FACTORS AFFECTING THE TIMING AND SUCCESS OF SOCKEYE SALMON SPAWNING MIGRATIONS

by

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ABSTRACT

Migration timing is a conserved life-history trait. To address the hypothesis that reproductive hormones are principal determinants of migration timing, I physiologically biopsied over 1000 sockeye salmon and monitored their subsequent behaviour with acoustic and radio telemetry as they migrated from the Pacific Ocean toward and into the Fraser River, and then onward to distant spawning areas. Links between physiology, behaviour, and survival were examined.

Circulating testosterone was found to be positively correlated with the rates of river entry in Late-run females but not in males, despite having concentrations that were equal if not higher than those of females. The notion of protandrous migration, in which males synchronize their activities to the reproductive and migratory schedules of females, was postulated as the basis for this difference. Once in river however, successful males and females were those that (1) took longest to enter the river, and (2) had high somatic energy, low testosterone, and low gill Na^+,K^+-ATPase activities.

An experimental test of the effect of reproductive hormones on the regulation of migration timing proved inconclusive. Relative to controls, GnRH and (or) testosterone treatment did not influence rates of ocean travel by males. Unfortunately, no females were examined. Nevertheless, significant, positive correlations between initial testosterone and travel times were found irrespective of hormonal treatment, which was unexpected but consistent with the previous studies.

In an experimental simulation of an 'early' migration, normally timed Late-run sockeye exposed to typical 10 ºC river temperatures and then released to complete migration were 68% successful. In contrast, salmon held at 18 °C and released were half as successful. The expression of a kidney parasite was near maximal in the 18 °C fish and undetectable in the 10 °C fish. Only gill Na^+,K^+-ATPase activity differed between groups, with a drop in the 18 °C fish. Though no clear stress, reproductive, or energetic differences were observed between groups, the ultimate effect of high temperature treatment was high disease expression, slowed migration speeds, and high migration mortality.

Changes in reproductive schedules, due to changes in latitudinal ocean distributions, are discussed as potential causes of early migration by Late-run sockeye.

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LIST OF ABBREVIATIONS & ACRONYMS

11-KT	11 ketotestosterone
ADP	adenosine diphosphate
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BC	British Columbia
Cl^-	chloride
DD	degree days
DFO	Department of Fisheries and Oceans, Canada
DNA	deoxyribonucleic acid
E_2	17β estradiol
Early-summer run	early-summer runs of Fraser River sockeye salmon
FL	nose-to-fork length
FOC	Fisheries and Oceans Canada (aka Department of Fisheries and Oceans)
FSH	follicle stimulating hormone (formerly known in fish as GtHI)
GnRH	gonadotropin-releasing hormone
GnRHa	gonadotropin-releasing hormone analog
GSE	gross somatic energy
GV	germinal vesicle
h	hour
HPA	hypothalamo-pituitary-adrenal axis
HPG	hypothalamo-pituitary-gonadal axis
HPI	hypothalamo-pituitary-interrenal axis (teleostian analog of the HPA axis)
Hsp70	heat shock protein 70
Htc	hematocrit
JDF	Juan de Fuca Strait
JS	Johnstone Strait
K^+	potassium
Late-run	Late summer runs of Fraser River sockeye salmon
LD	lipid drops
LH	luteinizing hormone (formerly known in fish as GtHII)
MANOVA	multivariate analysis of variance

MJ	mega joule
Na^+	sodium
NaCl	sodium chloride
NSERC	National Science and Engineering Research Council, Canada
NSOG	northern Strait of Georgia
POA	pre-optic area of the hypothalamus
POH	post-orbital to hypural length
POST	Pacific Ocean Shelf Tracking program
PSC	Pacific Salmon Commission, Vancouver
QCI	Queen Charlotte Islands (Haida G'waii)
SCN	suprachiasmatic nucleus
SEM	standard error of the mean
SOG	Strait of Georgia
SSOG	southern Strait of Georgia
Summer-run	mid-summer runs of Fraser River sockeye salmon
T	testosterone
UBC	University of British Columbia
VP	vitelline plaque

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CO-AUTHORSHIP STATEMENT

This dissertation is a central component of a multi-disciplinary research program. Though the design of experiments, analysis of data, and the writing of chapters and manuscripts herein were initiated by me, this was done within the framework of the broader research program led by Drs. Scott Hinch, Tony Farrell, David Welch, and Steve Cooke, from each of whom I received considerable guidance. Accordingly, they are listed as co-authors of the manuscripts developed from Chapters 2-6. Likewise, those who provided additional expertise and methodological support, and most importantly field support, are also acknowledged as co-authors. Ultimately, responsibility for the quality of the analyses and the writing is my own.

Chapter 2: Behaviour and physiology of sockeye salmon homing through coastal waters to a natal river.

Authors: Glenn T. Crossin, Scott G. Hinch, Steven J. Cooke, David W. Welch, Sonia D. Batten, David A. Patterson, Glen Van Der Kraak, J. Mark Shrimpton, and Anthony P. Farrell

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Chapter 3: Baseline physiology of sockeye salmon intercepted on the high seas at an early stage of homeward migration, and the effects of gonadotropin-releasing hormone and testosterone on homing behaviour.

Authors: Glenn T. Crossin, Scott G. Hinch, David W. Welch, Steven J Cooke, David A. Patterson, Ulrike Klenke, Yonathan Zohar, Melinda Jacobs, Lucas B. Pon, Paul M. Winchell, and Anthony P. Farrell

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Authors: Glenn T. Crossin, Scott G. Hinch, Steven J. Cooke, Michael S. Cooperman, David A. Patterson, David W. Welch, Kyle C. Hanson, Ivan Olsson, Karl K. English, and Anthony P. Farrell

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CHAPTER 1

INTRODUCTION

The First Migration

The wise daughter of the chief had a dream. When she woke she cried and cried for it seemed the answer to her people's woes, but being young, she could not articulate her vision. Neither her father, who was a powerful chief, nor the wisest men of the tribe could help her. The little girl continued to cry. The chief was perplexed.

An old medicine man came to the chief. He spoke, "The Raven, who lives among the Cedar and the Hemlock, is my good friend. He is very wise and knows many things that the wisest among us know not. Let me bring him to the Council Fire, that he may counsel us".

The chief gave his permission, and the old medicine man set off deep into the forest. He returned later that evening with the Raven perched on his shoulder. The great bird croaked as he spoke, and only the wisest could follow his talk-trail…

"What the daughter of the chief has seen is a giant fish, known as Salmon. In this moon, they are to be found far from here at the mouth of the mighty river. Because those of your tribe are my friends, living here within the great sweeping arms of Tsuga, Tree Mother, I will fly swift and far to bring one of these fish to your village, and the people will flourish".

Before the chief could thank it, the black bird was high in the air. It flew far and fast, following the bends of the great river until at last it reached the sea. Its keen eyes saw, far below, many Salmon swimming together in shoals at the mouth of the river. The Raven dove quickly and, by chance, caught the little son of the Salmon Chief in his talons. Rising high in the air, with the fish held firmly in his claws, the Raven flew back to the distant village of his friends.

Salmon Scouts, leaping high from the water in great flashing arcs, saw the direction in which the Raven flew. A horde of Salmon, led by their chief, swam rapidly in pursuit. Speedily the fish swam upstream, but the fast-flying bird reached the village far ahead of them. The Raven cautioned that many Salmon would be sure to swim to their village, in pursuit of the Salmon chief's son. The village chief ordered his people to weave a huge net. This they did swiftly, and when the Salmon came, many were caught in the net and the people wept with joy. But in deference to the grieving Salmon, some fish were spared, and these swam off and searched the forest streams for the Salmon chief's son, but none could find him. In grief, they spawned in the shallows beneath the beseeching arms of Tsuga, in hopes that their young might one day continue the search and find him. And in sadness they died. But ever since, year after year, the Salmon return and continue their search, and year after year, the people celebrate…

…And so it was, at the dawn of ages, when, according to Haida mythology, the first salmon migrated up the virgin rivers of the Pacific Northwest in pursuit of their Chief's abducted son, and where in grief they first spawned beneath the sweeping arms of *Tsuga* and *Thuja*, the Hemlock and Cedar, the Tree Mother and the Tree of Life. And so it was that the seeds of our fascination with the migration of salmon were planted, a fascination that has woven its way like

vines, to this day, through lore, through art, through philosophy, through science. As with so much of Nature, the root of this fascination is difficult to voice, but the desire to understand the workings of the natural world, its intricacies and mechanisms, drives the passions of the writer, the artist, the theologian, the scientist.

In science, the first steps toward understanding often begin by defining the terms of that being studied. Regarding 'migration', one can find scores of different definitions in the scientific literature (see Dingle 1996). The one I find most instructive for the study of salmon was put forth by Endler (1977) in which migration was defined as "…the relatively long-distance movements made by large numbers of individuals in approximately the same direction at approximately the same time…usually followed by a regular return migration." I have adopted this definition because it is free of jargon, fairly direct, and because it conforms well to the life-history of salmon. It is the latter half of this definition, the return migration, that corresponds to the spawning migrations of salmon, and it is this specifically that is the focus of this dissertation in which I examine some of the physiological mechanisms and environmental factors that affect both the timing and success of migrations. The model focus of this work is the spawning migration of sockeye salmon (*Oncorhynchus nerka*, Walbaum in Artedi, 1871).

Background

At some point in their life-history, all animals must contend with physiological and behavioural constraint imposed by resource limitation (Stearns 1992). At such times, animals must balance the demands for metabolism, growth, and reproduction within the context of a limited energy budget (Calow 1985). There are two related life-history events during which energy is most severely taxed: migration and reproduction, events which for many of the world's temperate species often occur in tandem (Dingle 1996).

For animals powering reproductive migrations with finite somatic energy reserves (i.e. capital breeders), and for those having only one life-time opportunity to reproduce (i.e. semelparity), the timing of life-history transitions can have profound effects on fitness. For example, when animals initiate migrations, time and energy are diverted away from somatic growth and variously toward somatic storage, active and standard metabolism, and reproductive development. As the opportunities to migrate and reproduce commonly lie within a narrow phenological window, one in which environmental conditions are optimal, reproductive migration is at its core an energetic undertaking, one that necessitates an optimal synchronization of migration and reproduction timings (Alerstam and Lindström 1990; Dingle 1996; Prop et al.

2003). Time and energy have thus become strong determinants in the evolution of life history variation (Calow 1979; Hendry et al. 2004).

The accrual and deposition of somatic energy reserves are key to the mechanics of migration, and thus key to fitness. Critical too is an efficient metabolism for parsing this energy to tasks both central (e.g. gamete production) and peripheral (e.g. secondary sexual character development, courtship behaviour) to reproduction. In his book on the subject, Dingle (1996) notes that in preparation for migration, birds, fish, and amphibians commonly 1) accrue somatic fat, 2) re-structure enzyme systems via endocrine messengers to store and mobilize energy to various bins, and 3) modulate their behaviour along migratory and reproductive lines. Fat is the primary fuel used by most migrant animals, and its efficiency as a fuel source is well known. When a gram of fat is oxidized it yields 9 kilocalories of energy while the oxidation of carbohydrates and protein yield only about half this amount. Thus, long distance migrants like the Monarch butterfly (*Danaus plexippus*) deposit nearly 125% of their pre-migration mass as added fat in preparation for migration, and animals as varied as the aphid (*Aphis fabae*), the ruby-throated hummingbird (*Archilochus colubris*), and the sockeye salmon (*Oncorhynchus nerka*) can accrue >50% of their mature mass as fat (Burgner 1991).

Though energy is clearly important for powering migrations, the rate at which it is expended can be heavily influenced by rates of reproductive development. In sockeye salmon for example, it has been estimated that upwards of 50% of the somatic energy expended by females during upriver migration is directed toward egg production (Crossin et al. 2004), and rates of egg production are highly conserved, varying little interannually (Patterson et al. 2004). Thus, to understand patterns of energy utilization, it is important to view it in the context of reproductive development, which is controlled by endogenous rhythms.

The endocrinology of reproductive cycles and correlations with seasonal migrations

Reproductive migrations, which are directed movements, tend to be triggered by predictable environmental stimuli, the most common of which are associated with the change of seasons. Photoperiod is perhaps the most predictable of these stimuli, and many animals have evolved precise circannual rhythms to synchronize migratory and reproductive processes as well as other developmental phenomena (e.g. diapause in insects, smoltification in juvenile salmonids), to ensure that events occur at optimal ecological times. Reproductive cycles are under strict endocrine control, and many of the hormones involved in maturation (e.g.

gonadotropin-releasing hormone [GnRH], testosterone [T] and its derivatives) have pleiotropic effects on migratory behaviour (see Dingle 1996), indicating co-evolved functionalities.

Though there is considerable variation in the seasonal pattern of GnRH secretion among vertebrates, the mechanisms are relatively conserved. GnRH is a high-order neuropeptide which is released in pulses from nerve terminals in the median eminence located at the base of the hypothalamus. It is the first in a cascade of hormonal messengers that surge through the hypothalamo-pituitary-gonadal (HPG) axis that spur the production of gametes (Figure 1.2). GnRH is secreted from cell bodies in the pre-optic area of the hypothalamus (the POA) into blood portals that connect to the adjacent pituitary gland where it activates receptors in the anterior region to stimulate the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). In teleost fishes, GnRH nerve bodies directly enervate the anterior pituitary to stimulate gonadotropin release. The gonadotropins then enter general circulation and are detected at target organs. In females, FSH and LH bind to receptors in the thecal and granulosa cells of the ovary to stimulate testosterone production and eventual aromatization to 17β estradiol (E_2), the principal estrogenic hormone involved in vitellogenesis. Activation of LH receptors in the Leydig cells of the testes stimulates testosterone production in males. The mechanisms by which testosterone and its derivatives influence migratory behaviour are not known, but presumably they act on the central nervous system and brain.

Many studies have documented correlative links between GnRH surges and the initiation of reproductive migrations (birds- Dawson et al. 2001; salmon- Ueda et al. 2000). As mentioned above, photoperiod is a major stimulus shaping the evolution of migration tactics. In mammals, ambient photoperiod is perceived by retinal photo-receptors and harnessed to elicit an HPG activation via the suprachiasmatic nucleus (SCN). As its name indicates, this centre lies just above the area where the optic nerves running from the retinas cross (i.e. the optic chiasm) before joining the POA. It is also well established as the principal endogenous pacemaker that regulates circadian and circannual secretions of melatonin- a hormone that maintains biological rhythmicity by orchestrating seasonal changes in behaviour and physiology via SCN binding and its direct neural connections between the optic nerves, the POA and the pineal gland, (Gorman and Lee 2002). As a pacemaker, the pineal gland very predictably secretes melatonin at

Hypothalamo-pituitary-gonadal axis

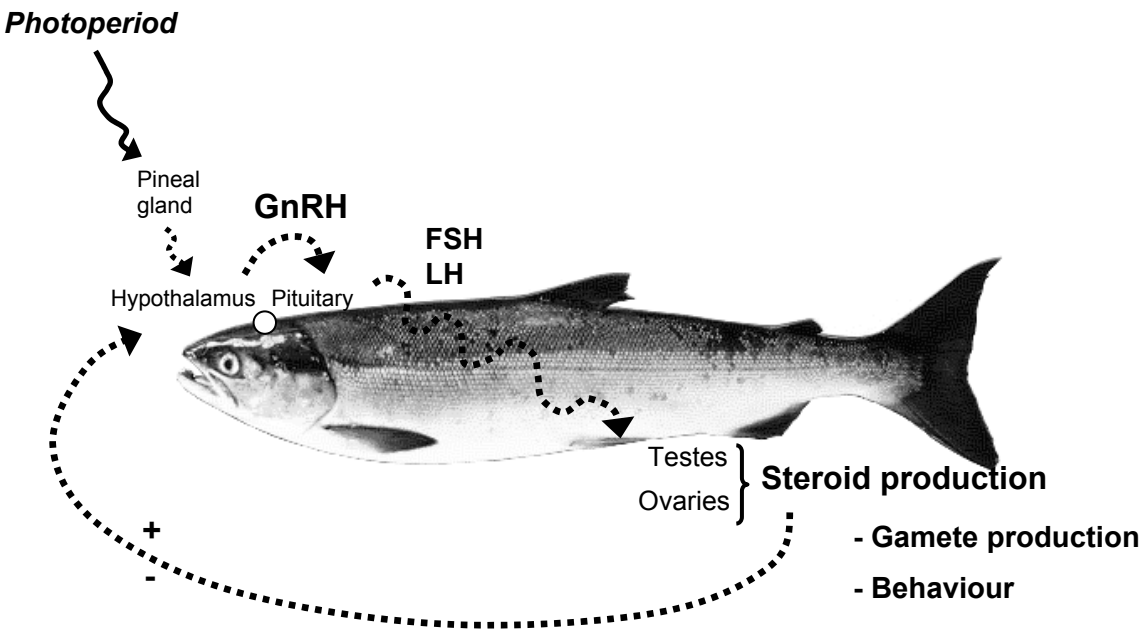

Figure 1.1: A sockeye salmon and the reproductive cascade along the HPG axis resulting from photoperiodicly induced surges of gonadotropin-releasing hormone. In salmon, increasing photoperiod decreases the night-time secretion of melatonin in the pineal gland via photoreceptor cells found in and near the pineal, thus providing a physiologic measure of the seasons that can be entrained. Seasonal increases in GnRH secretion ensue leading to the production of testosterone and its derivatives, and ultimately to gamete production. These sex steroids can exert pleiotropic effects on behaviours via central nervous system and brain centre binding. Steroidal feedback to the hypothalamus can either sustain or halt the release of the gonadotropins FSH and LH depending on species and relative stage of maturity.

night-time, but as it is neurally associated with the SCN, which enervates the optic nerve and the POA (where GnRH cell bodies lie), pineal secretion of melatonin can be modified by seasonal changes in photoperiod. To the best of our current knowledge, thus are the links between the SCN, the pineal gland and the hypothalamus that lead to the GnRH secretion and HPG axis activation that accompanies vernal migrations (review in Gorman and Lee 2002)

In non-mammalian vertebrates however, photoperiod is not entrained by way of SCN projection as in mammals. For example, in white crowned sparrows (*Zonotrichia leucophrys*) that were blinded with light-proof shields covering the eyes, gonadal growth proceeded normally

in response to normal daylight cycles (Yokoyama et al. 1978). However, shielding the brain from light brought gonadal development to a halt, even when their vision was restored. This suggested the presence of extra-retinal photoreceptors in the brain that were responsible for entraining photoperiodic information to the HPG axis. This was subsequently confirmed in many other birds, fishes, reptiles (reviewed by Foster and Soni 1998).

