA_B1	NKA_B1	< 0.001 ***	1.403	Immune response	-	F-CGTCAAGCTGAACAGGATCGT R-CCTCAGGGATGCTTTCATTGGA	Immune
CRP/SAP like pentraxin	PTX_ONMY	0.066	0.738		-	F-CAACGTCTCAAAGCCCATTT R-GCCTCGTTCTTGCTCAGAGT	Immune

Gene name	Assay_name	p-value	Fold change	General function	Accession number(s)	Primers sequences F	Category
Retinoid-inducible gene	RIG-I	< 0.001 ***	0.742	Interferon responses typical of anti-viral activity	-	F-ACAGCTGTTACACAGACGACATCA R-TTTAGGGTGAGGTTCTGTCCGA	Immune
Serum amyloid protein a (SAA)	SAA_ONMY	0.092	0.891		-	F-GGGAGATGATTCAGGGTTCCA R-TTACGTCCCCAGTGGTTAGC	Immune
Hyperosmotic protein 21	SHOP21	0.008 **	-0.532	part of ubiquitin-ligase complex inducible upon exposure to thermal and osmotic stress	CA054269	F-GCGGTAGTGGAGTCAGTTGGA R-GCTGCTGACGTCTCACATCAC	Immune
Cold inducile RNA binding protein	CIRP	< 0.001 ***	1.512	stress; osmoregulation	CA044962,CB499769,CK990431,CB503706	F-AAGCTGTGATTGTGCTCTAAAGAC R-TCCCACTTAGCATTCCATCCTTG	gill_Osmo, stress
Na^+K^+-ATPase alpha 1b	NKAA1B	< 0.001 ***	0.820	osmoregulation	CK879688	F-GCTACATCTCAACCAACAACATTACAC R-TGCAGCTGAGTGCACCAT	gill_Osmo
Na^+K^+-ATPase alpha 1c	NKAA1C	< 0.001 ***	-0.468	osmoregulation	CK885259	F-AGGGAGACGTACTACTAGAAAGCAT R-CAGAACTTAAAATTCCGAGCAGCAA	gill_Osmo
Na^+K^+-ATPase alpha 3	NKAA3	0.033 *	0.724	osmoregulation	CK170270	F-GGAGACCAGCAGAGGAACAG R-CCCTACCAGCCCTCTGAGT	gill_Osmo

Table 5.6 Comparisons of biological attributes of four stocks of sockeye salmon. Bolded p-values indicate significance at $p < 0.05$.

variable by stock	mean ± stdev (N)	p-value
Plasma glucose (mmol L^{-1})		**0.028**
Chilko	6.6 ± 1.43 (42)	
Early Shuswap	5.6 ± 1.01 (46)	
Harrison	6.3 ± 1.17 (4)	
Late Shuswap	6.2 ± 1.24 (124)	
Plasma lactate (mmol L^{-1})		0.090
Chilko	6.4 ± 2.52 (42)	
Early Shuswap	5.9 ± 4.41 (46)	
Harrison	6.0 ± 3.08 (4)	
Late Shuswap	6.2 ± 3.10 (124)	
Plasma Na^+ (mmol L^{-1})		0.479
Chilko	165.5 ± 9.51 (42)	
Early Shuswap	164.0 ± 9.20 (46)	
Harrison	164.8 ± 11.09 (4)	
Late Shuswap	166.1 ± 10.65 (124)	
Plasma Cl^- (mmol L^{-1})		0.618
Chilko	143.6 ± 8.20 (42)	
Early Shuswap	142.9 ± 13.82 (46)	
Harrison	141.1 ± 6.68 (4)	
Late Shuswap	144.8 ± 10.44 (124)	
Plasma osmolality (mOsm kg^{-1})		0.839
Chilko	353.9 ± 18.93 (42)	
Early Shuswap	350.4 ± 30.34 (46)	

variable by stock	mean ± stdev (N)		p-value
Harrison	351.8 ±	20.12 (4)	
Late Shuswap	353.9 ±	22.78 (124)	
Plasma cortisol (ng mL^{-1})			0.368
Chilko	250.7 ±	208.69 (42)	
Early Shuswap	203.7 ±	212.26 (46)	
Harrison	275.8 ±	310.73 (4)	
Late Shuswap	215.3 ±	216.54 (124)	
Plasma testosterone (ng mL^{-1})			**< 0.001**
Chilko	14.0 ±	7.22 (42)	
Early Shuswap	19.45 ±	13.08 (46)	
Harrison	7.40 ±	1.63 (4)	
Late Shuswap	10.0 ±	5.48 (124)	
Plasma 17β-oestradiol (ng mL^{-1})			**0.003**
Chilko	4.0 ±	4.44 (42)	
Early Shuswap	6.5 ±	6.93 (46)	
Harrison	1.5 ±	2.30 (4)	
Late Shuswap	3.2 ±	3.78 (124)	
Gross somatic energy (MJ kg^{-1})			**< 0.001**
Chilko	8.9 ±	0.37 (42)	
Early Shuswap	8.1 ±	0.57 (46)	
Harrison	8.2 ±	0.25 (4)	
Late Shuswap	8.7 ±	0.41 (124)	
Fork length (cm)			0.148
Chilko	57.0 ±	2.20 (42)	
Early Shuswap	58.3 ±	2.47 (46)	
Harrison	57.3 ±	3.88 (4)	

variable by stock	mean ± stdev (N)	p-value
Late Shuswap	58.5 ± 2.47 (124)	

CHAPTER 6: CONCLUSIONS

6.1 Overview

This thesis sought to gain a better understanding of how environmental conditions and individual fish physiological state influence homing sockeye salmon behaviour and survival in coastal marine waters. This objective was accomplished through a series of studies that used a variety of research approaches and combined multiple disciplines, the detail of which are discussed hereafter.

In chapter 2, a quantitative literature synthesis was used to summarize the greater body of literature on homing anadromous salmon in the marine environment and identified major gaps. Among my findings, I identified similar patterns that occur across species and life stages such as diel patterns and consistent swim speeds. A major research gap I identified was the need for the combined study of how environmental and physiological factors influence of salmon behaviour and survival. To address this gap, I recommended an approach combining tag types with environmental monitoring and physiological assessment, which I incorporated into empirical studies of chapters 3, 4 and 5. Using the most extensive data set to date on the marine thermal experience of homing sockeye salmon from recovered animal borne thermal data loggers, in chapter 3 I demonstrated that sockeye salmon spend a portion of their time migrating in upper surface waters of the coastal marine environment that likely contain cues necessary for navigation, but their thermal experience was not related to an individual's prior physiological state. Chapter 4 combined biotelemetry, oceanographic monitoring and physiological biopsies to show that sockeye salmon migration rate was influenced by environmental conditions including salinity and wind-induced currents that affect

conditions in upper surface waters sockeye salmon were found to occupy in chapter 3. Although I was not able to establish definitive physiological links with sockeye salmon behaviour using blood physiology in chapters 3 and 4, in chapter 5, I found links between individual fish physiological state and marine survival by examining gene expression of gill tissue and physiological indices from blood plasma and relating these to survival using biotelemetry. Biological processes related to marine survival included immune response, stress, metabolic state and osmoregulation.

Results from this thesis advance our fundamental understanding of the biology of anadromous salmon homing migrations through coastal waters, and can be applied to direct future research. Furthermore, data from chapters 3 and 4 can be applied towards forecasting movement patterns within a given year and forming predictions on how anadromous salmon will respond to climate related changes. In the following sub-sections, I draw conclusions based on the combined results from previous chapters in this thesis and provide management implications where applicable. I then conclude the thesis with my recommendations for future research.

6.2 Behavioural mechanisms and cues

Chapter 3 revealed the diel vertical movements that sockeye salmon make into the upper 10 meters of the water column. Similar diel vertical patterns were also identified across species and life stages of anadromous salmon in chapter 2, and are likely related to a combination of multiple factors including feeding, thermoregulation, predator avoidance, making osmoregulatory adjustments, and navigation that vary across life stages and locations. For homing sockeye salmon in the SoG, the benefits associated with

migrating in upper surface waters must outweigh the potential risks, including increased risk of exposure to predators and exposure to what is considered above metabolically optimal water temperatures (Eliason et al., 2011). Locating freshwater entry points is essential for the securing reproductive success of sockeye salmon, which only have one opportunity to spawn in a lifetime. Therefore, obtaining olfactory cues to locate freshwater entry points is perhaps the most important benefit homing sockeye salmon could gain from migrating in surface waters within estuaries.

The idea that homing salmon use olfactory cues in surface waters for navigation specifically in coastal marine systems has been presented in various reviews (Dittman & Quinn, 1996; Keefer & Caudill, 2014; Quinn & Dittman, 1990) albeit with very little empirical evidence for support [for exceptions see (Davidsen et al., 2013; Ueda, 2011)]. My thesis results provided empirical evidence that sockeye salmon respond to the distribution of freshwater influenced surface water by wind-induced currents during their coastal migration (chapter 4). I arrived at this conclusion based on my findings that migration rate was related to wind patterns upon fish arriving in the northern SoG. Although these results were correlative, and therefore not necessarily causative, I attributed the association between wind patterns and migration rate to wind-induced currents exposing homing sockeye salmon to freshwater olfactory cues, which in turn advanced reproductive maturation through a neuroendocrine response (chapter 4). This neuroendocrine response, which was previously shown in homing chum salmon (Ueda, 2011), may be analogous to the mechanism associated with the initiation of reproductive maturation in the open-ocean caused by changing photoperiod, and may be another example of migrating salmon using environmental cues to facilitate physiological

preparations and migration timing. Therefore I suggest that sockeye salmon rely, at least in part, on olfactory homing during their coastal homeward migration.