In some birds, the pineal gland itself contains light-sensitive cells that regulates melatonin secretion in response to light penetrating the skull (Takahashi et al. 1989), and in other birds and fish, the pineal gland and the eyes can function jointly as circadian pacemakers independently from the SCN. In salmon, the transmission of day-length to the hypothalamus and GnRH cell bodies is not fully understood, though it is likely to involve direct neural connections between extra-retinal photoreceptors in and around the pineal gland and the hypothalamus (Foster and Soni 1998). Recent studies are revealing *Oncorhynchus* as a unique among the genera of *Salmonidae* in that the secretion of melatonin in *Oncorhynchus* is not regulated by circadian rhythms but entirely by photoperiodic cues (Iigo et al. 1997, 2007). The evolutionary and ecological significance of this is unclear.

Though the precise physiologic pathways that regulate the seasonal activation of the HPG axes of mammals and non-mammals have yet to be fully elucidated, it seems that animals use the highly rhythmic secretions of melatonin in the pineal gland as an endogenous, hormonal representation of day-length, or more precisely, night-length. As both day- and night-length reflect the seasons of the year (and geographic latitudes), decreasing melatonin levels in spring can stimulate GnRH cell bodies into reproductive modes, thus allowing animals to adapt their physiology and behaviour to seasonally changing selective pressures (see Dingle et al. 1996).

Animals in the wild however animals appear to use various cues to 'fine-tune' the timing and extent of migration and reproduction relative to current conditions (Wingfield 1983). Animals may use two different cues to phase these relationships (Wingfield 1983; Wingfield and Kenagy 1991). The first uses predictive signals to initiate reproductive cycles in advance of the changing season (i.e. photoperiod). The second integrates information about the immediate environment as it relates to their own condition (i.e. food and temperature), which can either accelerate or decelerate gonadal development. For 'all-or-nothing' breeders like Pacific salmon, the response is to either initiate reproduction or defer until the next year if critical size and energy thresholds are not being met, though this later strategy is risky and most sockeye for example mature, migrate and spawn at 4 years of age (>95%) rather than 5 years (<5%) (Burgner 1991).

Once reproductive migrations have been initiated, migratory behaviour appears to be strongly influenced by reproductive physiology. Many researchers have sought to understand the role of reproductive hormones on such behaviours. Rowan (1925) showed experimentally that changes in photoperiod and castration could induce an out-of-season migratory behaviour in dark-eyed juncos (*Junco hyemalis*) and crows (*Corvus brachyrhynchos*). Similarly, the removal of gonads in sparrows (*Zonotrichia* spp.), which reduced sex steroid production, eliminated the pre-migratory deposition of somatic energy (i.e. lipid) reserves usually observed in preparation for spring migration, and made the birds sedate whereas they would normally be restless (Wingfield et al. 1990a), a behaviour linked to testosterone (Wingfield et al. 1990b). Interestingly, when this experiment was repeated prior to autumnal migration, which is not associated with reproduction, there was no effect of castration on pre-migratory physiology or behaviour, suggesting mechanisms other than steroid production in the regulation of migratory behaviour during non-breeding seasons (Wingfield et al. 1990a). In salmon, injections of a GnRH analog (GnRHa), which lead to testosterone production, lead to an increase in the number of times a mature adult sockeye would attempt to leap over a waterfall (Plate et al. 2003). GnRHa also lead to an increase in upstream swimming speeds and homing rates in adult salmon experimentally treated in freshwater (Andrew Dittman, Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, WA, unpublished data.; Sato et al. 1997).

Once environmental stimuli have been perceived and transduced into the HPG axis to activate reproductive development and migratory behaviour, somatic energy becomes an important fitness related trait for sustaining migratory locomotion, gamete production, secondary sexual characteristic development, mating, and parental care. Scores of studies have described the importance of endogenous and exogenous energy sources to the success of migration and reproduction (see overview in Dingle 1996; for sockeye salmon see Crossin et al. 2004). Animals must complete these tasks while buffering against environmental unpredictability (e.g. weather, temperature), and against other risks experienced en route to breeding areas like predation.

This dissertation thus has two general aims. The first is to characterize the behavioural physiology of a reproductive migration across a broad landscape. Using physiological sampling methods and bio-telemetry, I examined the reproductive, homeostatic and stress physiologies of over 1000 sockeye salmon and explored mechanistic links to migratory behaviour. Salmon were sampled and tracked over the course of migrations, from their onset in the North Pacific Ocean

to their conclusion in the lakes and streams throughout the Fraser River watershed- a spatial scale covering nearly 2000 km. The principal focus of my analyses is to understand the physiological bases of river entry timing, and how ocean physiology and entry timing can collectively influence the success of upriver migrations. Given their potent effect on migratory behaviour, the sex steroids will be examined, but given the inherently stressful nature of migrations, I will examine the modulating influence of stress physiology on reproductive and behavioural patterns.

Given the highly adaptive nature of migration timing, the second aim is to examine the consequences associated with migrations occurring outside of historic norms. Particular attention will be paid to the environmental factors that might not be otherwise encountered when salmon migrate abnormally, maladaptively early. For example, mean summer-time temperatures in the Fraser River have been getting progressively warmer over the last 20+ years (see Patterson et al. 2007). All salmon have a range of temperatures over which they function optimally, but when water temperatures rise, standard and active metabolism increase non-linearly such that progressively higher temperatures shift aerobic performance from the optimal thermal-neutral zone into the less-optimal range where swift drops in aerobic scope can occur. If temperatures go too high, aerobic scope crashes and cardiac collapse occurs (Farrell 2002). In the wild, a suite of other thermally related complications can afflict migrating salmon, like an increased susceptibility to water-borne diseases. I will examine how the progressive warming of the Fraser River (Patterson et al. 2007), coupled with the early migration phenomenon, influences the survival of salmon migrating through the Fraser River. To set the context, I will provide a brief overview of the life-history of Pacific salmon, and an overview of a recent, seemingly maladaptive change in migration timing afflicting sockeye salmon in the Fraser River in British Columbia Canada.

A brief overview of the life-history of migrating adult Pacific salmon

Several aspects of the life-history of sockeye are useful for examining the links between physiology and behaviour during spawning migrations. First, like all Pacific salmon, they undertake exceptional migrations from the open North Pacific Ocean to distant freshwater spawning locales scattered throughout the Pacific Northwest and eastern Asia (Figure 1.3), and they do so without the benefit of exogenous energy sources. Energetic and physiological patterns during migration, and the associated changes in behaviour, can thus be examined

without having to account for newly consumed energy. Second, their migrations are generally predictable. Because they support valuable fisheries, managers and fisheries scientists keep

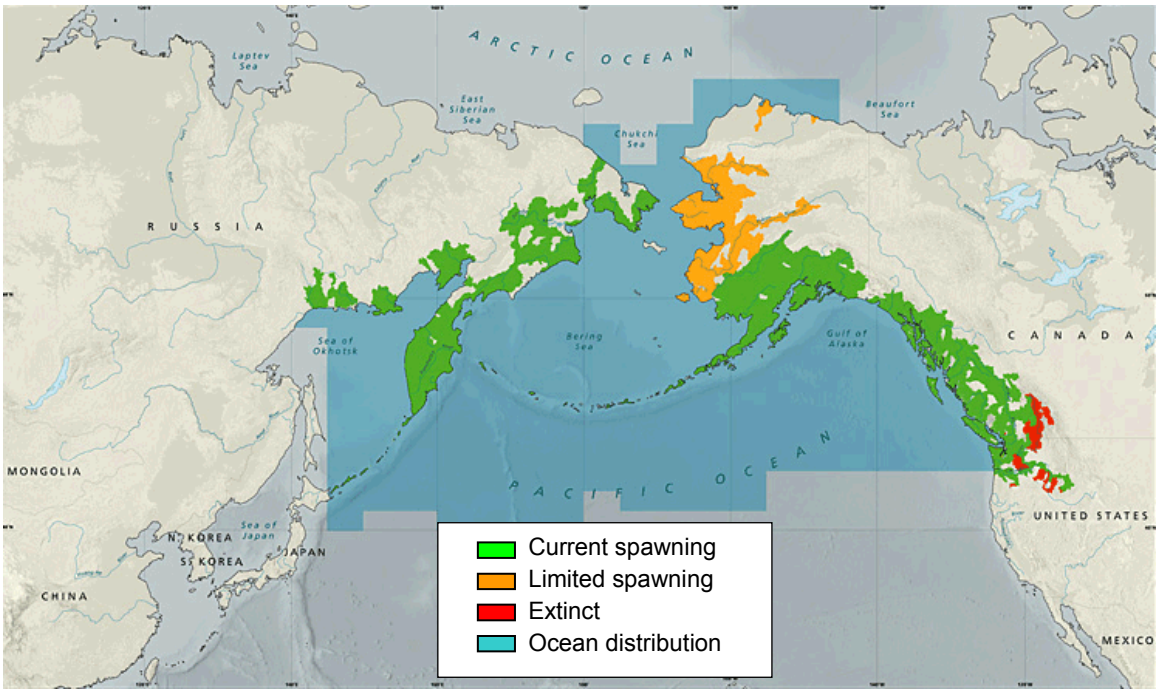

Figure 1.2: Map of the present spawning distributions of sockeye salmon throughout the Pacific rim (Image reproduced with permission from the State of the Salmon program, Wild Salmon Center, Portland, Oregon).

careful records of sockeye return river-entry timings, which for a given population typically varies by only a week around historical averages (i.e. 25 years; Cooke et al. 2004). Third, sockeye are semelparous, investing all of their energy into a single homeward, migratory, reproductive event.

The mechanisms triggering the homeward migration of sockeye salmon, and indeed all Pacific salmon, is a topic not very well understood (reviewed in Hinch et al. 2006), but recent research has found it to be closely linked to the initiation of gonadal maturation. Ueda et al. (2000) found experimentally that gonadotropin releasing hormone (GnRH) stimulates gonadal maturation, and that its onset corresponds with a shift from open ocean foraging to homeward migration. The environmental trigger for GnRH secretion in salmon has not been studied, but given the high conservatism of the mechanisms of GnRH secretion across taxa, photoperiod is likely the key factor (Quinn and Adams 1996). Other factors however may have the potential to override photoperiod as a stimulus. For example, salmon that fail to reach a minimum size and

energy threshold at maturity will opt for an additional year of growth at sea before initiating spawning migrations (Burgner 1991), a seemingly indispensable insurance policy for an animal that has a fixed energy budget and has only one lifetime opportunity to reproduce.

Once sockeye enter continental shelf waters and approach the estuaries of natal rivers, they must restructure their osmoregulatory physiology in preparation for freshwater entry. Based on samples taken from maturing sockeye intercepted 700 km and 250 km away from the mouth of the Fraser River (Hinch et al. 2006, Cooke et al. 2006), it is clear that fish are preparing for this transition well before their arrival at river mouths. Gill Na^+,K^+-ATPase activity (the principle enzyme facilitating ionic homeostasis whilst traversing salinity gradients) is lower at 250 km than at 700 km away yet both locales are high salinity areas. Also, sex steroid levels suggest that reproductive development is also well underway at both locations. For example, serum 17β estradiol (E_2) increases from 2.5 to 5 $ng \cdot ml^{-1}$ as salmon approach the natal estuary and remain at this level when the fish enter the river. Gonado-somatic index also increases, indicating that vitellogenesis is active (as per Ueda et al. 2000).

The timing of freshwater entry in sockeye is a highly conserved life-history characteristic, one that shows strong associations with temperature. Run-timing is but one link in an adaptive chain of traits designed to minimize exposure to thermal stress and difficult flow regimes en route to spawning areas (Hodgson and Quinn 2002). Because run-times are highly adaptive, significant deviations from historic norms may reduce fitness but this has not been tested empirically. Once migration has been initiated, salmon are 'swimming' against a biological clock and have a limited amount of time and energy with which to find natal streams and spawn before their inevitable death. Once the reproductive rhythm has been initiated, egg production appears to progress along a fixed trajectory that cannot be reversed (Patterson et al. 2004).

One of the central principles of evolutionary biology is that life-history traits cannot evolve independently from one another but in fact are embedded in a matrix of correlations (Stearns 1992). These correlations are known as trade-offs and reveal the constraints that shape the evolution of life-history variation (Reznick 1992; Rose and Bradley 1998; Zera and Harshman 2001). The most common hypothesis explaining the negative correlations observed among traits centres around the allocation of limited energy resources to competing functions, especially during migration. Reproductive trade-offs during migration have been documented in many animals (Calow 1985; Stearns 1992), including salmon (Hendry and Berg 1999; Kinnison et al. 2001; Crossin et al. 2004). These generally occur as a direct result of energy limitation and

typically involve some reduction in reproductive output like egg size and number (Smith and Fretwell 1974; Fleming and Gross 1990). The magnitude and scope of trade-offs depend on the strategies and tactics employed during migration and reproduction. Fish that reproduce more than once in their lifetime are known to sacrifice reproductive effort for characteristics that benefit future reproductive events either directly (e.g. future reproduction [Gustafsson and Part 1990], and future survival [Hutchings 1994; Clutton-Brock et al. 1982]), or indirectly (e.g. somatic growth [Wootton 1994; Jokela and Mutikainen 1995]). But for semelparous sockeye, egg size and number, secondary sexual characteristics, and body size are all sacrificed in favour of somatic energy storage and metabolic fuel efficiency when upriver migrations are long and arduous (Hendry and Berg 1999; Kinnison et al. 2001; Lee et al. 2003; Crossin et al. 2004).

Fraser River sockeye salmon and the recent problem of early migrations

The Fraser River drains approximately one third of the province of British Columbia and is the single, largest producer of wild salmon in Canada. Of its five salmon species, sockeye is the most commercially valued and the second most numerically abundant. Over 150 stocks of sockeye have been identified and these spawn throughout the watershed in locales ranging from 80 to 1200 km from the ocean (Gilhousen 1990). Stocks, and populations within stocks, vary in their adult morphology, energetics, fecundity, and spawning abundance (100s to 1,000,000s) (Crossin et al. 2004). Up-river adult spawning migrations commence in July with 'Early-run stocks'. 'Summer-run stocks' then follow in August, and 'Late-run stocks' enter in September and October (Woodey 1987). Late-run and Summer-run stocks reach the Fraser River from the ocean at the same time, but Late-run stocks usually remain in a holding behaviour in the estuary for several weeks before initiating up-river migration. However, since 1996, large segments of the Late-run stocks (including the world famous Adams River stock) arrived in the estuary at the normal time but commenced their river migration three-six weeks earlier than normal (Cooke et al. 2004), at the same time as the summer-runs. Associated with this abnormal behaviour has been extraordinarily high river migration mortality (up to 90-96% in some years, at least 50-60% in each year; Cooke et al. 2004). In contrast, prior to 1995, *total* freshwater mortality for Late-run stocks rarely exceeded 20%. This 'early' migration behaviour has also been observed in other salmon species including pink (*O. gorbuscha*), chum (*O. keta*) and chinook (*O. tshawytscha*) indicating that the phenomenon is not restricted to sockeye, however, data on these other species are limited and it is not known whether river mortality rates are elevated in these other species (M. Lapointe, Pacific Salmon Commission, pers. comm.).

Since this early migration phenomenon began, the total number of adult Late-run sockeye starting up-river migrations has ranged among years from ~ 75,000 to 6,300,000 with an annual average of ~ 1,500,000; from 1996 to present in excess of 4,000,000 adults have started migrations but not reached spawning grounds (Pacific Salmon Commission, unpub. data.). The costs to the fishery of this abnormal behaviour and ensuing premature mortality have been substantial. Not only have present day catches and future production of Late-run stocks been reduced, catches of summer-run sockeye, which co-migrate with the Late-run stocks, have also been restricted to minimize incidental by-catch of Late-run stocks (M. Lapointe, Pacific Salmon Commission, pers. comm.). These exceptionally high mortality rates constitute a severe conservation crisis and put the sustainability of Fraser sockeye and their fisheries in doubt. Biological extirpation is an imminent possibility for some stocks. Cultus Lake sockeye, one of the smallest Late-run stocks, was recently listed as 'endangered' by the Committee on the Status of Endangered Wildlife in Canada.

Coincident with early migration and high mortality of Late-run sockeye salmon is a high rate of *Parvicapsula minibicornis* infection. This freshwater kidney parasite infects all adult salmon when they migrate into the Fraser River (Jones et al. 2003). It has been speculated that early migrations, which increase freshwater residence times, may allow this and other parasites to inflict lethal damage (e.g. renal dysfunction; Wagner et al. 2005) whereas previously, sockeye would spawn before parasite expression reached lethal levels. Early migrating fish that survive the migration still spawn at about the same time as normally timed migrants (Wagner et al. 2005). Early migration also exposes salmon to thermal regimes to which they are not adapted. Research on swimming performance and oxygen consumption rates in sockeye salmon has yielded stock-specific optima at temperatures that correspond to mean temperatures encountered historically during their upstream migrations (Lee et al. 2003). Exposure to temperature regimes above their thermal optima, which occurs when Late-run sockeye salmon migrate earlier than usual into the Fraser River, would increase metabolic stress and rates of somatic energy expenditure. Bioenergetic modeling exercises have identified temperature to be the principle driver of energy exhaustion in migrant sockeye salmon during years of high flows and slowed passage (Hinch and Rand 1998). Typically, organisms can tolerate deviations around their thermal optima. Extreme changes however can induce high levels of stress, and potentially put at risk a suite of other metabolic, endocrine, and behavioural processes related to fitness. For example, temperatures encountered in 2004 by early-timed Weaver Creek sockeye, a Late-run stock, were high enough that stock-specific metabolic scope was reduced to zero during portions

of the run, accounting for at least 30% of the migration mortality of that stock that year (Farrell et al. 2008). When environmental conditions deviate significantly from historical averages, one should expect behavioural and physiological changes to occur in migrating animals in response to those deviations. The severity of the environmental change and the response of the animal in question will determine the effect, if any, on fitness.

The Fraser River has seen a significant departure in summer temperatures and flow regimes in recent years, changes that have occurred simultaneously with the observed change in sockeye migration behaviour. Specifically, the Fraser River has experienced a >1°C average increase in peak summer water temperature over the past 40 years with eight of the past 10 summers being the warmest on record (Patterson et al. 2007). In two recent extreme temperature years, 1998 and 2004, ~5 million adult Fraser River sockeye across all stocks disappeared during upstream migration and high temperatures were implicated as a principle factor (Macdonald et al. 2000; Williams 2005). Because of the abnormally early-timed migrations and a warming Fraser River, Late-run sockeye now experience temperatures 4-5°C higher than they did historically. Most Late-run sockeye also now encounter river temperatures >19°C during a portion of upstream migration. Such a temperature encounter has profound evolutionary significance because no sockeye stock anywhere in the world is known to have initiated river migration at 20°C (Hodgson and Quinn 2002).

Though constituting a conservation crisis, this early migration and high mortality phenomena offers a unique opportunity to study the mechanisms driving Pacific salmon migrations, particularly the endocrine and metabolic mechanisms that underlie behaviour, and the controlling effect of temperature. By examining individuals embarking on early and later migrations, and measuring different indicators of migratory 'preparedness' (e.g. energetic, hormonal, and metabolic variables), one can examine on an individual basis the correlations between timing and migration behaviour, physiology, fate, and fitness, and relate findings to environmental conditions encountered en route.

Thesis objectives and overview of subsequent chapters

There are two main objectives of this dissertation. The first was to describe the physiological mechanisms that underlie variation in river entry timing by homing sockeye salmon. A wide body of literature has examined the role of reproductive hormones on various behaviours exhibited by migrant animals. My goal was to characterize patterns of steroid hormone secretion (testosterone, 17β estradiol) in relation to river entry timing and rates of

migration in general by adult sockeye. However, other physiological factors like stress and energetics can exert a modulating influence the timing of river entry. Accordingly, patterns were also examined relative to homeostatic (e.g. plasma ions) and stress (lactate, glucose, cortisol) physiologies, and energetics (gross somatic energy).

The second objective was to examine the consequences associated with variation in river entry timing with regard to environmental conditions encountered in river. River temperature is one of the most variable environmental conditions encountered annually by homing salmon (Hodgson and Quinn 2002). Water temperatures encountered by migrating sockeye in the Fraser River can vary from 12 to 22 ºC (about a 2-fold change), and salmon populations may have adaptive thermal performance optima to match the prevailing temperature regime encountered during 'normal' migration times (Lee et al. 2003). The effects of temperature on fish physiology and behaviour are well known, and temperature is universally regarded as the master factor affecting all levels of biological organization in aquatic organisms (Magnusson et al. 1979). Given its profound influence on the evolution of river migration timing in Pacific salmon, and its influences on fish physiology, I will examine the role of river temperature on migration success.

In Chapter 2, I report the results of a survey study conducted in 2003 to examine baseline measures of reproductive (e.g. sex steroid levels), homeostatic (e.g. plasma ions), and stress (e.g. lactate, glucose, cortisol) physiology from sockeye captured during their coastal migration, ~250 km from the Fraser River. I used acoustic biopsy telemetry to relate physiological condition of individuals at capture to their subsequent migratory behaviour and fate during their approach to and entry into freshwater. This descriptive study identifies physiological factors which seem to influence timing and behaviour of river entry and sets the stage for subsequent research.

In Chapter 3, I report the results of a survey study conducted in 2006 which describes for the first time the baseline physiologies of adult sockeye captured near the start of their directed homeward migration in the high seas. Very little is know about the oceanic life-history of salmon, due mostly to the difficulty of studying salmon in the high seas, and virtually nothing is known about their ocean physiology. When salmon enter natal rivers, they exhibit a considerable amount of inter-population life-history variation in morphology and physiology, which is due to energetic constraints imposed by migration. This study examines whether this variation is detectable in fish near the beginning of homeward migrations when energy is not yet limiting. I also conducted an experiment with these sockeye after their baseline physiologies were examined. Sockeye were injected with time-released microspheres of GnRH administered either alone or in combination with testosterone, and relative to control and sham-injected

sockeye, I assessed travel times to reach the Fraser River using acoustic telemetry to test the hypothesis that reproductive development was the driving factor of migratory rates.

In Chapter 4, I report the results of a laboratory experiment from 2006 involving sub-adult pink salmon to examine the effects of exogenous implants of GnRH either alone or in combination with testosterone on reproductive and osmoregulatory physiology, and on survival. This study was conducted for two reasons. First was to provide a physiological point of reference from which to interpret the results of the hormonal-migration telemetry experiment conducted in the previous chapter (Chapter 3). Secondly, and more generally, I sought to glean insights to the synergistic effects of hormonal co-treatment on reproductive development via HPG activation but also to examine potential GnRH activation of the HPA axis and potential effects on osmoregulatory ability.

As stated in Chapter 2, the acoustic receiver array used to monitor sockeye movements was situated only in the Fraser River estuary extending to a locale only 80 km up river. This limitation in study design meant that large spatial gaps existed in the 2003 study, thus I could not assess the behaviour and fate of fish, or their physiological correlates, during most of their post-release coastal migration and their river migrations to spawning areas. In Chapter 5, I report the results of a physiological telemetry study on >500 sockeye, which is nearly three times as many telemetered in Chapter 2. Chapter 5 examines how initial physiology influences the timing of river entry, and how baseline physiology correlates with the ultimate success of migration.

In Chapter 6, I report on a study conducted in 2004 where I experimentally tested the consequences of altered migration timing on migration success by altering one of the key environmental variables associated with altered timing of adult migrants - thermal experience. Late-run sockeye which enter the Fraser River at historically normal times generally spawn and die before accruing ~500 freshwater degree days (DD). Most abnormally early migrants accumulate significantly higher DD totals. I captured normal-timed migrants, brought them to a laboratory, exposed them to one of two thermal regimes, and released them after 3 weeks to continue their migration. This is the first study to experimentally text the hypothesis that early migrants exposed to modestly high thermal conditions will suffer significantly higher levels of migration mortality.

In Chapter 7, I summarize the key results from this dissertation, highlight some of the physiological trends that occur as salmon migrate homeward to spawning areas. I also speculate on the greater relevance of my results and how they might be used to gain ultimate insights to the

Late-run sockeye problem. I end with a discussion of the effects of human handling on fish behaviour and survival.

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CHAPTER 2

Behaviour and physiology of sockeye salmon homing through coastal waters to a natal river.[1]

Introduction

The seasonal migrations of adult Pacific salmon (*Oncorhynchus* spp.) from the North Pacific Ocean to distant freshwater spawning locales rank among the most dramatic displays of behaviour in the animal kingdom. Yet, despite years of study, our understanding of the cues and mechanisms guiding ocean salmon to natal rivers is rudimentary (reviewed by Hinch et al. 2006). The key stimulus initiating homeward migration and maturation appears to be a change in photoperiod which is perceived by salmon and which activates the hypothalamic-pituitary-gonadal axis, thus setting reproductive development in motion (Ueda and Yamauchi 1995; Ueda et al. 2000). Additional endocrine, visual, and chemosensory systems likely assist them in navigating coastal margins to the mouths of natal rivers (Quinn 1980; Quinn et al. 1989).

Upon arrival at the natal river mouth, the timing of river entry is an important life-history decision made by salmon that is both species and population (or stock) specific. Ultimately, entry timing affects when fish arrive on the spawning grounds and dates of spawning, as well as subsequent rates of embryonic and fry development (Burgner 1991). Timing is also influenced by river temperatures and discharge rates, the distance to and elevation of spawning grounds, and the physical characteristics of the spawning environment (Gilhousen 1990, Brett 1995; Hodgson and Quinn 2002; Lee et al. 2003). For any given salmon species, and population within species, interannual variations in entry timing are usually small. For example, timing by populations of sockeye salmon (*O. nerka*) rarely deviate by more than a week interannually (Cooke et al. 2004), though there has been a recent, unprecedented change in entry timing of some populations returning to the Fraser River in British Columbia which has pushed entry dates far beyond baseline levels of variation.

Since 1995, as many as 60-90% of the individuals from populations entering the Fraser River in late summer (classified as the 'Late-run stock group' by fisheries managers) have advanced their entry date by as much as eight weeks from historic averages. Given the highly adaptive nature of river-entry timing by homing salmon, and to migrant animals in general, such

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behaviour is highly anomalous and negative fitness consequences are expected (Dingle 1996; Bêty et al. 2004; Cooke et al. 2004). Indeed, over four million Late-run sockeye have perished *en route* to spawning grounds since changes in river-entry timing were first detected over a decade ago, thus constituting a conservation crisis. By migrating early, sockeye enter the Fraser River at a time when temperatures are generally warmer than those historically encountered by the population and to which various phenotypes have adapted (Lee et al. 2003). Such exposures exert physiologic stress and accelerate rates of parasite disease development, which can alter performance and place salmon at considerable risk (Chapter 6- Crossin et al. 2008).

Efforts to characterize the physiological mechanisms underlying this recent change in entry timing require information gleaned from individual homing fish. My supervisor's research group (Professor Scott G. Hinch) has developed protocols for the non-lethal biopsy of salmon which, when coupled with telemetry, provide mechanistic insights to migration behaviour. Despite recent evaluations that have characterized potential mechanisms of mortality in river-migrating sockeye (Cooke et al. 2006a,b; Young et al. 2006), the endogenous mechanisms affecting their behaviour in coastal areas and the timing of river entry remain poorly understood (Hinch et al. 2006). Therefore, I examined whether energetic condition, reproductive state, and osmoregulatory preparedness were key factors affecting the decision of homing sockeye to leave the marine environment and initiate upriver migrations.

For Pacific salmon, which are semelparous animals relying on fixed endogenous energy reserves to power migration and reproduction, time and energy are of fundamental importance to the successful completion of upriver migration and thus are key to fitness (Brett 1995; Hendry and Berg 1999; Hendry et al. 2004; Crossin et al. 2004). There is a wide body of literature addressing the adaptive significance of somatic energy to homing salmon (Brett 1995; Hendry and Berg 1999; Kinnison et al. 2001; Crossin et al. 2004). There is also the recognition of a broad, direct relationship between migration timing and seasonal increases in circulating sex steroids in migrant animals (Dingle 1996). Previous work has shown that senescing sockeye with low somatic energy and high levels of circulating sex steroids tend to initiate upriver migrations earlier than less reproductively mature individuals, and often die *en route* to spawning grounds as a result (Cooke et al. 2006a,b; Young et al. 2006). Building from these studies, I used telemetric and physiological techniques to examine the mechanisms underlying individual behaviours and entry timing.

Homing salmon were intercepted from six major spawning populations approximately 215 km from the mouth to the Fraser River and non-lethally sampled to assess their energetic

and osmoregulatory states, and blood biochemistry (see Cooke et al. 2005). Salmon were then fitted with acoustic transmitters and released, allowing us to monitor individual and population specific behaviours as fish homed through the coastal marine environment and into the Fraser River. I examined correlations between these physiologic variables and observed behaviours (migratory path and timing), and predicted that salmon entering the river early would (1) have lower somatic energy levels, and (2) higher circulating levels of reproductive steroid hormones relative to individuals entering later. Because homing salmon must remodel their gill architecture to maintain ionic balance when moving from salt to freshwater, I also predicted that early entering individuals would have (3) lower gill Na^+,K^+-ATPase levels.

Methods and Materials

Biopsy and tagging techniques

On August 20-22 and August 26-27 of 2003, sockeye salmon (N=178) were captured by 55 ft purse seine vessel near Brown's Bay, at the southern end of Johnstone Strait, British Columbia (Figure 2.1), following methods detailed in Cooke et al. (2006a,b). This vessel also served as the platform for fish biopsy and acoustic transmitter implantation. Surface water temperatures at time of capture and release were 10-13 °C. Upon completion of each seine set, the purse seine was brought along the starboard rail of the vessel, and while still in the water, individual salmon were dipnetted and transferred to a large flow-through holding tank on boat's deck. Approximately 10-12 fish were taken from each seine set, and once onboard, were processed sequentially and released as quickly as possible (ranging from 5-50 min). In the collection of physiological samples, I used protocols for the non-lethal, unanaesthetized sampling of sockeye salmon (see Cooke et al. 2005) that were pre-approved by the University of British Columbia Animal Care Committee in accordance with the Canadian Council of Animal Care.

Individual salmon were removed from the holding tank and placed ventral side up in a padded V-shaped trough that was provided with a continuous supply of ambient seawater from a tube positioned near the salmon's head. Two people restrained the salmon while a third collected the biopsy. Typically, fish were confined to the trough for less than three minutes during which time nose-to-fork length (FL, cm) was measured, tissues were biopsied and an acoustic transmitter inserted. Biopsies included the removal of (a) a 0.5 g clip of adipose fin for DNA stock identification, (b) a 3 mL blood sample from the caudal vein (using a 1.5", 21 gauge vacutainer syringe; Houston 1990) for assessing plasma chemistry, and (c) a < 4 mm clip of six

to eight gill filament tips (~0.03 g) along the first gill arch (McCormick 1993) for assessing gill Na^+,K^+-ATPase activity. Gill tissue and centrifuged plasma samples were stored on dry ice for several hours to days until transfer to a –86 ˚C freezer. A hand-held micro-wave energy meter (Distell Fish Fatmeter model 692, Distell Inc, West Lothian, Scotland, UK) was placed on the left side of the fish in two locations to quantify gross somatic energy levels (GSE, $MJ \cdot kg^{-1}$) (Crossin and Hinch 2005). Vemco V16-H3 acoustic transmitters (16 mm diameter, 51 mm length, 25.0 g in air; Vemco Inc., Shad Bay, NS, Canada) were pushed down the esophagus into the stomach with a plastic applicator. Fish were immediately returned over the side of the boat and monitored until they swam away. Fish were on-board for less than one hour. The release site was approximately 215 km from the mouth of the Fraser River.