In addition to olfaction, my thesis also identified other potential cues that could be used for navigation and orientation in estuaries including celestial cues (based on moon phase differences discussed in chapter 3) and coastlines (or depth associated with coastlines; chapter 4). Indeed, the coastal system may be where sockeye salmon transition from relying predominately on geomagnetic cues, which are thought to increase the precision for location of coastal areas from the open-ocean (Lohmann et al., 2008; Putman et al., 2013). This notion is certainly plausible because the accuracy needed to locate a specific river entry point is likely at a finer-scale than can detected with magnetic cues alone. Furthermore, this need for gaining navigational cues in surface waters must outweigh the increased costs associated with experiencing elevated temperatures and increased predation risk.

A large question remains as to how relationships between the environment and behaviour will alter under future climate change scenarios that predict an earlier spring freshet (Morrison et al., 2002) and changing wind patterns in coastal systems (IPCC, 2007). Earlier spring freshet could result in sockeye salmon encountering weaker concentrations of freshwater cues in surface waters of coastal systems due to lower freshwater discharge given the timing of migration remains the same. Based on evidence from my thesis, encountering weaker olfactory signals could influence sockeye salmon migration timing in the estuary, but whether these effects would be substantial enough to result in reduced fitness is not known. Nonetheless, sockeye salmon may have to adjust their migration timing accordingly. Evidence that anadromous salmon may be able to

adjust their migration schedules comes from previous studies that have shown long-term shifts in migration timing in response to environmental variables such as temperature and freshwater discharge (Juanes et al., 2004; Quinn & Adams, 1996), as well as from chapters 3 and 4 of this thesis, which showed a large amount of variability in behaviours of sockeye salmon both within- and between- individuals.

One example of a shift in migratory behaviour within a single population is the early river-entry of a segment of Late Shuswap sockeye salmon in comparison to historic norms, which I addressed in chapters 3 and 4 of this thesis and is reviewed in Hinch et al. (2012). Data from my thesis indicated that these early migrants were already migrating earlier prior to reaching the mouth of the Fraser River (chapter 4), and therefore, the factors contributing to this behaviour span from prior life stages, which was also suggested in Hinch et al. (2012). In addition to sharing similar river entry timing with Summer Run fish stocks, my thesis identified that early migrating Late Shuswap fish were also behaving similarly to Summer Run fish while in the estuary based on their thermal experience (chapter 3), a finding that was previously unknown. Despite these additional findings, we still know very little about the causes of the early river entry behaviour of Late Shuswap sockeye salmon that persists to this date.

An important consequence of the early river entry behaviour was increased in-river mortality for 'early' migrants (Cooke et al., 2004a). More recent data suggests that mortality of 'early' migrants is variable year-to-year (Les Jantz, Department of Fisheries Oceans Canada; personal communication), likely due to differences river temperatures between years. Importantly, the 'early' Late Run fish belong to the Late Shuswap (e.g. Adams) population, which comprises the majority of sockeye salmon in the dominant run

that occurs every 4 years. Due to their prevalence in the dominant run, a substantial quantity of Adams fish are typically harvested in commercial fisheries within the coastal system. Thus, depending on management goals, data from this thesis could be used to time commercial fisheries openings for either targeting or avoiding 'early' Late Run fish. For example, managers may want to target 'early' Late Run fish in years with exceptionally high Fraser River temperature, thereby generating commercial revenue from fish that would otherwise not contribute to spawning. These types of management decisions are obviously extremely complex because, for instance, targeting 'early' Late Run fish would also result in catching Summer Run sockeye salmon that are co-migrating in the ocean. Nevertheless, data on movements and behaviours among and within stocks from my thesis has application towards management of Fraser River sockeye salmon, which are in need of a better biological understanding and management options.

6.3 Mechanisms of marine migration success

Based on my findings in chapters 3 and 4, there was not enough variability in environmental conditions experienced between individuals in marine waters that would give me reason to hypothesize that environmental conditions would influence marine survival. However, previous research has successfully related physiological condition of homing salmon with marine survival using blood plasma variables (Cooke et al., 2006a; Crossin et al., 2009a). Hence, to build on previous research, in chapters 3 and 4, I sought to establish physiological links with thermal experience and migration rate of homing sockeye salmon using data from blood plasma variables. However, I had very little success relating blood plasma physiology to behaviour in chapters 3 and 4.

Owing to the large number of molecular processes that can be observed with microarray technology and the previous success with homing salmon (Evans et al., 2011; Jeffries et al., 2012; Miller et al., 2011), in chapter 5, I used microarray analysis and qRT-PCR on gill tissue samples to successfully link gene expression patterns of homing sockeye salmon with marine survival. This was the first instance where gene expression has been specifically linked to marine survival of a homing anadromous salmon. The genomic signature correlated with marine survival was related to infection, immune response, stress and osmoregulation, and was similar to a signature previously linked to freshwater survival in Miller et al. (2011) (e.g. the MRS). However, the relationship between fish that had the MRS and survival was opposite between chapter 5 and Miller et al. (2011). This lead me to conclude that the MRS had different effects on survival between marine (examined in chapter 5) and freshwater [examined in Miller et al. (2011)] environments possibly due to the attenuation of disease resistance of fish when experiencing elevated temperatures in freshwater or to temporal differences between different study years. Regardless of its influence on survival, this genomic signature, which has now been identified in multiple studies, using multiple tissue types and microarray platforms, is the overwhelming signal present during the marine and freshwater migration of homing salmon.

In addition to linking gene expression with marine survival, I also found that fish with higher plasma lactate had a lower probability of marine survival (chapter 5). This finding is consistent with previous studies (Cooke et al., 2006a; Cooke et al., 2006b; Crossin et al., 2009a), and plasma lactate emerges as perhaps the most consistent blood plasma variable related to survival among studies. Interestingly, plasma lactate was not

related to gene expression, even though stress was identified as a biological process associated with gene expression, which was related to marine survival. These results from my thesis demonstrated that the combined approach of using both genomic techniques and blood plasma sampling with telemetry provided consensus between approaches in addition to finding novel results. Funding permitting (molecular techniques are costly), future studies should consider this combined approach for answering research questions, especially in observational studies such as what was presented in this thesis (discussed further in future work below).

6.4 Capture, sampling and tagging effects

The application of tags for studying behaviour and survival was a common theme throughout chapters 2, 3, 4 and 5 of my thesis. In addition to tagging, my research also used fisheries capture and tissue biopsies to address research questions. A major assumption of any study that captures, handles, inserts a transmitter, and releases animals into the wild is that the capture/sampling/tagging (CST) event (or the tag itself) has no adverse effects on behaviour or survival after release, and thus tagged individuals are representative of the larger population. In chapter 2, I found that testing or accounting for CST effects was a major gap in the literature, and remains a major hurdle for tagging research being incorporated into management decisions. Based on this, I attempted to account for CST effects using a variety of approaches in subsequent chapters.

In chapter 3 of my thesis, fish were tagged with thermal data loggers that recorded hourly temperature readings. By examining thermal profiles from recovered data loggers, I identified two stages of thermal behaviour, a stage of consistent thermal experience

immediately after release, followed by a stage of variable thermal behaviour. Ultimately, I attributed the consistent thermal stage to the homogenous environment fish were released into along with the possibility of CST related effects. Although this finding only suggested the possibility of CST effects, it demonstrated the potential application of different technologies, such as data loggers, for examining and accounting for CST effects in field studies.

In chapters 3, 4 and 5, I used tissue biopsies as a means of examining physiological condition of individual fish. To account for potential effects of tissue biopsies, I also released control fish that were not biopsied and then tested for behavioural and survival differences between control and biopsied fish. Chapter 4 was the only instance where I found effects of the biopsy procedure, and results from this chapter indicated that fish exposed to tissue biopsies took longer to reach the first detection location. However, tissue biopsy resulted in a minor delay of ~ 6 hrs for a portion of migration that averaged ~2.6 days (e.g., 12% longer), and there was a large amount of individual variability within both tissue biopsied (0.7 – 8.6 d) and non-biopsied (0.9 – 5.1 d) groups. In addition, there were no differences between biopsy and control fish after this initial section of migration, which suggests fish had either recovered from tissue biopsies or the effects were no longer detectable. I conclude that the effects of tissue biopsy are likely minimal compared to the overall capture event, but nevertheless, these results highlight the importance of incorporating controls into study designs, especially when studying behaviour immediately after release.