Laboratory assays

Population identity was determined for individual fish using both DNA and scale analyses (Beacham et al. 1995, 2004). DNA correctly assigned baseline populations to lake of origin about 80% of the time (Beacham et al. 2004), and a parallel radio telemetry study conducted in 2003 found that DNA correctly classified fish to the Stuart Stellako, Chilko/Quesnel, and Late-run stock groups about 94% of the time based on where radio tagged fish were last detected in spawning locations (English et al. 2004). Numbers tagged for each population and tagging details are presented in Table 2.1.

Plasma testosterone (T) and 17β-estradiol (E_2) levels were measured by radioimmunoassay (McMaster et al. 1992) and used to assign fish sex as secondary sexual characteristic were not fully expressed at that point of migration. Sex was assigned by plotting T against E_2 which generates two distinct clusters. Plasma ion (K^+, Cl^-, Na^+), glucose, lactate, and osmolality were quantified by procedures described by Farrell et al. (2001). Gill tissue Na^+,K^+-ATPase activity was determined by kinetic assay (McCormick 1993).

Acoustic receiver array and transmitters

Acoustic receivers were placed in an array on the seabed of the Strait of Georgia (SOG) on 11-13 August, 2003 (see Welch et al. 2006). In total, 21 Vemco VR-2 receivers were deployed in four lines extending off the mouth of Fraser River (Figure 2.1). VR-2 receivers are internally recording data loggers, which record the tag ID code (serial number) and the date and time that an acoustic tag is detected. The array of receivers extended north to south 36 km, and each line extended 7-9 km out into the Strait from the near-shore, creating a rough grid of approximately 250 km^2. The receiver lines were positioned to cover the four main entrances to the Fraser River. Three additional VR-2s were positioned at the Pitt River confluence, Albion,

and Mission, BC, which are 43, 65, and 83 km respectively upriver from the mouth. The later most receivers at Mission lie at the tidal boundary; thus fish moving beyond this move into fully fresh and non-tidally influenced river water. With the tags used in this study, the VR-2s could detect a fish passing within approximately 1 km under ideal conditions, though the achieved range varies with environmental conditions.

For analytical purposes, I divided the movements of fish from Browns Bay to Mission into three distinct migratory segments and examined travel or holding times in each. Specifically, I determined four population-specific travel times: (1) travel time from Brown's Bay to first detection on the SOG array at the Fraser River mouth (segment 1), (2) holding time (first to last detection) in the SOG array (segment 2) which was used as a surrogate for holding time, and (3) travel time from the Pitt River confluence to Mission, BC (segment 3) (see Figure 1 and 4). Using segment distances I calculated migration travel rates. By examining the number and temporal sequence of VR-2 receivers visited by homing sockeye, I estimated the migration trajectories of all fish through the SOG array so that I could better visualize movement patterns. Graphic representation of these patterns were generated for individuals from two Late-run (Adams and Weaver) and two Summer-run (Chilko and Horsefly) populations. These four individuals were deemed representative in that the number of VR-2s that each visited was similar to the mean number visited by their population.

Statistical analyses

All physiological data were log(10)-transformed to reduce heteroscedasticity. As only half of the fish in this study were physiologically biopsied, I assessed whether the physiological sampling methodology exerted any deleterious affects on salmon behaviour and survival by contrasting travel times and survivorship (measured as a detection at the last in-river VR-2 station at Mission) from the point of release to Mission, between biopsied and non-biopsied fish using analysis of variance (ANOVA) and a Chi square contingency table analysis, respectively. I also used Chi square to examine whether the number of ocean losses was distributed evenly across the six main populations, or whether particular populations were more heavily impacted. I examined for any broad physiologic differences (variables: GSE, Na^+, K^+, Cl^-, osmolality, lactate, glucose, and gill Na^+,K^+-ATPase) between survivors and ocean losses using multivariate analysis of variance (MANOVA). Sexes were pooled for these analyses.

To explore physiological differences among populations at time of capture, MANOVAs were conducted on sex-specific data. Variables included in the models were: GSE, FL, Na^+, K^+, Cl^-, osmolality, lactate, glucose, gill Na^+,K^+-ATPase, T, and E_2 (females only). To identify the

relative importance of individual physiological variables underlying multivariate relationships, I conducted a series of 2-way analyses of variance (ANOVA) with population and sex as main effects, and their interaction. Three variables were sex-specific (female plasma 17β-estradiol, male plasma testosterone, female plasma testosterone) so only one-way ANOVA could be used for those. *A posteriori* tests were used to identify populations that differed when the ANOVA model was significant. To assess sex- and population-specific relationships between travel times (from point of release to specific locations) and physiological variables, I conducted a series of correlation analyses. Where correlations were significant, I used linear regression to describe relationships between the physiological factor (the dependent variable) and travel time of individuals (the independent variable) and used analysis of covariance (ANCOVA) to assess whether these relationships differed between sexes or among populations (the class variables).

All analyses were conducted using JMP 4.0 (SAS Institute, Cary, NC, USA). Because of multiple comparisons, I conducted Bonferroni corrections to minimize the potential for Type II errors (Rice 1989). I designated statistical significance at $\propto = 0.05$ and made Bonferroni corrections to minimize the possibility of false positives. However, due to the high conservatism of Bonferroni corrections (see Cabin and Mitchell 2000), I indicate significance at the $\alpha = 0.05$, $\alpha = 0.01$, and $\alpha = 0.001$ levels (See Tables 1 and 2), thus allowing readers to define for themselves which levels are most biologically meaningful (as per Cabin and Mitchell 2000).

Results

Six major Fraser River populations were identified from DNA analyses: Chilko, Horsefly, Stellako, Adams, Weaver, and Birkenhead. Chilko, Horsefly, and Stellako enter the Fraser River immediately after arrival from early August through September and are classified as 'Summer-run' populations (or stocks) by management agencies (M Lapointe, Pacific Salmon Commission, personal communication). Adams and Weaver delay entry for up to 6 weeks after arriving at the estuary (holding), entering in September and October, and are classified as 'Late-run' populations. Birkenhead behave somewhat intermediate and arrive in coastal areas with the Late-run Adams and Weaver populations, but migrate immediately into the Fraser River with Summer-run populations. For convenience, I grouped Birkenhead sockeye with the Summer-run populations. A summary of population identities, acoustic tag deployments, number of detections, and the total number and sex of biopsied individuals is presented in Table 2.1.

Salmon abundances at date of passage by Summer and Late-run populations through the marine area tagging site at Brown's Bay, and past a hydroacoustic counting facility in the lower

Fraser River (at Mission, BC) are presented in Figure 2.2. Data were collected from DFO and PSC test fisheries, and from the PSC hydroacoustic facility (M Lapointe, Pacific Salmon Commission, personal communication). Late- and Summer-run populations overlapped during passage through Johnstone Strait, though the peak of abundance was slightly earlier for Summer-run fish (Figure 2.2a). By contrast, Fraser River entry was bi-modal, with Summer-run fish entering earlier than Late-run fish, though some Late-run fish entered at the same time as Summer-run fish (Figure 2.2b).

I classified the telemetered fish into one of several 'fate' categories (Table 2.1). Fish detected on at least one SOG receiver were termed 'ocean-detected' (79% of total releases) and fish detected on at least one river receiver were termed 'river-detected' (84% of total releases). The higher percentage of river-detected fish than ocean-detected fish indicates that about 6% of fish entering the river went undetected the ocean, probably because four SOG receivers near the main entrance to the Fraser River were lost and never recovered (Figure 2.1). Fish that were detected in the ocean but not subsequently in the river were termed 'failed river entrants' (5% of total releases) and fish that were never detected on any receiver after release were termed 'unaccounted' (11% of total releases). By using three sets of paired receivers, my ability to detect fish once they entered the river was very high. In fact, of the 150 fish detected in river, 135 (or 90%) were detected at all three receiver stations, 13 (or <9%) were detected at two of the three stations, and only 2 (or >1%) were detected at a single river station. The raw detection files downloaded from the receivers indicate that each fish was detected multiple times as it made its way past a receiver on a given date, but once detected they were never detected again at a later date, suggesting directed upriver movement.

Of the fish that reached the last up-river receiver station, I found no difference between those biopsied (N=74) and not biopsied (N=76) in either their travel time (ANOVA, P=0.091) or their survivourship (Chi square, P=0.726); ultimately, 83% of biopsied and 85% of non-biopsied fish were detected at Mission. These results suggest that the biosampling procedure exerted no deleterious effects on salmon behaviour, survival and travel times, which is consistent with previous studies (Cooke et al. 2005; Cooke et al. 2006a,b). I also found no difference between the proportion of fish that survived to Mission versus the number of ocean losses within each of the six main populations (Chi square test, P=0.756), suggesting that the small percentage of mortality observed was distributed evenly across populations.

I combined 'failed river entrants' with 'unaccounted' into a category of 'ocean-losses' (N=14), and used this new group to compare the physiology of those that disappeared in the

ocean with those that successfully reached Mission (N=74). I found no differences in plasma ions, metabolites, somatic energy, and gill Na^+,K^+-ATPase between ocean-lost fish and successful migrants (MANOVA, F=0.667, P=0.274, N=86). Because the MANOVA was non-significant, I did not run subsequent ANOVAs for each individual variable. I must caution, however, that the lack of statistical significance may have been a result of a low sample size in the ocean-losses category. Results from a parallel telemetry study which occurred at the same time, on the same populations of co-migrating sockeye, using the same tagging platforms and using similar biopsy protocols as the present study, but which had more than twice the sample size as the present, found that fish which disappeared during migration (i.e. ocean losses) were characterized by statistically higher plasma Na^+, osmolality and lactate levels than surviving fish (Cooke et al. 2006a).

Sex-specific MANOVAs indicated a significant population-level physiological difference among females (Wilks λ, P<0.002, N=46), but not among males (Wilks λ, P=0.217, N=40). Table 2 lists population means and standard errors for all of the physiological variables measured. Two-way ANOVAs revealed significant population level differences in gross somatic energy levels (Table 2.2: F=7.88, P<0.01, N=85), and significant population differences in plasma testosterone concentrations among females (Table 2.2, F=5.59, P<0.01, N=46). Tukey's tests revealed that gross somatic energy was greatest in Adams and Chilko fish, lowest in Weaver, Birkenhead and Stellako fish, and moderate in Horsefly fish. Female testosterone was greatest in Birkenhead and Horsefly, lowest in Adams, Weaver and Stellako, and moderate in Chilko. No population differences were observed for the remaining variables (plasma Na^+, K^+, Cl^-, glucose, lactate, T, E_2, and gill Na^+,K^+-ATPase). A significant effect of sex was observed in plasma Cl^- concentrations (Table 2, F=7.35, P<0.01, N=85) with values higher in females (147.7 ± 0.68 $mmol \cdot L^{-1}$) than in males (144.8 ± 0.73). None of the interaction terms in any of the two-way ANOVAs were significant (all P>0.06).

I calculated population-specific rates of travel and (or) holding times through the four segments of the homing migration. Through segment 1, populations differed in travel time (population P=0.025, N=66; Figure 2.3a), and males migrated faster than females (sex P=0.017, N=66; 4.53±0.3 for males vs. 5.53±0.3 days for females). However, through all other segments, populations differed in travel times but males and females did not (segment 2, population P<0.001, sex P=0.655, N=66; segment 3, population P=0.020, sex P=0.959, N=62; segment 4, population P<0.001, sex P=0.552, N=67; Figure 2.3b,c). The Late-run Adams and Weaver populations spent significantly more time holding at the river mouth (segment 2) and migrated at

slower rates over an approximately 40 km stretch of the Fraser River (segment 3) than the four Summer-run populations (Birkenhead, Chilko, Horsefly, Stellako). By dividing the distance traveled through segments 1 to 2 by travel time for each individual, the estimated coastal migrations rates were ~20.9 km·day^{-1} for Late-run populations and ~33.2 km·day^{-1} for Summer-run populations. River migration rates (segment 3) were ~27.7 km·day^{-1} for Late-run populations and ~40.1 km·day^{-1} for Summer-run populations.

There were few significant correlations between travel rates and holding times and the physiological and energetic variables. Correlations between individual travel and holding time estimates and physiology are summarized for each sex in Table 2.3. The strongest correlations were between female testosterone levels and holding time in segment 2 ($r=-0.485$, $P<0.01$, $N=36$) and travel time through segment 3 ($r=-0.524$, $P<0.01$, $N=34$), both of which were significant after Bonferroni correction. Female testosterone levels were also correlated with time from Pitt River confluence to Mission ($r=-0.324$, $P<0.02$, $N=48$). These data suggest that entry timing and migration speed were related to testosterone levels in females. Indeed, both Late- and Summer-run females showed the same negative relationship between entry date and testosterone levels (Figure 2.4). Neither the intercepts nor the slopes of these linear regression relationships differed (ANCOVA, $P=0.587$). The pooled regression equation is $y=-132.93x + 5E+06$, $r^2=0.288$, $N=39$. Other significant correlations in females at $\alpha=0.05$ were identified between travel time and plasma K^+, osmolality, and glucose. In males, no Bonferroni significant correlations were observed, but correlations were observed at $\alpha=0.05$ between travel time and plasma Cl^-, osmolality, glucose, and lactate (Table 2.3).

Males and females showed similar migratory patterns through the SOG array, and the movements of four representative fish are presented in Figure 2.5. The two Summer-run individuals (a male and a female, Figure 2.5a,b) covered a much smaller area than the two Late-run individuals (also a male and a female, Figure 2.5c,d) who were detected over a much broader area. These patterns are consistent with the travel and holding times presented in Figure 2.3 and indicate that the Summer-run fish entered the river quickly relative to the Late-run fish that held for a much longer time before entering the river.

Discussion

Though the timing of sockeye salmon migrations into freshwater are largely population-specific adaptations to environmental factors (river temperature and discharge rates), I found that within a population's particular window of river entry, individual variation in timing by female

sockeye was associated with circulating testosterone levels, but not with somatic energy levels or measures of osmoregulatory preparedness. Both Late- and Summer-run females with high testosterone levels entered the river significantly faster than those with lower levels, which supports my prediction and is consistent with results from a parallel study in which the holding time at the river mouth prior to entry was inversely related to female 11-ketotestosterone and 17β estradiol levels (Cooke et al. 2008). These results show that Late-run females entering directly, thus forgoing the two to six week period in the Strait of Georgia that is typical for Late-run fish, have hormone levels that resemble Summer-run females which do not hold. Furthermore, these results lend support to the idea that the recent migration of Late-run salmon into the Fraser River far in advance of adaptive norms, and their consequent high mortality (Cooke et al. 2006a,b; Young et al. 2006), is related in part to an advance of the reproductive schedule of females. However, neither Late- nor Summer-run males show correlations between testosterone levels and entry timing, despite having circulating levels that were in most cases higher than that of females. This raises fundamental questions about their behavioural ecology and about the cues initiating freshwater entry which will be addressed below. Predictions about somatic energy and osmoregulatory state as drivers of freshwater entry however were not strongly supported by the data for either sex.

Homing sockeye salmon exhibit a considerable degree of life-history variation. At the mouths of natal rivers, somatic energy densities, size at maturity, ovary mass and egg number, and the expression of secondary sexual characteristics all vary by population in response to the distances fish must swim upriver to spawning grounds (Brett 1995; Hendry and Berg 1999, Kinnison et al. 2001; Crossin et al. 2004). Presumably, these patterns have evolved, in part, as a means to optimize the partitioning of finite somatic energy reserves between the competing demands of active and standard metabolism, and reproductive development. Additionally, the behaviour of homing salmon varies at the mouths of natal rivers. Variation in river entry dates can be inferred from the variation in spawning dates observed among populations. And indeed, records kept by fisheries managers clearly indicate differences in run-timing and in holding time at the river mouth, though at present this variation can not be ascribed to specific populations. Summer-run populations traveling furthest up the Fraser River (i.e. Chilko, Horsefly, Stellako), and the Birkenhead population tend to initiate migration in early summer, ahead of the summer peak in river temperatures. Those traveling shorter distances (Late-run Adams and Weaver populations) avoid peak river temperatures by holding for up to 6 weeks in estuarine waters at the river mouth. Interestingly, both behavioural tactics ensure that salmon migrate into the river

when temperatures are near the temperature optimum for their evolved performance (e.g. oxygen consumption rates; Lee et al. 2003).