It is extremely difficult to control for the capture event in studies examining free-swimming fish, which must be captured from the wild. However one way to examine for

capture-related effects is to incorporate multiple capture methods into a single study. In chapters 3, 4 and 5, I tested for differences in behaviour and survival of fish captured using two different commercial fishery gear types, purse seine and troll fishing. These two capture types are analogous to two treatments with varying stress levels, and I had predicted that the purse seine capture event would be more stressful than the troll capture event for a number of reasons including increased overall duration of the purse seine capture event and crowding in the purse seine net causing injury and an oxygen deficit. In chapter 5, I found that fish captured by purse seine had elevated levels of stress hormones compared to troll captured fish, which appeared to be related to differences in the time course of blood sampling between capture methods rather than a difference in the overall magnitude of the stress response. Coinciding with this, there was no effect of capture method on behaviour or survival after release in chapters 3, 4, and 5. Further examination of fisheries-specific stress responses could be accomplished by holding troll-captured fish for the same amount of time prior to sampling as purse seine fish and compare blood parameters. I was not able to examine differences in survival between the two capture methods in freshwater, where I would predict effects of injury and stress to become exacerbated due to increased water temperatures and stress, and declining immune defenses.

A potential issue of tagging research, which was discussed at length in chapter 2, is the introduction of bias into survival estimates due to CST related mortality or due to tag regurgitation. To examine whether survival estimates were effected by CST related mortality or were biased due to tag regurgitation, I integrated a net pen holding experiment into the 2010 tagging study, the data being presented in chapter 5. There was

no mortality or tag regurgitation during the ~32 hr holding experiment leading me to conclude my survival estimates are reasonable approximations to natural survival rates. However, the net pen holding experiment was not designed to test for delayed mortality that might occur > 32 hrs after release or to test for other mortality causing factors such as predation that could be elevated in impaired fish after release into the wild.

Indeed predation may play a key role in post-release mortality from a CST event, and a recent experimental study demonstrated that seals are even able to learn to use acoustic signals from tags to locate fish (Stansbury et al., 2014). These results are in no way surprising and are akin to 'classical conditioning' that was demonstrated in the famous Pavlov's dog study [reviewed in Clark (2004)]. The ultimate question in context or my own research is whether seals use these signals in the wild to locate and feed on tagged fish? This question is difficult to test, but it basically boils down to a numbers game. There is no question that seals can detect the signal frequencies used in my studies, but the tags I used were intentionally programmed to emit a signal randomly every 60-120 seconds. Furthermore, a seal would need to encounter enough tagged fish in the wild in order to learn to use a tag signal to find food. The chances of a seal encountering enough tagged fish over time to learn to use the signal is extremely low given the expansive study area and relatively few fish released with tags (i.e., 400) compared to the larger fish population (i.e., millions). In addition, a seal would need to be within close enough proximity to actually detect a tag signal. Seals are able to detect swim wakes from a large distance way (Dehnhardt et al., 1998), likely a trait they developed in response to hunting in turbid waters. Therefore, given the immense numbers of salmon that can be present in a location at one time, and the low chances of seals encountering

multiple acoustic tags across time and space in a close enough proximity to detect the signal it would simply not be an energetically efficient means of gathering food. Most importantly, I think studies such as Stansbury et al. (2014) are necessary for examining the potential of tag-induced predation, a concept that is underappreciated in the literature.

6.5. Future directions

6.5.1 General directions

Biological processes influencing animal migrations are complex with numerous potentially interacting variables. Large-scale observational studies, such as those presented in chapters 3, 4, and 5, are useful for identifying biological relationships under natural conditions that are otherwise difficult to examine in controlled laboratory experiments. Based on results from my thesis, I would propose a series of complementary studies involving laboratory-holding experiments, field surveys, and field tagging studies. Laboratory-holding studies would experimentally test hypothesis about relationships between environmental conditions (i.e., temperature, salinity, oxygen, olfactory cue concentrations) and sockeye salmon physiology [for examples see (Cooperman et al., 2010; Eliason et al., 2011; Jeffries et al., 2012; Jeffries et al., 2014; Wagner et al., 2006)], and would serve as groundwork for testing hypotheses under field conditions. Below I propose future research based on finding from my thesis, arranged in order from more specific studies to more general areas of focus that need to be addressed.

6.5.1 Test of the osmoregulation hypothesis

In my thesis, I found no evidence that osmoregulatory state of individual fish was associated with migration timing despite including osmoregulatory indices from blood plasma in models testing for these associations. However, the hypothesis that osmoregulation drives migration timing during the seawater to freshwater transition is in need of more directed examination. I would propose a laboratory holding study followed by a field telemetry study to examine the influence of osmoregulation on sockeye salmon behaviour.

The laboratory holding study would be similar to Cooperman et al. (2010), in which fish would be captured and then held in different salinity treatments (full strength seawater, isoosmotic water, and freshwater) and sampled for gill and blood tissues on multiple occasions throughout the study to monitor changes in osmoregulatory state. In addition to using blood plasma sampling, I would recommend using a molecular biomarker approach focusing on specific biomarkers identified in other studies, such as the sodium-potassium-ATPase (NKA) isoforms alpha 1a, and alpha 1b identified in (Richards et al., 2003; Shrimpton et al., 2005). NKA isoforms may be more representative of osmoregulatory state, but they have yet to be tested for homing adult salmon under laboratory conditions.

Following the laboratory holding study, I would use a tagging study to test hypotheses under field conditions. The methods for the proposed tagging study would be similar to my tagging study in 2010 (i.e., fish sampled in ocean and using physiological biotelemetry). However, in addition to examining blood plasma physiology, osmoregulatory gene biomarkers would be run on gill tissue and related to migration

timing and survival from telemetry data. A specific hypothesis I would test would be that expression of the freshwater specific osmoregulatory isoform (NKA alpha 1a) would be related to migration rate in the ocean. I would predict that fish with elevated expression of the freshwater specific NKA isoform would migrate faster into freshwater. Relating NKA isoforms to migration behavior during the seawater to freshwater transition is the next step for exploring the influence of osmoregulation on homing sockeye salmon behaviour.

6.5.2 Coastal olfactory homing

The use of olfactory homing in coastal homeward migrations is another gap in our understanding of the reproductive migration of salmon. To address this gap, I would propose a field survey approach similar to Ueda (2011) in which sockeye salmon are captured and sampled at different locations along their migration pathway. Sampling sites would vary by distance away from the river, such as Hydraguya, Johnson Strait, Northern SoG, Central SoG, the SoG at the Fraser River mouth and at sites within the Fraser River. At each location, fish would be destructively sampled and rosettes would be taken from the olfactory organ and qRT-PCR would be run to determine expression levels of specific genes related to olfactory homing that have been previously identified in freshwater studies (N. Bett, unpublished data). The level of expression of these olfactory genes could be examined across spatial scales and I would predict increases in expression of these genes after arrival in the SoG where freshwater cues first become present, with increasing expression thereafter.

A field manipulation experiment could accompany the field survey of olfaction described above. In such study, estuary water acquired from close to the Fraser River mouth could be transported in vessel to a location where Fraser River sockeye salmon would be perceived to be 'Fraser River water naïve', such as Queen Charlotte Strait (although it is debatable whether fish are truly 'Fraser River water naïve' at this point). Sockeye salmon could be captured and a subset of fish exposed to the transported water presumably containing the olfactory cues for a selected amount of time prior to being tagged and released. A control group of fish not exposed to the transport water would also be held for the same amount of time, tagged and tracked using telemetry. Salinity and temperature would also need to be controlled for across treatments. Additionally, at the experiment beginning and endpoint a subset of fish could be destructively sampled from the olfactory cue and control fish as described above in the field survey to test for changes in olfactory genes and reproductive state in response to the treatment with olfactory rich waters.

6.5.3 Estuarine currents and energy

In chapter 4, I found that sockeye salmon migrated faster when winds were in a direction that would create currents favoring migration towards the Fraser River. I suggested that the use of currents or tides may result in energetically efficient swimming. To specifically examine how sockeye salmon interact with estuarine currents or tides, I would propose a field tagging study using accelerometer tags such as in Wilson et al. (2014a). Sockeye salmon would be captured north of the SoG, tagged with accelerometers and released. To relate behaviour to currents and tide, an extensive

telemetry array would be deployed in the estuary near the mouth of the Fraser River similar to Crossin et al. (2007). A current meter would be attached at each receiver location to measure surface currents. Data on movements and swim speed could be paired with current readings to examine whether fish are using currents to reduce energy expenditure. Tags could be applied across a number of stocks with varying in-river migration difficulties to test for stock-related differences.

6.5.4 Pathogen progression

Based on results from chapter 5 and Miller et al. (2011), I would propose further studies examining the influence of stress and pathogens on behaviour and survival of homing sockeye salmon during the marine to freshwater transition. I would first propose a holding experiment similar to what was proposed above for examining osmoregulation. However, in addition to salinity treatments, ecologically relevant temperature treatments (i.e., low ~ 11 °C and high ~ 18 °C) could also be included in a factorial design. Fish would be sampled progressively (as proposed in 6.5.1) and immune and stress related gene biomarkers, and pathogen biomarkers could be run on gill tissue samples. This study design would help tease apart the effects of salinity and temperature on disease progression.

A field tagging study could follow the holding study, and would be similar to the 2010 tagging study in this thesis. However, instead of using microarray analysis on gill tissue samples as done in chapter 5 of this thesis, biomarkers specifically selected from the holding study could be used to compare migration rates and survival of tagged fish in both the SoG and in the Fraser River. In order to fully explore the effects of pathogens on

survival, more extensive telemetry arrays would be needed in the Fraser River to get a detailed resolution of in-river movements and survival. This study would help identify specific pathogens influencing survival and would help resolve survival differences observed between marine and freshwater environments in chapter 5 of this thesis and Miller et al. (2011).

6.5.5 Role of predation

Predation on salmon by seals may be a significant source of mortality during the estuarine migration, and is likely related to many behaviours. To address the role predation plays in influencing salmon behaviour and survival, I would propose studies that tag both seals and homing salmon within the SoG, and then movements and interactions can be monitored and compared. These types of studies are already underway in other parts of Canada and around the globe, many of which fall under the Ocean Tracking Network (OTN) umbrella (Cooke et al., 2011a). Organizations such as OTN provide a useful platform for collaborative, multidisciplinary research such as what is presented in this thesis.

6.5.6 Oceanographic links

Currently, oceanographic monitoring is limited across both space and time in the SoG, making it challenging to relate environmental conditions to fish movements. Oceanographic models that predict conditions at extremely fine scales already exist for the SoG, and these models have numerous applications for studying salmon movements, behavoiur and survival. However, in many cases predictive models would benefit from

validation through environmental monitoring and animal borne tags that measure environmental characteristics. Ultimately, a combination of methods may be needed to establish relationships, but this is an important step that needs to be taken in the SoG.

6.6 Conclusion

Through the use of multiple research approaches this thesis empirically established novel relationships between environmental conditions, physiological state and homing sockeye salmon behaviour and survival in marine waters. In addition to advancing the basic biological understanding of the homing migration of sockeye salmon in marine environments, this thesis is broadly applicable to other anadromous salmon, as well as to studies invoking a similar approach of physiological biotelemetry for studying animal movements.

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APPENDICES

Appendix A. Boolean search terms used in literatures searches in two data bases, the Web of Science, and Aquatic Science and Fisheries Abstracts.

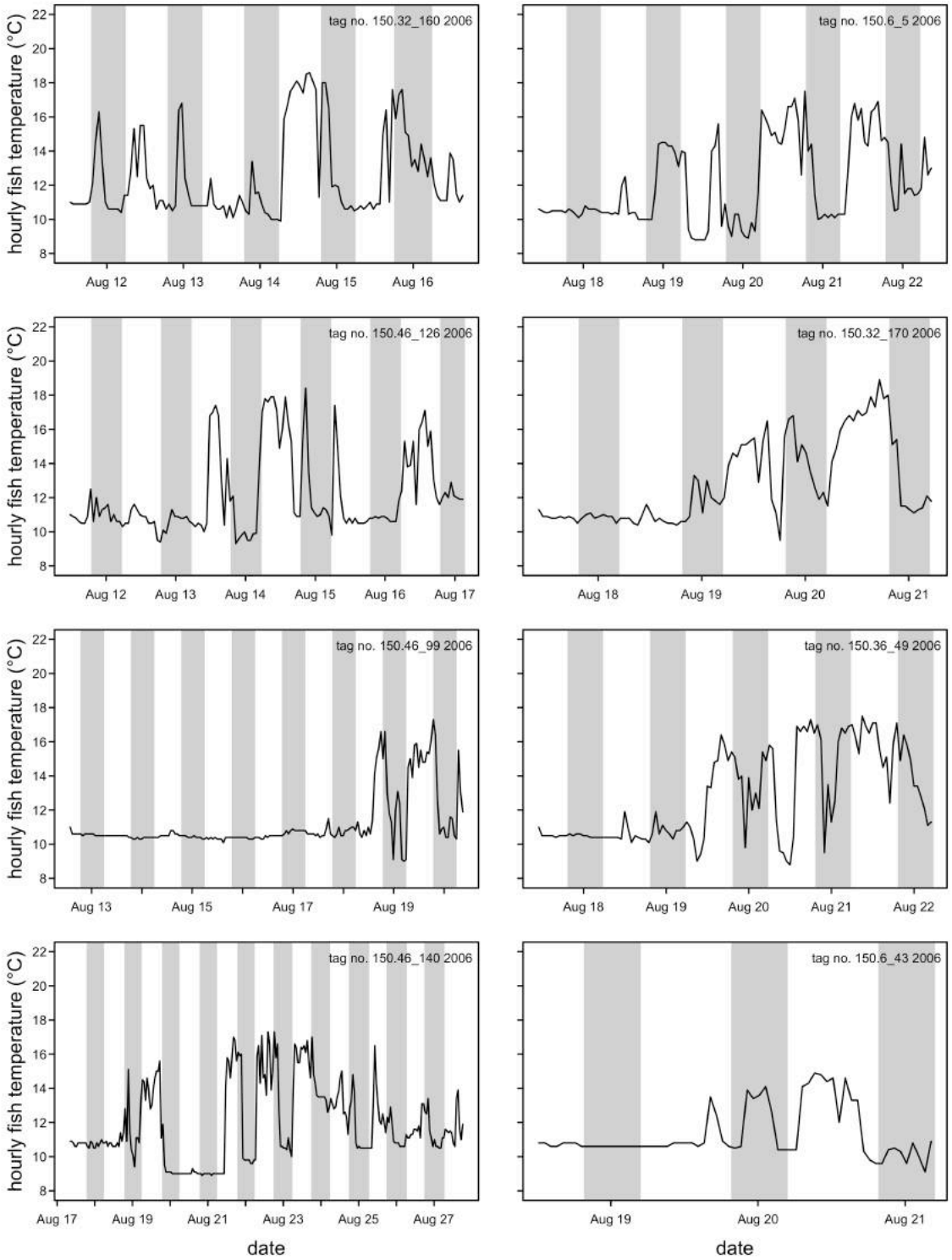
Web of Science Search Terms:

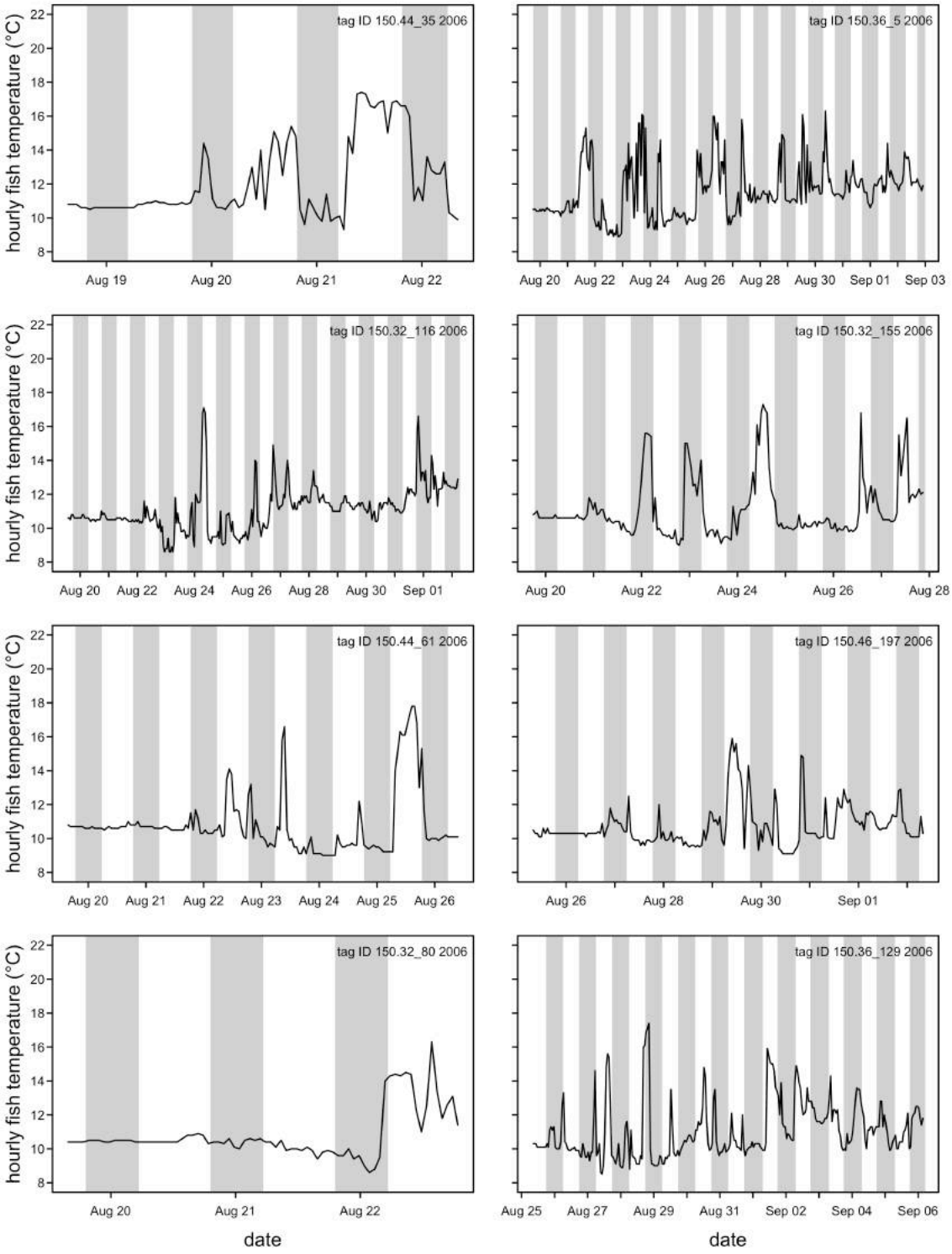
TS=(ocean* or marine* or coast* or estuar*) AND TI=(salmon* or oncorhynchus or salmo or steelhead or cutthroat) AND TS=(mortality or survival or dispersal or behavio$r or migrat* or movement or physiolog*) AND TS=(telemetry or tag* or track* or transmit* or "data logger") NOT TS=(salmonella* or paleo* or shark*) NOT TI=(cod or herring or mullet or oyster* or mussel* or bass or sunfish* or charr or shrimp or eel or gilthead or bream or turbot or alosa or trutta) NOT SO=(Food Chemistry or Aquaculture Nutrition or Aquaculture Research or Aquaculture Engineering)