The fundamental importance of somatic energy to successful upriver migration was the basis for the prediction that variation in river entry timing would depend in part on the availability of reserve energy. Gross somatic energy reserves did differ significantly among populations (Table 2.2), but were not significantly correlated with timing at any segment of the migratory route. Though ultimately a constraint to successful migration, somatic energy did not appear to constrain the entry timing of sockeye in this study. The spread of somatic energy levels observed in this study are within the normal range of values observed in Fraser sockeye salmon (Gilhousen 1991; Crossin et al. 2004).

I had also predicted that river entry timing and behaviour would reflect a degree of osmoregulatory preparedness, but gill Na^+,K^+-ATPase activities did not differ among populations and also did not significantly correlate with entry timing. All fish sampled at Brown's Bay had enzyme activities that were at a level similar to those reported earlier by Shrimpton et al. (2005) for homing sockeye at the same location. All fish sampled at Brown's Bay thus appeared physiologically prepared for the ionic challenges associated with freshwater entry. Despite this however, some individuals entered the river directly (i.e. within hours of first detection at the river mouth) while others held for more than a week before entering. Plasma osmolality was correlated with a longer travel time in males to the river mouth, and longer holding times in males and females, suggesting that gill ATPase activity at the salt-to-freshwater interface does not necessarily reflect subtle differences in an individual's internal osmotic composition. Alternatively, high osmolality may reflect a differential effect of handling stress.

Plasma glucose and lactate levels at capture were not significantly different among the six populations, suggesting that the handling of fish during tagging and sampling was consistent. Average glucose and lactate levels (6.5-7.5 and 8.5-10.5 $mmol \cdot L^{-1}$, respectively) were both above baselines values, suggesting some handling stress, but lactate was below a threshold level (>12 $mmol \cdot L^{-1}$ lactate; Jain and Farrell 1998) suggested for rapid recovery in rainbow trout. Furthermore, plasma glucose in males and females, and lactate in males, were positively correlated with travel times to, and holding times at, the river mouth. As these variables are commonly used to assess the stress response of fish, it is possible that the stress associated with purse-seine capture and subsequent handling at Brown's Bay affected subsequent swim performance *en route* to the river mouth and resulted in a potential delay across the six populations under study. Research on the same populations of homing sockeye had earlier

showed that high plasma lactate and cortisol levels were characteristic of homing sockeye that failed to enter the Fraser River (Cooke et el. 2006a). While I can implicate stress with a delay in ocean migration, I cannot attribute ocean losses to stress, though I caution that the high survival rate meant that low numbers of ocean losses may have precluded statistical resolution of such an effect.

Stress related increases in plasma cortisol, glucose and lactate can have a depressive effect on circulating sex steroid levels in fish (Pankhurst and Van Der Kraak 1997; Kubokawa et al. 1999). However, I did not see unusually low hormone titres. Furthermore, I did not see a significant correlation between female plasma testosterone and travel time from Brown's Bay to the SOG array, but I might have expected a longer travel time associated with lower hormone levels. Nevertheless, testosterone was a key correlate of holding time and river entry timing in females.

The final stages of ovarian maturation in salmonids is associated with pronounced changes in steroid hormone biosynthesis (Van Der Kraak et al. 1989; Devlin and Nagahama 2002: Nakamura et al. 2005). In late vitellogenic follicles, there is a marked reduction in the expression of aromatase which is responsible for the conversion of testosterone and androstendione to 17β-estradiol and estrone, respectively. Consequently, circulating androgen levels rise. There are also increases in the activity of other enzymes, most notably those involved in the synthesis of progestins that are involved in the maturation of oocytes. Thus, the observation that female fish with the highest testosterone levels were the first to initiate upriver migration is consistent with my prediction, and suggests strongly that those fish were in an advanced reproductive state relative to fish entering later. Interestingly, though hormone levels were somewhat higher in summer-run females (Figure 2.5), both Summer- and Late-run females showed the same inverse relationship between testosterone and entry timing.

In males, testosterone levels were generally higher than in females but were not significantly correlated with timing behaviour at any stage. In fact, I did not detect any clear physiological indicator of freshwater entry for males. Unfortunately, I did not measure 11-keto testosterone in this study, which is a derivative of testosterone and a powerful androgen with strong effects on behaviour. However, in a parallel study conducted with the same populations of fish at the same time and place as this study, 11-ketotestosterone levels did not correlate with holding behaviour or river entry in males (Cooke et al. 2008). Entry may thus be affected by some endogenous variable (or variables) that I did not measure in this study, or by some other exogenous factor. One interesting hypothesis to test is that males synchronize and initiate

freshwater entry and upriver migration in response to the entry schedules of females. Evolutionary theory predicts that female animals will time reproduction to coincide with optimal environmental conditions for the rearing of offspring (Lack 1954), and my results lend support to this idea. Accordingly, male sockeye should synchronize river entry to reflect the migratory schedules of females. Male sockeye, and indeed all male Pacific salmon, are known to arrive on spawning grounds before females (termed protandrous reproduction, or "protandry") in order to position themselves optimally for access to females (Morbey 2000), a tactic common among species in which competition for females during courtship is fierce (Myers 1981; Oring and Lank 1982). If male sockeye are timing their entry to that of co-migrant females, then they should swim faster than females to arrive on spawning grounds first. Telemetry studies on upriver migrating sockeye support this prediction (Hinch and Rand 1998; Standen et al. 2002) though it is most obvious in relatively fast and turbulent reaches (Hinch and Bratty 2000).

Additional support for the idea that males time their migration to that of females comes from pheromone studies in spawning salmon. F-type prostaglandins released in the urine of vitellogenic female rainbow trout (*O. mykiss*) act as releaser-type pheromones which positively influence male courtship behaviour, milt volume, and circulating testosterone and 17,20-dihydroxyprenenone levels (Olsén and Liley 1993). In this study, males generally had higher testosterone levels than females. As female sockeye homing into natal rivers are commonly in a vitellogenic state, my results suggest the possibility that homing females could be emitting a chemical cue that influences the entry timing of homing males, though certainly dilution factors are much larger in the marine area than in spawning systems. However, support for this notion can be drawn from Døving and Stabell (2003), who have proposed that the return to natal rivers by ocean homing salmon is facilitated by a conspecific recognition of amino acids, bile salts, steroid hormones, and prostaglandins, all of which salmon can detect at very low concentrations (Døving et al. 1980). Collectively, these compounds constitute what is known as the "home stream olfactory bouquet" (Carruth et al. 2002) and may be key to guiding salmon into and up natal rivers, but at present this hypothesis has not been tested.

In summary, from the point of release at Brown's Bay, male sockeye were faster than females when homing through nearly 200 km of the Strait of Georgia, where tides and currents are strong and variable. Upon arrival at the river mouth, clear differences in behaviour were detected among Late- and Summer-run populations, which is consistent with fishery records and which are likely tactics shaped by the mean temperature regimes encountered historically *en route* to spawning areas. Late-run sockeye milled about by the river mouth, covering a greater

area and visiting more acoustic receivers than Summer-run sockeye, and resided longer before heading upriver. The sexes did not differ in these patterns, nor did they in migration travel times in the lower, tidally affected reaches of the Fraser River. The key physiological variable related to freshwater entry of females was plasma testosterone. None of the physiological variables were correlated with river-entry timing in males, and in neither sex was energetic condition or osmoregulatory state strongly correlated with entry timing.

Table 2.1. Number of acoustic transmitters deployed, the number of physiologically sampled sockeye salmon, and the number of tagged individuals detected in the ocean and in the Fraser River in 2003. Numbers are reported by population. After release, fish were subsequently detected in the receiver array at the Fraser River mouth (ocean detections), and (or) in the river at the Pitt River confluence, Albion, and Mission receiver stations. Fish not detected anywhere are classified as 'unaccounted' individuals.

Tagging Summary	------ LATE-RUN ------		---------- SUMMER-RUN ----------					
Population	Adams	Weaver	Birkenhead	Chilko	Horsefly	Stellako	Other populations	TOTAL
Number tagged	36	18	31	47	28	10	8	178
Number that were biopsied (females, males)	16 (6,10)	12 (6,6)	19 (8,11)	22 (11,11)	10 (5,5)	6 (3,3)	3 (1,2)	88 (40,48)
Number detected in ocean (biopsied vs. unbiopsied)	30 (14,16)	15 (10,5)	23 (13,10)	37 (19,18)	20 (5,15)	10 (6,4)	6 (2,4)	141 (69,72)
% of total released	83%	83%	74%	79%	71%	100%	75%	79%
Number detected in river (biopsied vs. unbiopsied)	30 (13,17)	15 (10,5)	27 (17,11)	40 (21,18)	22 (7,15)	10 (6,4)	6 (1,5)	150 (75,75)
% of total released	83%	83%	87%	85%	79%	100%	75%	84%
Number failing to enter river (% of total released)	6	3	4	7	6	0	2	28 (16%)
# detected in ocean but not in river	2	0	1	3	2	0	1	9 (5%)
# unaccounted	4	3	3	4	4	0	1	19 (11%)

Table 2.2. Comparison of the physiological attributes of sockeye salmon (*Oncorhynchus nerka*) upon capture at Brown's Bay by population and sex. Analyses were conducted using ANOVA with population and sex as main effects, and their interaction. All variables were log(10) transformed prior to analysis. Significant correlations are * $P < 0.05$, ** $P < 0.01$. Bold faced values indicate significance at Bonferroni corrected α-values: 0.005 for females, 0.006 for males. Superscript letters indicate Tukey's test population differences in significant ANOVA models.

Variables	Population	Mean capture values and SEMs	Total N	Population	Sex	Interaction
Gross somatic energy ($MJ \cdot kg^{-1}$)	Adams	9.00 ± 0.22^{a}	85	**F=7.88, P<0.001**	F=3.70, P=0.06	F=0.92, P=0.48
	Weaver	7.82 ± 0.25^{c}				
	Birkenhead	7.78 ± 0.20^{c}				
	Chilko	9.01 ± 0.18^{a}				
	Horsefly	8.16 ± 0.27^{b}				
	Stellako	9.40 ± 0.35^{a}				
Plasma Na^{+} ($mmol \cdot L^{-1}$)	Adams	179.60 ± 1.97	84	F=1.61, P=0.17	F=0.22, P=0.64	F=1.59, P=0.17
	Weaver	184.17 ± 2.26				
	Birkenhead	182.16 ± 1.80				
	Chilko	177.40 ± 1.71				
	Horsefly	179.24 ± 2.47				
	Stellako	178.27 ± 3.19				
Plasma K^{+} ($mmol \cdot L^{-1}$)	Adams	0.76 ± 0.21	84	F=0.64, P=0.67	F=0.12, P=0.73	F=0.22, P=0.95
	Weaver	1.00 ± 0.24				
	Birkenhead	1.28 ± 0.19				
	Chilko	1.08 ± 0.18				
	Horsefly	0.84 ± 0.26				
	Stellako	1.07 ± 0.34				
Plasma Cl^{-} ($mmol \cdot L^{-1}$)	Adams	146.22 ± 1.10	85	F=1.03, P=0.41	F=7.35, P<0.01** females > males	F=0.20, P=0.96
	Weaver	148.73 ± 1.26				
	Birkenhead	145.08 ± 1.00				
	Chilko	146.08 ± 0.93				
	Horsefly	146.25 ± 1.38				
	Stellako	145.20 ± 1.78				

Variables	Population	Mean capture values and SEMs	Total N	Population	Sex	Interaction
Plasma osmolality ($mOsm \cdot kg^{-1}$)	Adams	353.69 ± 3.79	85	F=0.54, P=0.75	F=0.02, P=0.90	F=0.43, P=0.83
	Weaver	358.38 ± 4.35				
	Birkenhead	351.88 ± 3.47				
	Chilko	350.31 ± 3.22				
	Horsefly	353.40 ± 4.77				
	Stellako	357.17 ± 6.16				
Plasma glucose ($mmol \cdot L^{-1}$)	Adams	7.37 ± 0.26	85	F=1.05, P=0.40	F=2.95, P=0.09	F=0.69, P=0.64
	Weaver	6.97 ± 0.30				
	Birkenhead	7.05 ± 0.24				
	Chilko	6.68 ± 0.22				
	Horsefly	6.70 ± 0.32				
	Stellako	7.28 ± 0.42				
Plasma lactate ($mmol \cdot L^{-1}$)	Adams	9.40 ± 0.80	85	F=1.01, P=0.42	F=0.04, P=0.84	F=0.85, P=0.52
	Weaver	10.58 ± 0.92				
	Birkenhead	9.70 ± 0.73				
	Chilko	8.23 ± 0.68				
	Horsefly	9.17 ± 1.01				
	Stellako	8.99 ± 1.30				
Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	Adams	1.71 ± 0.14	85	F=0.62, P=0.69	F=0.12, P=0.73	2.24, P=0.06
	Weaver	1.62 ± 0.16				
	Birkenhead	1.63 ± 0.13				
	Chilko	1.81 ± 0.12				
	Horsefly	1.62 ± 0.18				
	Stellako	2.09 ± 0.23				

Variables	Population	Mean capture values and SEMs	Total N	Population	Sex	Interaction
Male plasma testosterone ($pg \cdot ml^{-1}$)	Adams	7470 ± 1418	39	F=0.86, P=0.52	n/a	n/a
	Weaver	6125 ± 1419				
	Birkenhead	8532 ± 1228				
	Chilko	6504 ± 1047				
	Horsefly	6079 ± 1553				
	Stellako	4297 ± 2005				
Female plasma testosterone ($pg \cdot ml^{-1}$)	Adams	5052 ± 603[a]	46	**F=5.59, P<0.001**	n/a	n/a
	Weaver	4908 ± 779[a]				
	Birkenhead	7925 ± 575[c]				
	Chilko	5812 ± 575[b]				
	Horsefly	7554 ± 853[c]				
	Stellako	4422 ± 1101[a]				
Female plasma 17β-estradiol ($pg \cdot ml^{-1}$)	Adams	4090 ± 949	46	F=1.46, P=0.23	n/a	n/a
	Weaver	5197 ± 1226				
	Birkenhead	5197 ± 905				
	Chilko	5533 ± 905				
	Horsefly	4048 ± 1343				
	Stellako	2547 ± 1733				

Table 2.3. Sex-specific correlation coefficients and p-values relating sockeye salmon gross somatic energy, body size, and plasma biochemistry to travel and holding times determined through acoustic telemetry. Asterisks signify statistical significance at α=0.05. Double asterisks signify significance at α=0.01. Bold faced values signify significance at Bonferroni corrected α-values: 0.0033 for females (11 variables), 0.0035 for males (10 variables).