ASFA Search Terms:

KW=(salmon* or oncorhynchus or salmo) and KW=(ocean* or marine* or coast* or estuar*) and KW=(mortality or survival or dispersal or behavio$r or migrat* or movement or telemetry or tag* or track* or physiolog* or temperature or salinity or fisher* or climate or predat*) and not TI=(cod or mykiss or herring or mullet or oyster* or mussel* or bass or sunfish* or charr or shrimp or eel or gilthead or bream or turbot or alosa or trutta) and not JN=(food chemistry or veterinary microbiology or nutrition or shellfish or ornithology) and not KW=(salmonella* or shark* or paleo*)

Appendix B. Plots of individual fish hourly temperature experience for all recovered tags.

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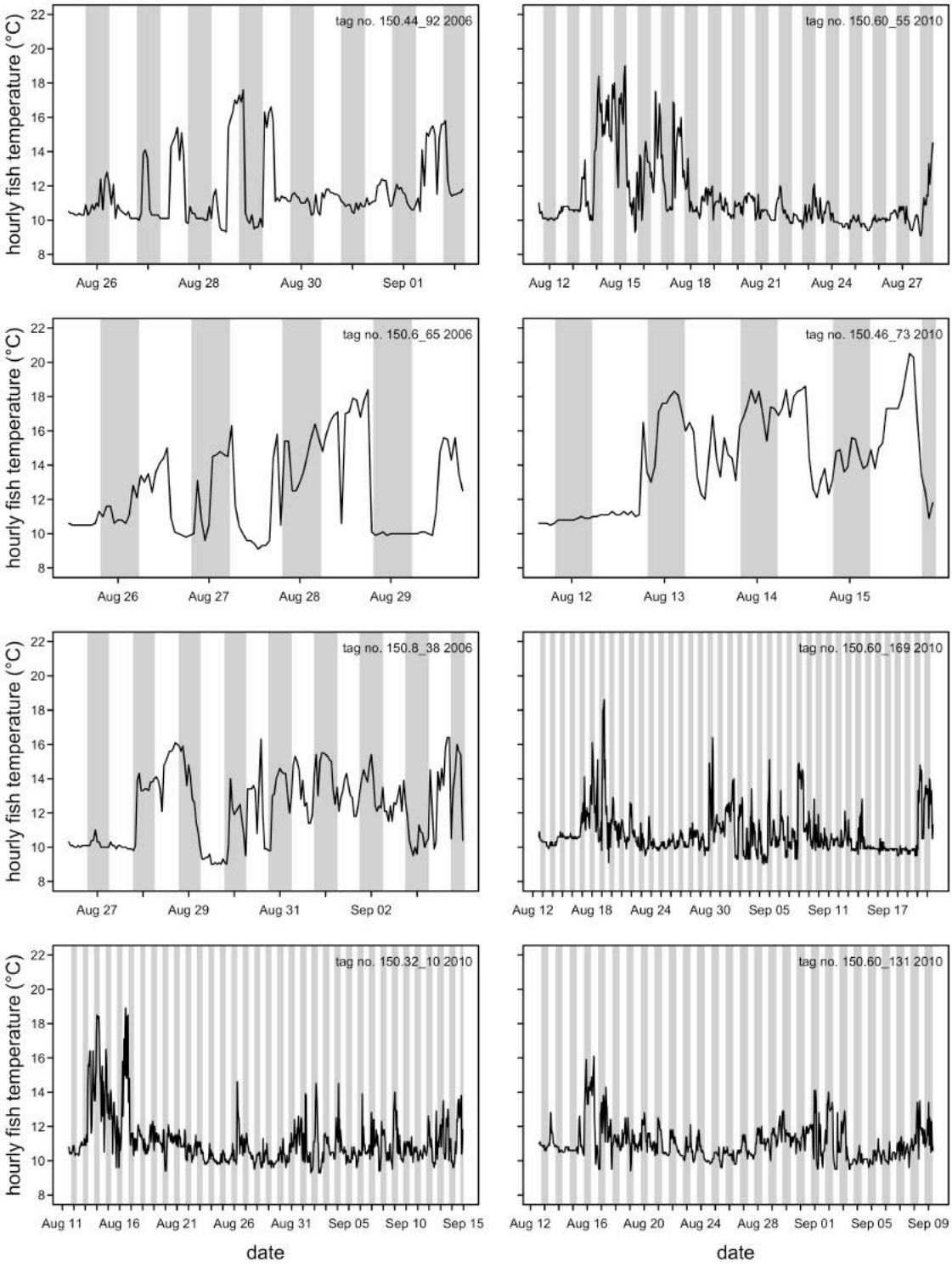
date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tag no. 150.8_38 2006
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tag no. 150.32_10 2010
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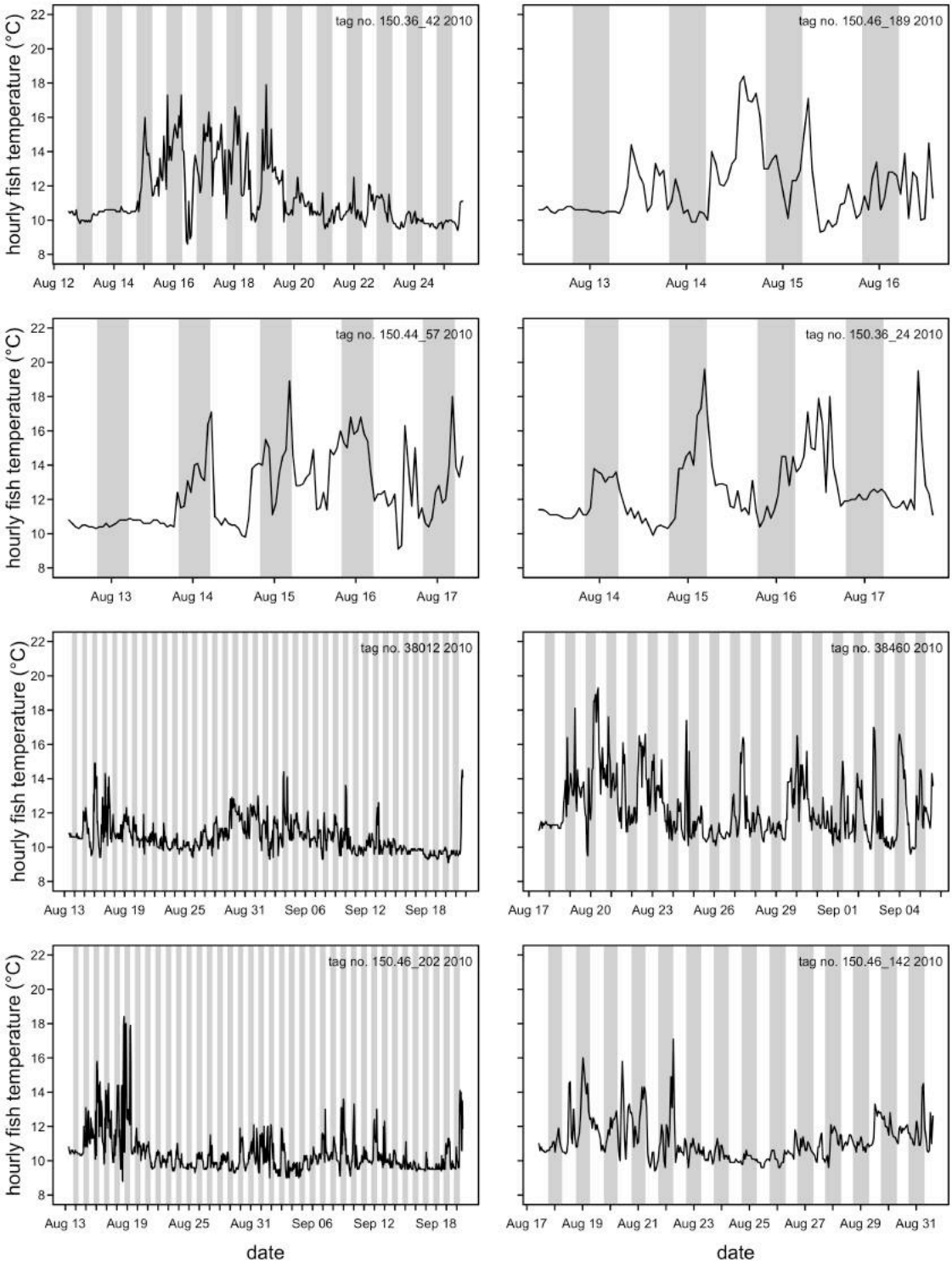
hourly fish temperature (°C)
date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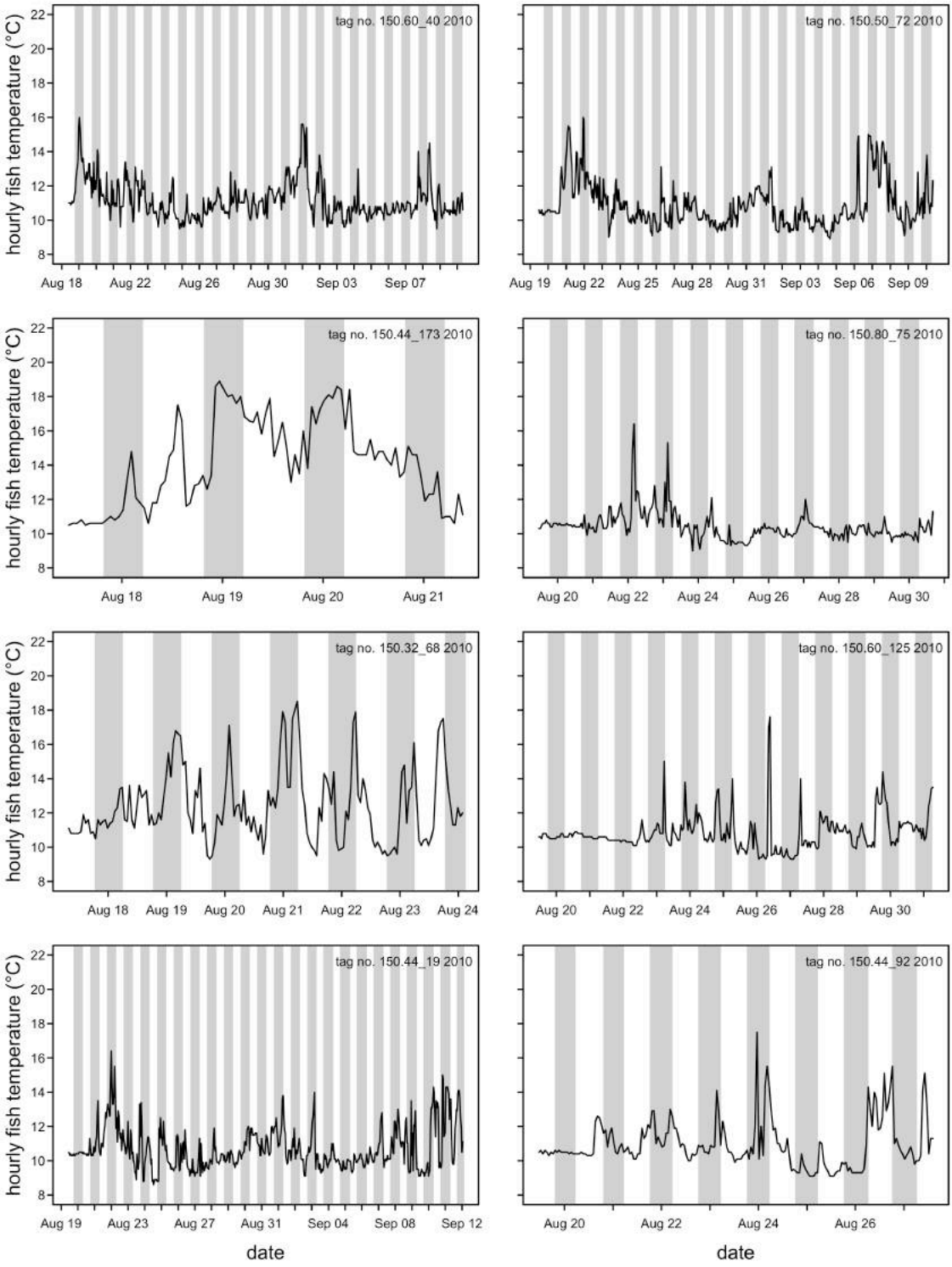
tag no. 150.44_57 2010
tag no. 150.36_24 2010
tag no. 38012 2010
tag no. 38460 2010
tag no. 150.46_202 2010
tag no. 150.46_142 2010
hourly fish temperature (°C)
date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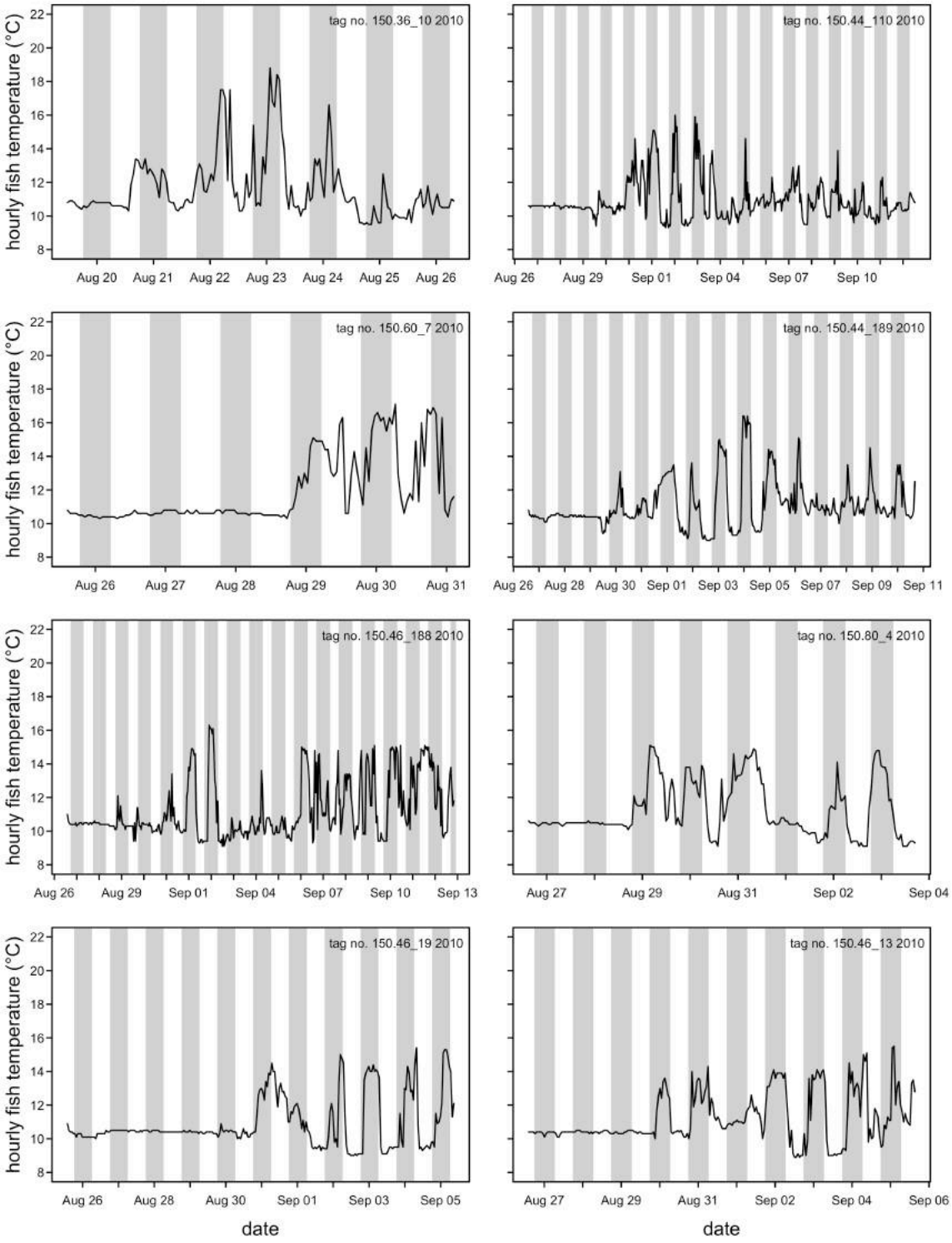
tag no. 150.50_72 2010
tag no. 150.44_173 2010
tag no. 150.80_75 2010
tag no. 150.32_68 2010
tag no. 150.60_125 2010
tag no. 150.44_19 2010
tag no. 150.44_92 2010
hourly fish temperature (°C)
date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tag no. 150.44_110 2010
tag no. 150.60_7 2010
tag no. 150.44_189 2010
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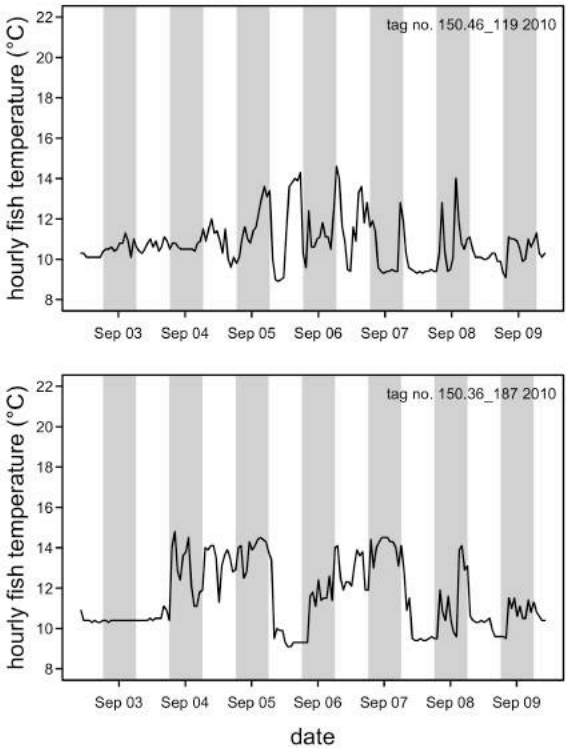
tag no. 150.80_4 2010
tag no. 150.46_19 2010
tag no. 150.46_13 2010
hourly fish temperature (°C)
date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tag no. 150.36_187 2010
hourly fish temperature (°C)
22
20
18
16
14
12
10
8
Sep 03
Sep 04
Sep 05
Sep 06
Sep 07
Sep 08
Sep 09
date