Sex	Variables	Time from Brown's Bay to SOG array	N	Holding time in SOG array	N	Time from last SOG array detection to Pitt River confluence	N	Time from Pitt River Confluence to Mission	N
Females	Gross somatic energy ($MJ \cdot kg^{-1}$)	0.080 (0.65)	36	0.010 (0.56)	36	0.171 (0.34)	34	0.033 (0.83)	48
	Nose to fork length (cm)	0.004 (0.98)	36	-0.101 (0.56)	36	-0.221 (0.21)	34	-0.054 (0.72)	48
	Plasma Na^+ ($mmol \cdot L^{-1}$)	0.321 (0.06)	35	0.292 (0.09)	35	-0.108 (0.55)	33	0.018 (0.91)	47
	Plasma K^+ ($mmol \cdot L^{-1}$)	0.157 (0.37)	35	0.046 (0.79)	35	-0.371 (<0.03)*	33	-0.089 (0.55)	47
	Plasma Cl^- ($mmol \cdot L^{-1}$)	0.071 (0.68)	36	0.209 (0.22)	36	-0.113 (0.52)	34	-0.023 (0.88)	48
	Plasma osmolality ($mOsm \cdot kg^{-1}$)	0.212 (0.22)	36	0.359 (0.03)*	36	-0.093 (0.60)	34	-0.001 (0.99)	48
	Plasma glucose ($mmol \cdot L^{-1}$)	0.342 (0.04)*	36	0.336 (<0.05)*	36	-0.024 (0.89)	34	0.34 (0.019)	48
	Plasma lactate ($mmol \cdot L^{-1}$)	0.165 (0.34)	36	0.302 (0.07)	36	-0.182 (0.30)	34	0.099 (0.50)	48
	Plasma testosterone ($pg \cdot ml^{-1}$)	-0.193 (0.26)	36	**-0.485 (<0.001)**	36	**-0.402 (<0.001)**	34	-0.325 (<0.02)*	48
	Plasma 17β estradiol ($pg \cdot ml^{-1}$)	0.062 (0.72)	36	-0.010 (0.95)	36	-0.048 (0.79)	34	0.059 (0.69)	48
	Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	-0.087 (0.62)	35	-0.160 (0.36)	35	-0.110 (0.50)	39	0.140 (0.35)	47
Males	Gross somatic energy ($MJ \cdot kg^{-1}$)	-0.205 (0.26)	32	-0.324 (0.07)	32	-0.350 (0.06)	29	-0.176 (0.35)	30
	Nose to fork length (cm)	0.007 (0.97)	32	0.108 (0.56)	32	0.435 (<0.01)**	29	0.182 (0.34)	30
	Plasma Na^+ ($mmol \cdot L^{-1}$)	0.281 (0.12)	32	0.283 (0.12)	32	0.255 (0.18)	29	0.083 (0.66)	30

Sex	Variables	Time from Brown's Bay to SOG array	N	Holding time in SOG array	N	Time from last SOG array detection to Pitt River confluence	N	Time from Pitt River Confluence to Mission	N
Males	Plasma K^+ ($mmol \cdot L^{-1}$)	0.208 (0.26)	32	-0.048 (0.80)	32	0.030 (0.88)	29	-0.174 (0.36)	30
	Plasma Cl^- ($mmol \cdot L^{-1}$)	0.471 (<0.01)**	32	0.425 (<0.02)*	32	0.127 (0.51)	29	-0.004 (0.98)	30
	Plasma osmolality ($mOsm \cdot kg^{-1}$)	0.478 (<0.01)**	32	0.351 (<0.05)*	32	0.195 (0.31)	29	-0.087 (0.65)	30
	Plasma glucose ($mmol \cdot L^{-1}$)	0.192 (0.29)	32	0.366 (<0.04)*	32	0.046 (0.81)	29	0.117 (0.54)	30
	Plasma lactate ($mmol \cdot L^{-1}$)	0.494 (<0.01)**	32	0.480 (<0.01)**	32	0.230 (0.23)	29	0.147 (0.44)	30
	Plasma testosterone ($pg \cdot ml^{-1}$)	-0.129 (0.48)	32	0.277 (0.13)	32	0.322 (0.09)	29	0.084 (0.66)	30
	Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	0.138 (0.45)	32	-0.212 (0.24)	32	-0.309 (0.10)	29	-0.155 (0.41)	30

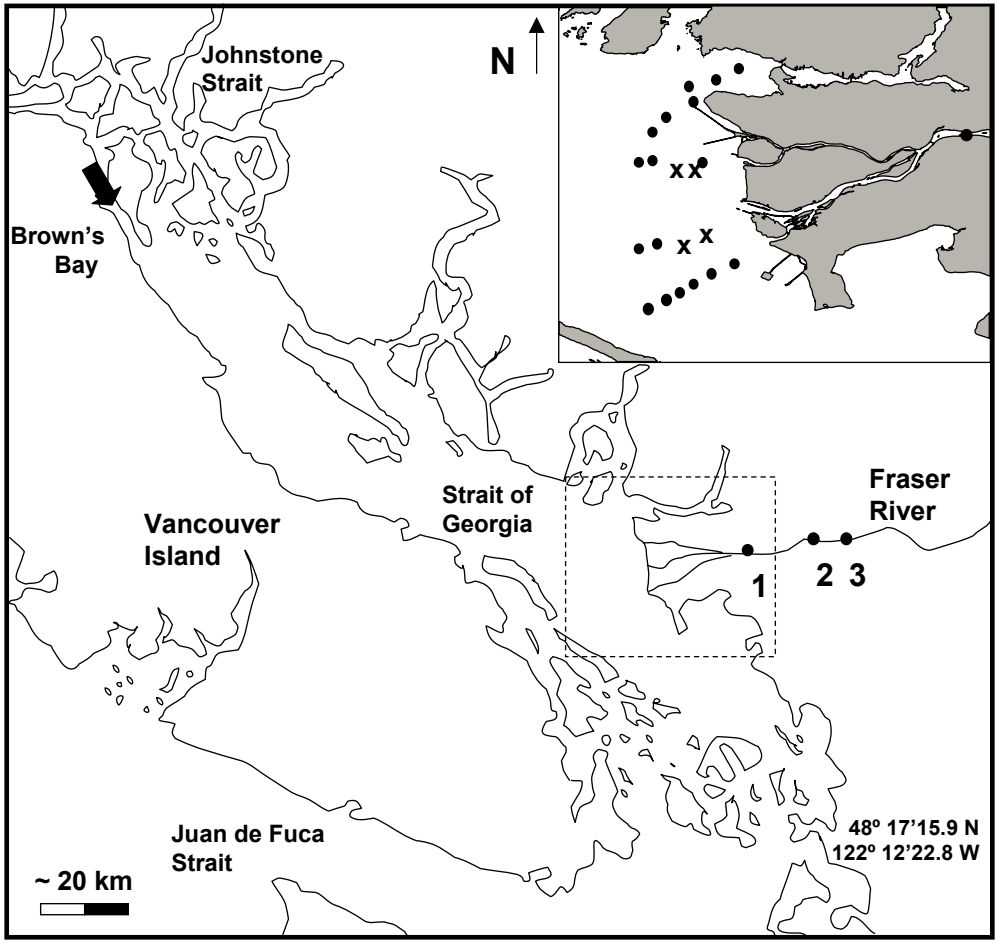

Fig. 2.1: Map of southwestern coastal British Columbia and Vancouver Island with an inset of the Fraser River delta showing the position of the acoustic receiver array positioned at the river mouth. Salmon were captured by purse-seine at Brown's Bay, biopsied for physiological samples, and fitted with acoustic transmitters. Fish were then released and eventually detected in the acoustic array at the river mouth (black circles) and at receiver stations positioned in the Fraser River at the Pitt River confluence (1), Albion (2), and Mission (3) (numbered black circles). Acoustic receivers that were deployed but not recovered are indicated with "x".

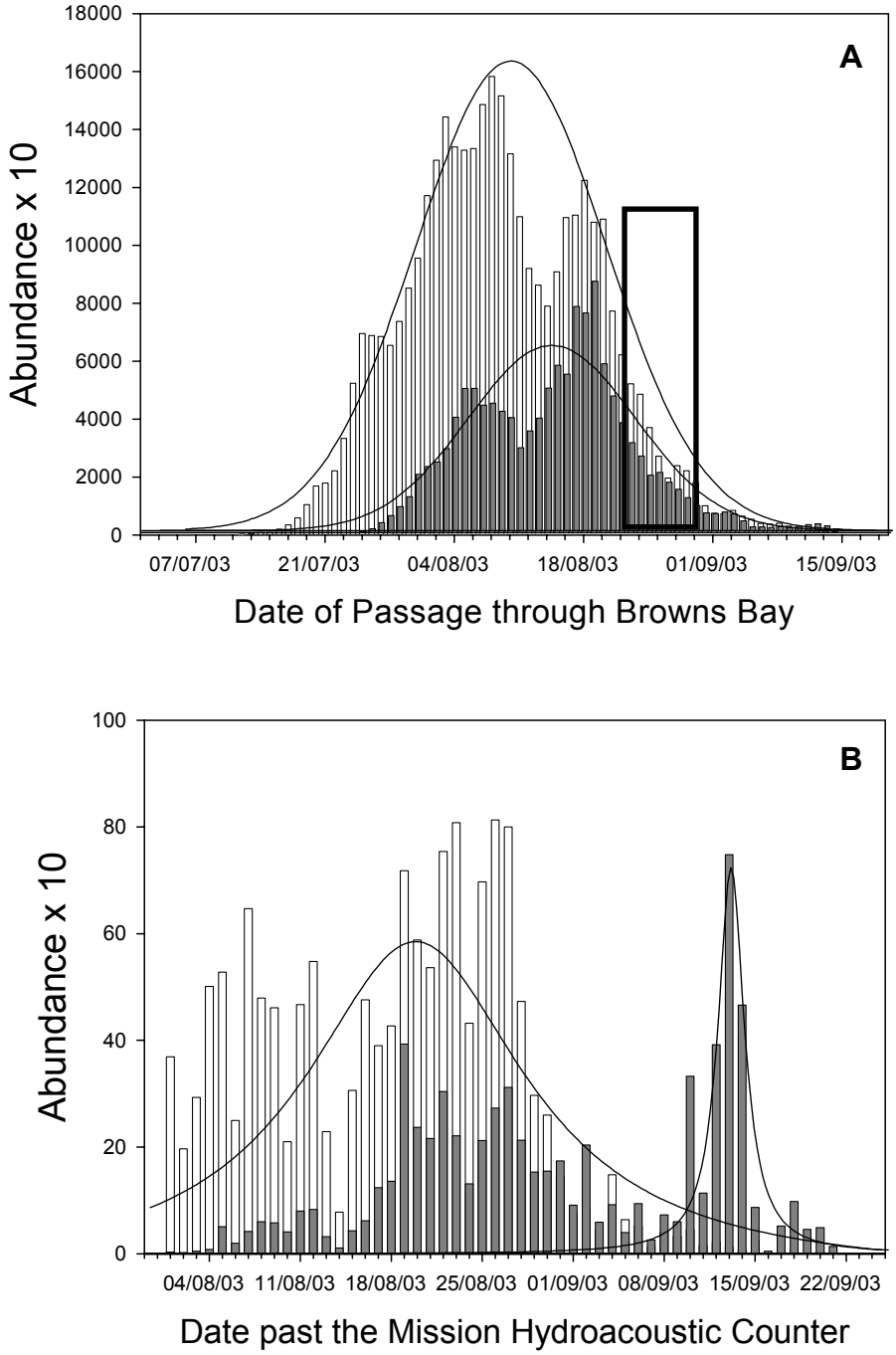

Fig. 2.2: Abundances of sockeye salmon and dates of passage through the marine area collection site in Johnstone Strait (A), and past a hydroacoustic enumeration facility approximately 80 km upriver from the Fraser River mouth (B). Dark bars are Late-run abundances, and light bars are Summer-run abundances. Rectangle in panel a indicates the dates during which fish from this study were sampled. Data from M. Lapointe, Pacific Salmon Commission, Vancouver, BC (unpublished).

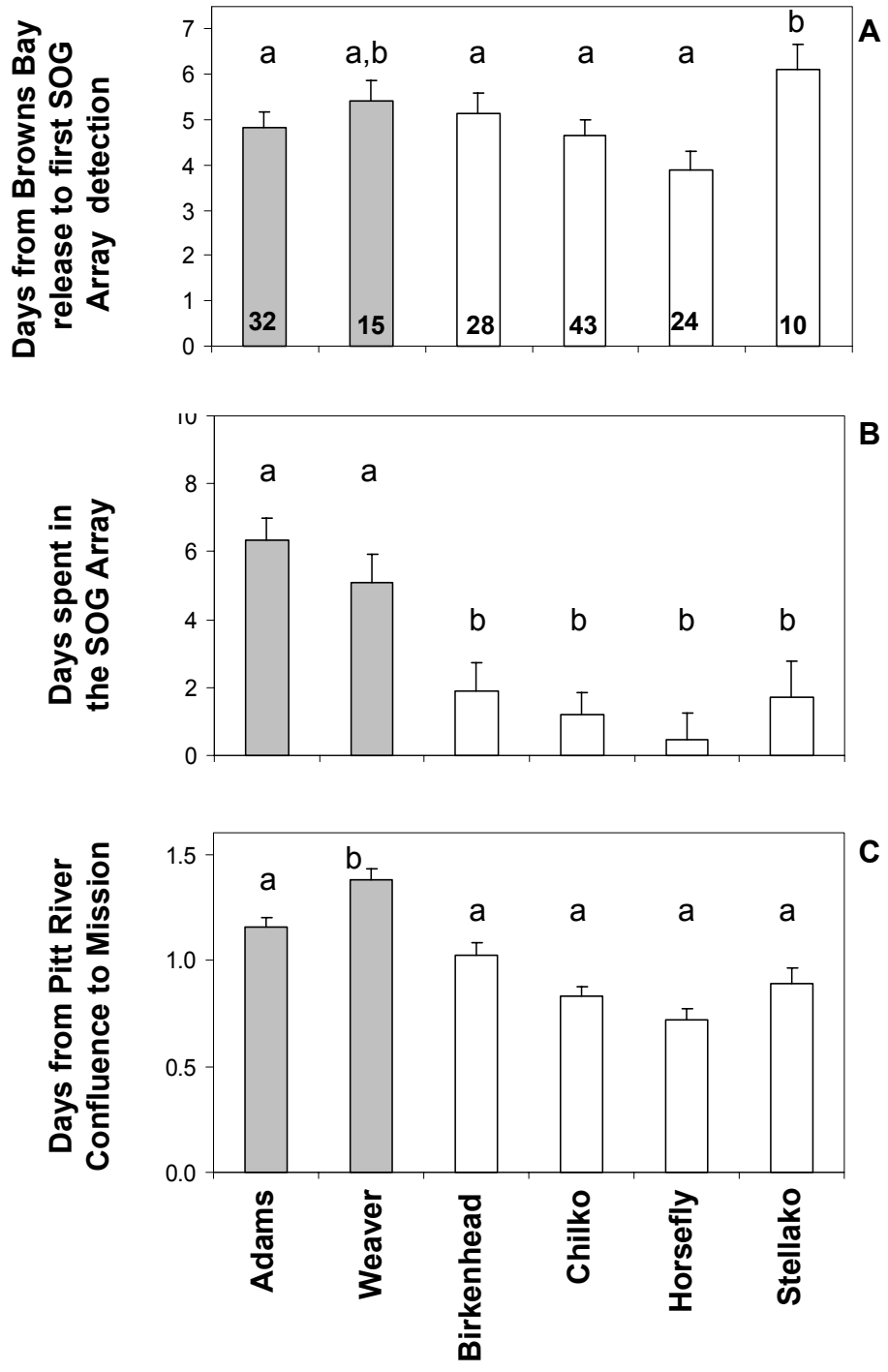

Fig. 2.3: Mean travel time from point of release at Brown's Bay to first SOG array detection (A), mean holding time in the SOG array (B), and mean travel time from the Fraser-Pitt Rivers confluence to Mission B.C. (C). Dark bars are Late-run populations, and light bars are Summer-run populations. Sample sizes (numbers of fish) are indicated at base of bars in panel A. Error bars signify +1 SEM. Different letters indicate significance at $P<0.05$.

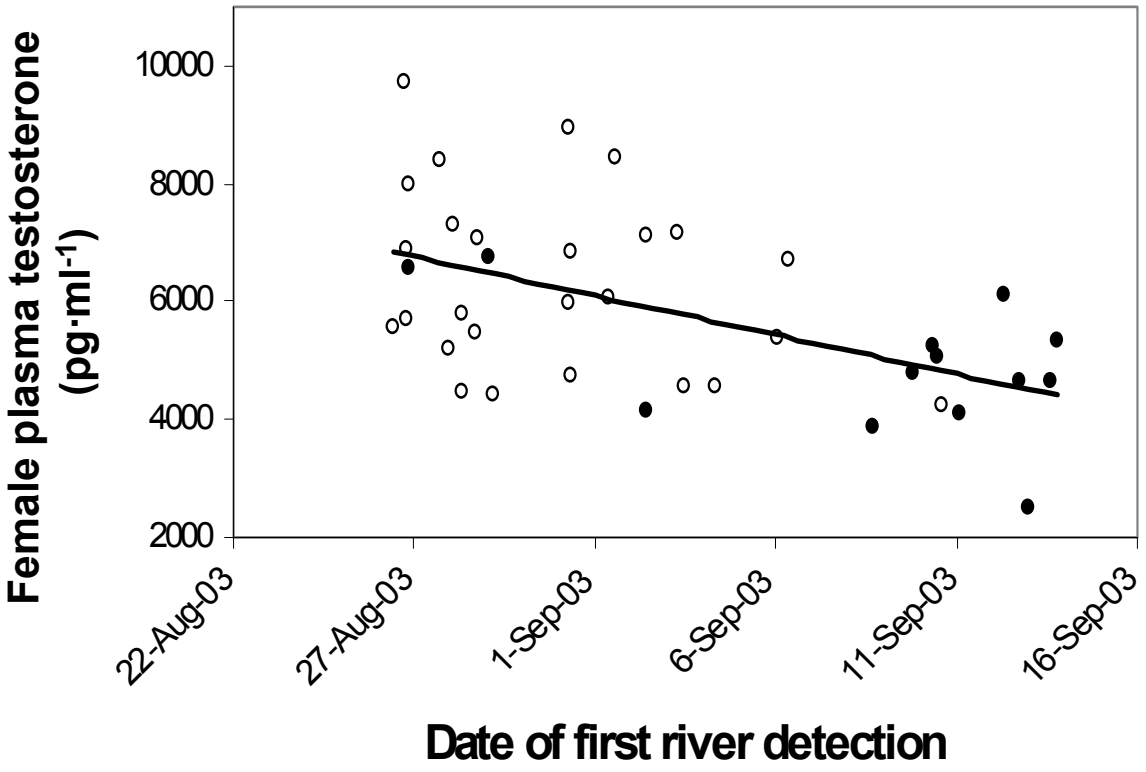

Fig. 2.4: Relationship between dates of river entry and plasma testosterone concentrations in female sockeye salmon. Fish were released at Brown's Bay, 215 km from the mouth of the Fraser River and river entry date was determined by telemetry. Open circles represent Summer-run populations, dark circles represent Late-run populations. The line is the best linear fit for the pooled data: $P<0.001$, $r^2=0.288$.