Appendix C. The 50 most up-regulated and 50 most down-regulated genes from PC1 of gene expression from microarray data based on relative fold changes between fish from the 15% most PC1 positive and 15% most PC1 negative groups. Positive and negative fold change values indicate genes were up- and down-regulated, respectively in the group of fish with a higher probability of marine survival relative to the group of fish with a lower probability of marine survival. P-values were calculated using t-tests and an fdr correction factor was applied. Functional links were inferred using RefSeq (http://www.ncbi.nlm.nih.gov/refseq/).

Accession (44k slide)	Uniprot ID	gene symbol	gene name	relative fold change	fdr-adjusted p-values	functional link
C025R103	Q764Q5	Abcb9	ATP-BINDING CASSETTE; SUB-FAMILY B (MDR/TAP); MEMBER 9	7.63	7.01E-22	metabolic processes
C051R159	O73723	actr3	ACTIN-RELATED PROTEIN 3	7.12	1.53E-22	structural component
C134R090	Q3UNZ6	Arhgap27	RHO GTPASE ACTIVATING PROTEIN 27	8.00	2.12E-22	metabolic processes
C026R108	Q9PTY0	atp5b	ATP SYNTHASE BETA CHAIN; MITOCHONDRIAL PRECURSOR	7.80	2.02E-15	metabolic processes
C103R003	Q4VAN1	BTN1A1	BUTYROPHILIN; SUBFAMILY 1; MEMBER A1	11.88	9.77E-16	immune response
C169R129	Q6AYC4	Capg	CAPPING PROTEIN (ACTIN FILAMENT); GELSOLIN-LIKE	10.30	2.90E-15	structural component
C103R108	Q9HA01	CCDC51	COILED-COIL DOMAIN CONTAINING 51	8.36	2.65E-19	unknown
C054R094	O14519	CDK2AP1	CDK2-ASSOCIATED PROTEIN 1	9.19	6.04E-20	cell proliferation
C232R127	Q7T339	chmp5	CHROMATIN MODIFYING PROTEIN 5	9.14	8.96E-18	cell proliferation
C100R143	Q53XX5	CIRBP	COLD INDUCIBLE RNA BINDING PROTEIN	6.87	5.66E-14	osmoregulation
C035R142	O96005	CLPTM1	CLEFT LIP AND PALATE ASSOCIATED TRANSMEMBRANE PROTEIN 1	6.90	4.93E-23	immune response
C210R135	P08125	COL10A1	COLLAGEN ALPHA-1(X) CHAIN PRECURSOR	9.66	8.85E-19	structural component
C144R010	Q96DA6	dnajc19	DNAJ (HSP40) HOMOLOG; SUBFAMILY C; MEMBER 19	8.57	3.80E-20	metabolic processes
C022R166	Q9NUQ9	FAM49B	FAMILY WITH SEQUENCE SIMILARITY 49; MEMBER B	9.87	1.96E-25	structural component
C100R113	Q6IX74	GADD45B	GROWTH ARREST AND D-DAMAGE-INDUCIBLE; BETA	7.27	8.84E-20	apoptosis/stress
C024R155	Q8NHV1	GIMAP7	GTPASE; IMAP FAMILY MEMBER 7	7.49	5.85E-15	immune response
C106R090	O13116	hcea	CHORIOLYSIN H	7.09	4.19E-14	immune response
C212R028	Q6IBK9	HCLS1	HEMATOPOIETIC CELL-SPECIFIC LYN SUBSTRATE 1	7.33	1.59E-16	protein binding
C226R095	Q96P57	HEBP2	HEME BINDING PROTEIN 2	8.19	1.50E-15	cell proliferation
C263R041	Q6NS40	HMGB3	HIGH-MOBILITY GROUP BOX 3	7.60	1.59E-14	cell proliferation

Accession (44k slide)	Uniprot ID	gene symbol	gene name	relative fold change	fdr-adjusted p-values	functional link
C160R035	Q90593	HSPA5	HEAT SHOCK 70KDA PROTEIN 5 (GLUCOSE-REGULATED PROTEIN; 78KDA)	10.12	1.44E-19	stress/protein folding
C078R113	O15116	LSM1	LSM1 HOMOLOG; U6 SMALL NUCLEAR RNA ASSOCIATED (S. CEREVISIAE)	6.94	8.09E-20	protein synthesis
C180R106	P16527	MARCKS	MYRISTOYLATED ALANINE-RICH C KISE SUBSTRATE (MARCKS)	7.51	7.09E-22	structural component
C172R007	Q719N3	Mmd	MONOCYTE TO MACROPHAGE DIFFERENTIATION-ASSOCIATED	7.51	2.96E-23	structural component
C152R082	Q16771	MPO	MYELOPEROXIDASE	8.93	5.17E-17	oxidative stress/apoptosis
C088R144	Q64119	Myl6	MYOSIN; LIGHT POLYPEPTIDE 6; ALKALI; SMOOTH MUSCLE AND NON-MUSCLE (PREDICTED)	9.03	5.00E-21	metabolic processes
C213R104	Q92542	NCSTN	NICASTRIN	9.09	1.39E-22	protein binding/metabolism
C188R126	Q9DCJ5	Ndufa8	NADH DEHYDROGENASE (UBIQUINONE) 1 ALPHA SUBCOMPLEX; 8	7.19	1.49E-22	oxidative phosphorylation
C202R022	Q7Z5B2	NFU1	HIRA INTERACTING PROTEIN 5	9.42	3.28E-24	metabolism
C145R059	O14822	PIGK	PHOSPHATIDYLINOSITOL GLYCAN; CLASS K	7.06	1.73E-21	protein binding/metabolism
C186R068	Q9W735	proml1	PROMININ-LIKE 1	7.83	3.01E-18	cell proliferation
C061R057	Q9BZD6	PRRG4	PROLINE RICH GLA (G-CARBOXYGLUTAMIC ACID) 4 (TRANSMEMBRANE)	7.52	1.70E-22	osmoregulation
C139R061	Q864Z2	RGS5	REGULATOR OF G-PROTEIN SIGNALLING 5	11.24	2.92E-19	apoptosis
C181R014	Q495B9	RHBDD1	RHOMBOID DOMAIN CONTAINING 1	9.17	4.99E-19	apoptosis
C224R086	Q9H8A9	RNF213	CHROMOSOME 17 OPEN READING FRAME 27	7.39	3.16E-17	protein binding/metabolism
C036R113	Q9CRZ9	Rpl13	RIBOSOMAL PROTEIN L13	8.72	2.22E-17	cell proliferation
C197R132	Q6IRL3	Rtn4	RETICULON 4	7.53	3.97E-18	cell proliferation
C041R138		Rv1301		9.58	2.86E-11	unknown
C133R064	Q6UVJ0	sass6	SPINDLE ASSEMBLY 6 HOMOLOG (C. ELEGANS)	7.34	9.06E-21	cell proliferation
C096R061	O00173	SDCBP	SYNDECAN BINDING PROTEIN (SYNTENIN)	8.71	2.24E-17	structural component
C031R048	Q96CT1	SERPINF1	SERPIN PEPTIDASE INHIBITOR; CLADE F (ALPHA-2 ANTIPLASMIN; PIGMENT EPITHELIUM DERIVED FACTOR); MEMBER 1	7.88	1.47E-13	cell proliferation
C075R132	Q8C1Y6	Srp54	SIGNAL RECOGNITION PARTICLE 54	7.49	1.18E-19	protein synthesis
C197R093	Q3TVN9	Stt3a	STT3; SUBUNIT OF THE OLIGOSACCHARYLTRANSFERASE COMPLEX; HOMOLOG A (S. CEREVISIAE)	8.20	1.88E-15	protein binding
C211R155	Q07283	TCHH	TRICHOHYALIN	9.48	2.31E-16	cell proliferation
C149R127	Q96IK2	TPM1	TROPOMYOSIN 1 (ALPHA)	8.40	1.45E-21	structural component
C163R120	Q9TR04	WASL	WISKOTT-ALDRICH SYNDROME-LIKE	11.88	7.78E-23	structural component
C115R081	O00308	WWP2	WW DOMAIN CONTAINING E3 UBIQUITIN PROTEIN LIGASE 2	8.55	6.39E-22	protein binding/immune