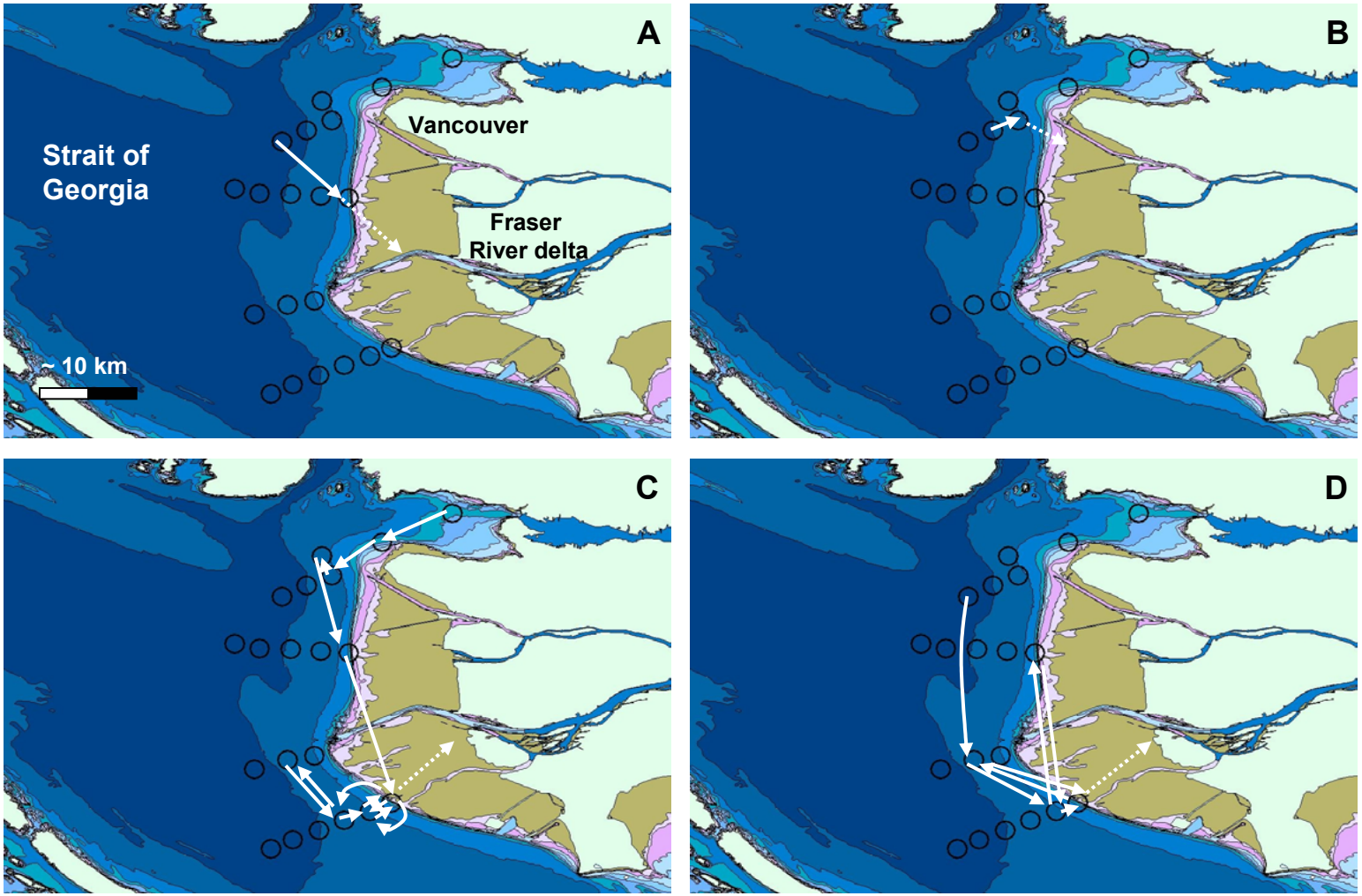

Fig. 2.5: Representative movements of sockeye salmon homing through marine waters to the Fraser River: (A) Summer-run Chilko male, first entered the array at 1956 h, August 25 and was last detected at 2307 h. August 26, a total of 0.13 days. The fish was detected upriver 0.89 days later at Mission. (B) Summer-run Horsefly female, first entered the array at 1913 h, August 25 and was last detected at 0242 h, August 26, a total of 0.51 days. The fish was detected upriver 0.72 days later at Mission. (C) Late-run Adams female, first entered the array at 1116 h, August 27 and was last detected at 1021 h, September 8, a total of 11.96 days. The fish was detected upriver 5.37 days later at Mission. (D) Late-run Weaver male, first entered the array at 1812 h, August 28 and was last detected at 1053 h, September 8, a total of 10.70 days. The fish was detected upriver 4.15 days later at Mission. Solid lines indicate the linear sequence of acoustic receivers visited by each fish and are not proportional to time. Dashed lines are projected directions of river entry.

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CHAPTER 3

Physiological profiles of Fraser River sockeye salmon and effects of exogenous GnRH and testosterone enhancement on migration rates at an early stage of homeward migration in the Gulf of Alaska.[2]

Introduction

The long-distance migrations of animals have long fascinated ecologists, and the spawning migrations of Pacific salmon (*Oncorhynchus spp.*) are among the longest known, traversing large swathes of the northern Pacific Ocean and extending vast distances into natal watersheds. Direct observation of migratory behaviours have been gleaned from positional telemetry in which individual salmon were tracked while homing through coastal waters to natal rivers (Døving et al. 1985; Quinn et al. 1989; Tanaka et al. 2000; Cooke et al. 2005, 2006a,b, 2008; Chapter 2- Crossin et al. 2007), and efforts to characterize the physiological mechanisms that underlie migratory behaviours have benefited from physiological biopsy at the time of transmitter implantation (Cooke et al. 2005, 2006a,b, 2008; Chapter 2- Crossin et al. 2007). Descriptive studies with Pacific salmon have shown that the shift from ocean foraging to homeward migration is tied to the photoperiodic activation of the hypothalamo-pituitary-gonadal axis (HPG; Ueda and Yamauchi 1995), a pattern that is observable in many taxa (reviewed by Dingle 1996). Experimental work with lacustrine sockeye salmon (*O. nerka*, also known as kokanee salmon) has shown that exogenous implantation of gonadotropin-releasing hormone (GnRH) results in the cessation of active foraging in the open lake environment, increases in circulating testosterone, and a premature migration to a natal inlet stream for spawning (Sato et al. 1997; Kitahashi et al. 1998). Additionally, injections of a GnRH analog (GnRHa), which leads to testosterone production, led to an increase in the number of times a mature adult sockeye would attempt to leap over a waterfall (Plate et al. 2003). GnRHa also led to an increase in upstream swimming speeds and homing rates in adult salmon experimentally treated in freshwater (Andrew Dittman, Northwest Fisheries Science Center, National Marine Fisheries

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Service, Seattle, WA, unpublished data; Sato et al. 1997). Thus, a principal driver of migratory rate and pattern in sockeye salmon is clearly the seasonal surge of testosterone, which in addition to its primary role in reproductive development has potent effects on migratory behaviour. Other studies (Hinch et al. 2006; Chapter 5- Crossin et al. in review-A) have shown that endogenous energy supply, which for homing salmon that ultimately stop feeding becomes finite, and the activity of gill Na^+,K^+-ATPase, an enzyme that enables salmon to maintain homeostasis when traversing salinity gradients, are strong correlates of migratory timing into natal rivers.

While these observations have all provided fascinating details about the behaviour of homing salmon, especially as it pertains to river-entry timing, most of these studies have focused on salmon either very near the marine-to-freshwater interface (Cooke et al. 2005, 2006a, 2006b, 2008; Crossin et al. 2007), or exclusively in freshwater (Young et al. 2006). In these studies, salmon had already stopped feeding and begun the programmed catabolism of their digestive tract that so characterizes their semelparous, capital breeding life-history. Moreover, energy stores and the partitioning of energy between the soma and the gonads reflected population-specific life-history variation with respect to river entry date and difficulty on river migration and reproductive development (Kinnison et al. 2001, 2003; Crossin et al. 2004). Specifically, populations with long and arduous freshwater migrations exhibit physiological and behavioural characteristics conducive to energy conservation (Brett 1995; Hendry and Berg 1999; Kinnison et al. 2001; Crossin et al. 2003, 2004). Homeward migration is initiated months before these observations, when salmon are on the high seas and still foraging. There location then makes capture difficult, which has resulted in very few studies of salmonid energetics, physiology and reproductive development far at sea (see review in Hinch et al. 2006). These gaps raise the important question of when in the sea migration does population-specific variation in energy storage and allocation to reproductive occur?

In this study, I had two principal objectives. First was to characterize baseline variation among populations (grouped by run timing) in energetic condition, reproductive state, and osmoregulatory preparedness is present in ocean salmon not yet reliant on endogenous reserve energy to fuel migration, i.e. far enough away from the river mouth that they are still feeding. Second was to test the hypothesis that reproductive hormone concentrations influence migration rates from high seas areas to the natal river. The experimental approach involved the capture of sockeye early in homeward migration as they made continental landfall near the Queen Charlotte Islands in the northeast Pacific Ocean (coastal British Columbia, Canada), over 850 kms from the Fraser River mouth. Upon capture by purse seine, salmon were non-lethally biopsied for

energetic condition, blood plasma, and gill tissues. These fish were divided into four groups that were either given an injection of GnRH or GnRH in combination with testosterone, or injected with saline (sham treatment) or left untreated (control). All salmon then received an acoustic transmitter implant and were released to resume migration. Their subsequent behaviour was monitored as they swam across several acoustic telemetry receiving lines positioned at intervals along the homeward trajectory. Regarding my first objective, I predicted that baseline energy stores would show little variation among run timing groups because all are actively foraging during this early stage of homeward migration (Hinch et al. 2006). However, because migration is activated by the photoperiodic stimulation of the HPG and other axes, I predicted that the hormonally mediated processes of reproductive development, energy partitioning between the soma and the gonads and osmoregulation would vary according to run-timing groups with those that enter the Fraser River first being more reproductively mature and more osmoregulatorily 'prepared' for freshwater entry. Regarding my second objective, I predicted that fish receiving exogenous hormone enhancement would exhibit faster rates of homeward migration relative to controls, irrespective of run-timing group.

Methods and Materials

Fish capture and sampling protocols

Post-smolt sockeye move off the continental shelf into the open ocean after about one year. For Fraser River sockeye, whose ocean distributions have been examined (French et al. 1976; Burgner 1991), their migration into the Gulf of Alaska is also dependent on current patterns and temperature and their subsequent open ocean migration is dominated by the direction and strength of the Alaskan Gyre (Healey 2000). After two years of ocean growth, Fraser River sockeye initiate the homeward migrations by swimming eastward to the coast where they make continental landfall at latitudes near the northwest coast of British Columbia, Canada (Thomson et al. 1992). Homeward migrating fish were targeted in this area along the northwest coast of the Queen Charlotte Islands (Fig. 1) using a commercial purse seine vessel chartered for this study. Capture efforts began on 28 July 2006 and continued until 3 August 2006. Seining was conducted in a manner to minimize capture stress and handling by using several tagging methods similar to those used in previous ocean-capture studies with adult sockeye (see Cooke et al. 2004, 2005, 2006a,b, 2008; Chapter 2- Crossin et al. 2007, Chapter 5- Crossin et al. in review-A). The seine net was brought alongside the rail of the vessel but kept open and in the water so that individual fish could be removed by dipnet and transferred swiftly

to a large tank on the quarter deck containing a continuous supply of fresh, ambient seawater. Ten to fifteen salmon were dipnetted from each seine set and all other salmon were promptly released. The biopsy and tagging procedures were then performed as expeditiously as possible.

Individual salmon were removed from the holding tank and placed ventral side up in a padded V-shaped trough that was provided with a continuous supply of ambient seawater from a tube positioned near the salmon's head. Two people restrained the salmon while a third collected the biopsy. All handling, biopsy and tagging protocols involving hormone implant research were approved by the University of British Columbia Animal Care Committee in accordance with the Canadian Council of Animal Care. The biopsies were conducted on unanaesthetized fish, making the results comparable with past studies for fish sampled closer to and in the Fraser River (Cooke et al. 2006a,b, 2008; Young et al. 2006; Chapter 2- Crossin et al. 2007; Chapter 5- Crossin et al. in review-A). Typically, fish were confined to the trough for less than three minutes during which time fork length (FL, cm) was measured, tissues were biopsied and an external dorsal tag affixed. Biopsies included the removal of (a) a 0.5 g clip of adipose fin for DNA stock identification, (b) a 3 ml blood sample from the caudal vein (using a 1.5", 21 gauge vacutainer syringe; Houston 1990) for assessing plasma chemistry, and (c) a < 4 mm clip of six to eight gill filament tips (~0.03 g) along the first gill arch (McCormick 1993) for assessing gill Na^+,K^+-ATPase activity. Gill tissue and centrifuged plasma samples were stored in liquid nitrogen for several days until transfer to a –86 ˚C freezer. A hand-held microwave energy meter (Distell Fish Fatmeter model 692, Distell Inc, West Lothian, Scotland, UK) was to be used to quantify gross somatic energy (GSE) concentrations as I have done in other studies (e.g. Crossin and Hinch 2005; Cooke et al. 2006, 2008; Chapter 2- Crossin et al. 2007; Chapter 5- Crossin et al. in review-A). However, the meter malfunctioned after the first day of sampling and was not used thereafter. An addition group of co-migrating sockeye salmon (N=36) were collected on a single day during the middle of the sampling period and sacrificed for measurement of GSE and gonad masses. These fish had their gonads removed prior to freezing the gonads and carcasses separately for transport to the laboratory, where samples were thawed, weighed and homogenized for proximate analysis and a GSE estimate (Crossin et al. 2004). Subsequent DNA analyses revealed that four fish were Early Summer-Run (males), eight were Summer-Run (3 males, 5 females), and 22 were Late-Run (10 males, 12 females).

My previous sampling of Fraser River sockeye along the west coast of the Queen Charlotte Islands found that feeding and energy accrual is on-going during this early stage of homeward migration (see Hinch et al. 2006). This meant that transmitters needed to be

surgically inserted into the abdominal cavity rather than inserted intragastrically as in other studies (Chapter 2- Crossin et al. 2007) so as not to interfere with feeding. Therefore, on completion of the biopsy, sockeye were transferred to an aenesthetic bath of MS222 (50 $mg \cdot L^{-1}$) for 1-2 minutes to achieve stage 3 anesthesia. Transmitter implantation took 1-2 min while sockeye were bathed in a dilute solution of MS222 (20 $mg \cdot L^{-1}$); sockeye resumed activity at the completion of surgery. Transmitters were inserted via a 2-3 cm incision in the ventral abdominal cavity, which was closed with silk sutures (see KRC 2007 for implantation specifications). After surgery, the sockeye divided into four treatment groups: 150 $\mu g \cdot kg^{-1}$ injection of a GnRH analog (GnRHa; see Mylonas et al. 1995; Mylonas and Zohar 2001; Chapter 4- Crossin et al. in review-B), 150 $\mu g \cdot kg^{-1}$ of GnRH plus 4 $mg \cdot kg^{-1}$ testosterone (T) injection, saline injection (sham), or untreated (control). Injections were delivered with a 1ml syringe fitted with a 21 gauge needle into the dorsal sinus between the epaxial musculature, just posterior to the dorsal fin. Sockeye had a numbered cinch tag inserted posterior to the dorsal fine, then were immediately returned over the side of the boat and visually monitored until they swam away. Fish were on-board for less than one hour and most were on-board for less than 10 minutes.