Accession (44k slide)	Uniprot ID	gene symbol	gene name	relative fold change	fdr-adjusted p-values	functional link
C156R132		zgc:66014		7.48	1.08E-20	unknown
C174R084	Q94987	zip	ZIPPER	8.02	4.92E-13	structural component
C035R047	Q6ZS36	ZNF551	HYPOTHETICAL PROTEIN FLJ38288	10.26	7.32E-23	protein synthesis
C085R143	Q8K0L1	Aldh6a1	ALDEHYDE DEHYDROGENASE FAMILY 6; SUBFAMILY A1	-2.63	3.04E-15	metabolic processes
C225R052	Q53GB6	ARPC1A	ACTIN RELATED PROTEIN 2/3 COMPLEX; SUBUNIT 1A; 41KDA	-2.64	1.44E-14	structural component
C063R015	P25489	atp1a1	SODIUM/POTASSIUM-TRANSPORTING ATPASE ALPHA-1 CHAIN PRECURSOR	-4.19	2.00E-14	osmoregulation
C152R123	P87362	BLMH	BLEOMYCIN HYDROLASE	-3.27	2.83E-14	immune response
C168R153	Q92051	cahz	CARBONIC ANHYDRASE	-2.57	3.00E-10	osmoregulation
C137R006	Q9W790	CCT1	TCP-1-ALPHA	-3.02	1.56E-13	cell proliferation
C164R044	Q9TRK3	CCT2	SIMILAR TO CHAPERONIN CONTAINING TCP1; SUBUNIT 2	-2.66	1.19E-19	protein binding
C155R006	P10247	Cd74	CD74 ANTIGEN (INVARIANT POLPYPEPTIDE OF MAJOR HISTOCOMPATIBILITY CLASS II ANTIGEN-ASSOCIATED)	-2.67	4.16E-13	immune response
C131R153	Q9YIB4	COL1A1	ALPHA 1 TYPE I COLLAGEN	-2.81	1.16E-13	cell proliferation
C192R027	Q3U5Z9	Copb2	COATOMER PROTEIN COMPLEX; SUBUNIT BETA 2 (BETA PRIME)	-3.32	6.34E-17	
C147R115	Q8WVW8	COPG2	COATOMER PROTEIN COMPLEX; SUBUNIT GAMMA 2	-2.66	2.36E-15	protein binding
C044R019	Q922W1	Ctnnb1	CATENIN (CADHERIN ASSOCIATED PROTEIN); BETA 1	-3.05	2.33E-16	cell proliferation/apoptosis
C117R025	Q86X36	DHX8	DEAH (ASP-GLU-ALA-HIS) BOX POLYPEPTIDE 8	-2.66	2.10E-15	protein synthesis
C074R137	Q92814	DYNC1H1	DYNEIN; CYTOPLASMIC 1; HEAVY CHAIN 1	-2.61	1.05E-12	protein binding
C183R117	P13639	EEF2	EUKARYOTIC TRANSLATION ELONGATION FACTOR 2	-3.06	8.89E-12	protein synthesis
C133R048	Q7T3B0	eif3m	ZGC:63996	-2.98	2.38E-11	protein synthesis
C198R079		engB		-2.75	0.008727798	
C134R157		FDPS		-2.79	7.10E-13	
C171R003	Q6ZME7	FKBP10	FK506 BINDING PROTEIN 10; 65 KDA	-2.62	3.02E-13	protein binding
C068R014	Q3TIS5	Fubp1	FAR UPSTREAM ELEMENT (FUSE) BINDING PROTEIN 1	-3.34	4.81E-12	protein binding
C015R009	O42259	gapdh	GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE	-2.93	3.21E-11	
C164R116	Q4R591	GPI	BRAIN CDNA; CLONE: QCCE-13068; SIMILAR TO HUMAN GLUCOSE PHOSPHATE ISOMERASE (GPI);	-2.65	8.98E-13	metabolic processes
C055R065	Q9H3E9	GRHPR	GLYOXYLATE REDUCTASE/HYDROXYPYRUVATE REDUCTASE	-2.61	2.41E-16	metabolic processes
C062R008	Q15690	GSTP1	GLUTATHIONE S-TRANSFERASE PI	-3.15	2.92E-06	metabolic processes
C111R089	Q5SZI6	HSPG2	PERLECAN	-2.96	0.000900994	structural component
C144R070	Q9UF40	JMJD2B	JUMONJI DOMAIN CONTAINING 2B	-2.58	3.53E-12	cell proliferation
C078R077	Q5VYJ3	JMJD2C	JUMONJI DOMAIN CONTAINING 2C	-2.69	4.06E-14	cell proliferation
C195R021	Q3T0J3	MRPL16	MITOCHONDRIAL RIBOSOMAL PROTEIN L16	-2.55	2.01E-11	protein synthesis

Accession (44k slide)	Uniprot ID	gene symbol	gene name	relative fold change	fdr-adjusted p-values	functional link
C136R034	P25847	MSH2	DNA MISMATCH REPAIR PROTEIN MSH2	-2.58	1.10E-17	cell proliferation
C212R037	O43776	NARS	ASPARAGINYL-TRNA SYNTHETASE	-2.65	8.10E-13	cell proliferation
C157R057	P15771	NCL	NUCLEOLIN	-2.65	1.77E-12	protein synthesis
C185R024	Q7YRC5	NOB1	LIKELY ORTHOLOG OF MOUSE NIN ONE BINDING PROTEIN	-2.63	4.80E-12	protein synthesis
C112R031	Q5U3S7	nosip	NITRIC OXIDE SYNTHASE INTERACTING PROTEIN	-2.71	1.23E-15	protein binding/metabolism
C133R067	Q86UR1	NOXA1	NADPH OXIDASE ACTIVATOR 1	-2.75	1.56E-13	protein binding/metabolism
C165R147	Q9EPH8	Pabpc1	POLY(A) BINDING PROTEIN; CYTOPLASMIC 1	-2.88	3.04E-13	protein binding/metabolism
C125R045	Q99778	PDIA6	PROTEIN DISULFIDE ISOMERASE FAMILY A; MEMBER 6	-2.71	1.50E-15	protein binding
C192R132	Q9NTT6	PGM3	PHOSPHOGLUCOMUTASE 3	-2.56	1.58E-14	metabolic processes
C098R108	Q99460	PSMD1	PROTEASOME (PROSOME; MACROPAIN) 26S SUBUNIT; NON-ATPASE; 1	-2.56	1.58E-11	protein binding
C053R002		rhcg1		-2.54	1.78E-09	
C066R073	Q5ZK03	SEC23A	SEC23 HOMOLOG B (S. CEREVISIAE)	-2.76	1.54E-10	protein synthesis
C066R005	Q7ZYA4	sf3b1	146KDA NUCLEAR PROTEIN	-3.01	5.32E-13	protein synthesis
C116R082	Q9P1I4	ST13	SUPPRESSION OF TUMORIGENICITY 13 (COLON CARCINOMA) (HSP70 INTERACTING PROTEIN)	-2.61	7.67E-18	immune response
C101R027	Q2YDL1	STXBP6	HYPOTHETICAL PROTEIN	-2.73	3.11E-10	
C079R055	Q9BW75	THOP1	THIMET OLIGOPEPTIDASE 1	-2.62	9.33E-19	protein binding/metabolism
C091R116	Q96G71	TNPO3	TRANSPORTIN 3	-2.55	2.20E-12	protein binding
C200R050	Q7ZUG2	tpt1	TRANSLATIONALLY CONTROLLED TUMOR PROTEIN	-3.24	5.80E-11	immune response
C060R022	P41383	TUB2	TUBULIN ALPHA-2/ALPHA-4 CHAIN	-2.58	1.35E-11	
C029R010	Q3T0L2	TXNDC4	THIOREDOXIN DOMAIN CONTAINING 4 (ENDOPLASMIC RETICULUM)	-2.53	1.39E-14	protein binding/metabolism
C022R065	P56399	Usp5	UBIQUITIN SPECIFIC PEPTIDASE 5 (ISOPEPTIDASE T)	-3.02	8.86E-19	protein binding
C050R018	P23787	vcp	VALOSIN CONTAINING PROTEIN	-2.65	6.79E-14	protein binding/metabolism