I investigated the procedures, protocols and potential effects of hormone implants with a laboratory study conducted prior to the field work (Chapter 4- Crossin et al. in review-B). Specifically, I examined the physiological effect of exogenous GnRH and testosterone (T) implants on reproductive and osmoregulatory physiology in pre-adult pink salmon (*O. gorbuscha*) held in saltwater to provide a point of reference for interpreting GnRH and T mediated changes in survival, migration rates and timing in the present study. Two-hundred and fifty pink salmon were divided into 4 treatment group (GnRH, GnRH+T, T, and control/sham). Survival during the 5-month long experiment was 97%, suggesting negligible effects, specifically of hormone treatment on survival (Crossin et al. in review-B).

Acoustic telemetry

Salmon carrying transmitters were detected on telemetry receiver lines in marine and freshwater environments (Figure 3.1; KRC 2007). The Queen Charlotte Strait (QCS) receiver line was positioned across Queen Charlotte Strait just north of Port Hardy. The Northern Strait of Georgia (NSOG) receiver line was positioned across northern Strait of Georgia between the towns of Comox and Powell River BC. The QCS line was approximately 460 km from the tagging locale in Rennell Sound. These lines were intended to detect fish migrating along the eastern side of Vancouver Island. Two other lines, the Lippy Point (LP) line and the Juan de

Fuca (JDF) line, were positioned to detect fish migrating along the west coast of Vancouver Island.

Laboratory assays

Both DNA and scale analysis were used for population identification of biopsied sockeye with procedures used commonly on Fraser River sockeye (Beacham et al. 1995, 2004). Plasma concentrations of testosterone (T) and 17β-estradiol (E_2) were measured by radioimmunoassay and used the ratio of the two hormones to determine fish sex, as secondary sexual characteristic were not fully expressed at time of handling. Plasma concentration of ions (K^+, Cl^-, Na^+), glucose, lactate, and osmolality were quantified by procedures described in Farrell et al. (2001). A kinetic assay was used to assess gill tissue Na^+,K^+-ATPase activity (McCormick 1993).

Statistical analyses

Because of small samples sizes within some populations, I pooled populations within run timing groups. I used MANOVA to explore physiological differences among groups at time of capture. Variables included in the MANOVA models were: FL, Na^+, Cl^-, osmolality, lactate, glucose, gill Na^+,K^+-ATPase. If the sexes did not differ, these variables were pooled for subsequent MANOVA contrasts among run timing groups. Excluded from MANOVA models were variables known to vary fundamentally between the sexes (e.g. T, E_2, FL). I followed MANOVA analysis with a series of one-way analyses of covariance (ANCOVA) with population as the main effect and Julian date as a covariate to identify the relative importance of individual variables underlying multivariate relationships and to account for any variation due to sampling date as fish were captured over a six-day period. *A posteriori* tests were used to identify populations that differed when an ANCOVA model was significant. ANCOVA, with FL as covariate, was used to compare travel times among treatment groups for each physiological variable. I conducted a series of correlation analyses to assess relationships between travel times (from point of release to specific locations) and physiological variables. I conducted a series of ANCOVAs to compare the fate of fish (i.e. those successfully reaching the first acoustic lines versus those that did not) with respect to physiological variables. Finally, ANCOVA, with FL as covariate, was used to compare travel times among treatment groups.

All analyses were conduced using JMP 4.0. Because of multiple comparisons, I conducted Bonferroni to minimize the potential for Type II errors. I designated statistical significance at $\alpha=0.05$ and made Bonferroni corrections to minimize the possibility of false

positives. However, due to the high conservatism of Bonferroni corrections, I indicate significance at α=0.05, 0.01, and 0.001 (See Tables 3.1 and 3.2), thus allowing readers to define for themselves which levels are most biologically meaningful. Prior to analyses all physiological data were $\log_{10}$ transformed to reduce heteroscedasticity.

Results

Fish capture

In total, I biopsied, tagged and released 196 sockeye salmon: 59% were from Late-run populations bound for the Fraser River (N=116), 19% were from Fraser River Summer-run populations (N=38), and 17% were from Fraser River Early Summer-run populations (N=33). Only 5% of the fish (N=6) were from populations bound for other watersheds (i.e., central British Columbia coast, or Puget Sound in Washington State) or were unidentifiable. Six populations comprised 93.5% of all the Fraser River samples, with Adams sockeye dominating the total (59%). I limited statistical analyses to the largest six populations: Scotch (Early Summer-run), Birkenhead (Summer-run), Chilko (Summer-run), Horsefly (Summer-run), Stellako (Summer-run) and Adams (Late-run), and due to small samples sizes for some, populations were pooled within run-timing groups. For population specific physiological measures, see Hinch et al. (2007).

Baseline physiology

I found no significant differences among the three run timing groups in plasma Na^+, Cl^-, lactate, glucose, cortisol and gill Na^+,K^+-ATPase activity (MANOVA, Wilks' λ F=1.701, P=0.054, N=164). The only significant physiological difference among run-timing groups was in male testosterone (P=0.003, N=113; Table 3.1), though plasma Cl^- showed a modest difference among timing groups (P=0.03, N=174). Only gill Na^+,K^+-ATPase activity and testosterone had significant associations with Julian date for both sexes (ANCOVA, gill Na^+,K^+-ATPase activity P<0.001, [T] P<0.001; Table 3.1). GSE levels estimated from co-migrating sockeye did not differ significantly between Summer- and Late-run females (ANCOVA, P=0.127; Table 3.1). I did not collect any Early Summer-run sockeye. Early Summer-run males had lower GSE than either Summer- or Late-run males (ANCOVA, P = 0.018; Table 3.1). Ovary mass did not differ between Summer-run (mean ± SEM, 65.8 g ± 9.4) and Late-run females (77.0 g ± 6.0) (ANCOVA; group P=0.314, FL P=0.075, N=17). However, testes mass did differ among timing groups (ANCOVA, group P=0.026, FL P=0.073, N=16). Early Summer-run

males had heavier testes (58.3 g ± 11.3) than either Summer-run (28.4 g ± 15.7) or Late-run males (15.0 g ± 7.1).

Hormonal treatments

In total, 60% of the sockeye received hormonal injections (GnRH or GnRH+T) and 40% received a sham injection or were controls without knowing the population for any individual. Subsequent DNA analyses revealed that 26 Scotch sockeye (Early Summer-run) were treated as follows: 6 GnRH, 6 GnRH+T, 6 sham and 8 control; 6 Birkenhead sockeye (Summer-run) were treated as follows: 2 GnRH, 1 GnRH+T, 1 sham and 2 control; 11 Chilko sockeye (Summer-run) were treated as follows: 3 GnRH, 2 GnRH+T, 2 sham and 4 control; 14 Horsefly sockeye (?-run) were treated as follows: 2 GnRH, 9 GnRH+T, 2 sham and 1 control; 7 Stellako sockeye (?-run) were treated as follows: 3 GnRH, 2 GnRH+T, 0 sham and 2 control; and 110 Adams sockeye (Late-run) were treated as follows: 39 GnRH, 28 GnRH+T, 11 sham and 32 control.

Travel times and fate

All detected fish were males and detections occurred between August 9-17. In total, 7% of tagged Fraser River sockeye (N=13) reached the northern end of Vancouver Island (Figure 3.1) and similar numbers were hormone-treated (3 GnRH, 4 GnRH+T) and non-treated (4 sham, 2 control). Eleven sockeye were detected on the Queen Charlotte Strait (QCS) receiver line, and 1 was detected on the Lippy Point lines). Another fish (GnRH-treated) by-passed detection on the Lippy Point line but was detected further south on the Juan de Fuca line. Of the 12 fish detected on the QCS and Lippy Point lines, 31% (N=4: 2 hormone treated (1 GnRH, 1 GnRH+T) and 2 non-treated (both sham) were subsequently detected in the Fraser River. At least one fish from all seven populations of Fraser sockeye and from all four treatment types were detected, but the small sample size required that I pool run timing groups as well as hormone-treated groups (GnRH and GnRH+T)' and non-treated' groups (sham and control) for subsequent analyses, focusing fish detected at Queen Charlotte Strait and at Lippy Point (i.e., the first acoustic lines at ~480 and 490 km, respectively, from the tagging site in Rennell Sound; Figure 3.1) and comparing them with undetected salmon. I found no difference in body length-corrected travel time between hormone-treated and non-treated fish (ANCOVA, P=0.138; mean travel time=11.9 days ± 0.7; mean migration speed=41.0 km·day^{-1} ± 0.8). A negative significant correlation ($p < 0.05$) was found between travel time and plasma testosterone concentration (Pearson's r=-0.813, P=0.0013, N=12) and a significant positive correlation with plasma Cl^- concentration (Pearson's r=0.817, P=0.0012, N=12) (Figure 3.2).

Comparison of physiology in detected and not detected sockeye

I found no differences in physiological or size measures between detected (N=12) and non-detected ones (N=183) sockeye, although Julian date was significant for some of the variables (Table 3.2). I explored the physiological stress aspect by examining the data distribution for cortisol and lactate concentrations using box and whisker plots (Figure 3.3). Generally, non-detected fish had more variable cortisol and lactate concentrations, with 20-30% greater range and with the 25th to 75th percentiles overlapping for non-detected and detected fish. I also present similar plots created for cortisol and lactate concentrations from biopsied fish captured and released in Johnstone Strait and which were subsequently either detected or not detected entering the Fraser River (data from Chapter 5). The data from Chapter 5 provide a relevant contrast as they are independent from those in the present study, they represent much larger sample sizes in terms of detected versus not detected, and fish handling approaches were similar in many regards though they were less invasive as they did not involve anesthesia or surgical implantation of transmitters, and handling time was probably less for each fish. The 25th to 75th percentile range for not detected and detected groups showed nearly complete overlap for both variables (Fig. 3). The only difference was that not detected fish had more lactate concentration data points that exceeded the 95th percentile.

Discussion

Baseline physiological patterns

By intercepting Fraser River sockeye salmon at sea, over 850 kms away from the mouth of their natal river system and early stage of homeward migration, I found that testosterone concentrations varied among run-timing groups in a way that was similar to that observed near the river mouth shortly before upriver migration (Cooke et al 2006b). In contrast, population-specific trade-offs between somatic energy and egg production, as found in previous studies of adult Fraser sockeye at the start of river migration (Crossin et al. 2004), were not found. Trade-off between somatic energy and reproductive investment occurs in many animals (Stearns 1992), and has been commonly observed in salmon at the start of upriver migrations (Hendry and Berg 1999; Kinnison et al 2001; Crossin et al. 2004). Possibly low sample sizes (N=35) limited my ability to detect differences among groups. Equally likely is that active foraging precludes this difference. Circulating T and E_2 in females certainly did not differ among the run-timing groups, which probably underlies the lack of difference observed in ovary mass.

In complete contrast, circulating T levels in males were significantly different among

run-timing groups, being positively correlated with river entry timing. Early Summer-run males had the highest (at least twice as high) T concentrations, and T concentrations were progressively smaller in Summer-run, then Late-run fish. Similarly, testes mass was heaviest in Early-summer and the lightest in Late-run males.

The physiological concentrations of all other stress (lactate, glucose, cortisol) and osmoregulatory (Na^+, osmolality, gill Na^+,K^+-ATPase) variables did not differ, which is consistent with observations from other years and sites where homing Fraser sockeye were sampled (Cooke et al. 2006a, 2006b; 2008; Hinch et al. 2006). Plasma chloride concentration for Late-run sockeye was higher than Early Summer and Summer run sockeye, but the biological relevance of this difference is unclear.

Ocean migration rates and effects of reproductive hormone enhancement

I also explored the hypothesis that GnRH and testosterone influence salmon migration rates to a natal river. On one hand I predicted that reproductively more advanced sockeye would travel faster through the marine environment. Previous studies have shown correlations between reproductive hormone levels and migration timing that support this position (Cooke et al. 2006, 2008), but experimental work had been lacking. Therefore, experimental hormonal manipulations might delineate proximate mechanisms affecting migratory speeds and timing. Despite the small number of fish that were actually detected at acoustic receiver stations, marine travel rates were similar for equal numbers of non-treated and hormone-treated fish. It was impossible to assess whether or not run-timing of the population played a role marine migration rate.

Regardless, travel times were strongly correlated with initial T and plasma chloride concentrations, but no other measured variable; salmon with relatively high T (as predicted) and low chloride levels traveled the fastest. Thus, it was surprising that the hormone treatments had no detectable effect. In contrast, as sockeye migrate towards freshwater environments, plasma chloride levels decline (Hinch et al. 2006). It is thus possible that the positive correlation between plasma Cl^- concentration and travel time reflects ionoregulatory preparedness, and perhaps motivation, for migration towards natal rivers. The observed range in initial chloride levels was very similar to that observed from Fraser sockeye captured in the same locale in 2003, the only other year for which data are available (Hinch et al. 2006).

The low detection rate of acoustic tagged sockeye in this study was unexpected given previous successes using similar protocols. The excellent survival of pre-adult pink salmon (97%) after similar hormone treatments (Chapter 4- Crossin et al. in review-B), and the similar

detection rate for untreated and hormone-treated fish here suggest that the treatments per se were not a major problem. Instead, my fish collection procedures at sea could have been a factor, imposing significant stress on some individuals, which then suffered post-release mortality. However this should have been revealed by the physiological variables, especially cortisol and lactate concentrations, which did not differ between detected and undetected fish. In fact, some of the undetected fish had some of the lowest lactate and cortisol concentrations and several of the detected fish had levels of lactate exceeding 12 $mmol \cdot L^{-1}$, which laboratory studies suggest is a critical survival threshold (Jain and Farrell 2003). The handling procedures used here were certainly more invasive and time consuming than previous biopsy telemetry studies (Cooke et al. 2004, Cooke et al. 2006, Cooke et al. 2008, Chapter 2- Crossin et al. 2007) (e.g. they involved operating in rough seas, and using anesthesia and surgical procedures). Even so, intra-individual variation in cortisol and lactate concentrations for the undetected fish was remarkably similar to that from a companion biopsy telemetry study occurring that same year, later in the season with the same populations of sockeye (Figure 3.3).

Interception fisheries are an equally plausible factor for the low detection rate. Commercial and Native fisheries were very active in locales throughout the migration route during the period that I released the tagged fish. However, only a few of the fish (4%) were reported as being captured. Even so, significant under-reporting of transmitters in salmon telemetry studies is common (see Robichaud and English 2007). This return percentage was similar to that derived from other release platforms in other years (Chapter 5- Crossin et al. in review-A). Certainly, commercial and Native fisheries were very active during the migration of the tagged fish in locales along their migratory route. Daily exploitation rates in northern Vancouver Island waters near the QCS acoustic line and onward into Johnstone Strait (see Figure 3.1) during the time that I estimate tagged fish were migrating through ranged from 0-70% with a daily average of 45% during the first two weeks of August. This represents a total harvest in excess of 1.1 million sockeye (unpublished data from Jim Cave, Pacific Salmon Commission, Vancouver, BC). This estimate does not include First Nations fisheries which were also occurring or fisheries occurring seaward of the QCS line. Considering that I tagged only 196 sockeye over a very narrow window of sampling dates, and given their tendency toward schooling, it seems very possible that many of the non-detected fish may have been removed by the fishery. Commercial harvest estimates further along the migration route through Johnstone Strait and into the Strait of Georgia were 51% between Aug 17-26. Assuming a 10 day migration from QCS to the Strait of Georgia, this exploitation rate can explain the loss of 8

of the 11 migrating past QCS. Detection efficiency of the acoustic lines was quite high (~98%; KRC 2007), so non-detection due to technical shortcomings is an unlikely explanation.

It is quite possible that the low detection of sockeye in this study is due to the cumulative effects of fisheries harvest, initial physiological state, predation, and other environmental stressors. Whatever the cause, I was not able to fully evaluate the hypothesis that advanced maturation via exogenous hormone treatments leads to faster rates of migration. However, the novel information from the baseline physiological measures revealed interesting patterns of GSE and ovarian investment that are consistent with results of other studies conducted much closer to natal rivers. That actively foraging female sockeye are investing toward egg production in a similar manner as when they are not feeding suggests that rates of development are endogenously programmed (see Patterson et al. 2004), as evidence by the consistently significant and positive influence of Julian date on circulating sex steroid concentrations and negative influence on gill Na^+,K^+-ATPase activities.

Table 3.1. Least squares means (+/- SEM), sample sizes, and P values for ANCOVAs of physiological differences, with Julian date as the covariate, among run-timing groups of homing Fraser River sockeye salmon (*Oncorhynchus nerka*) captured in Rennell Sound, Queen Charlotte Islands in 2006. Data were pooled except where gender symbols are indicated. The direction of the relationship between Julian date and a given physiological variable, when significant, is indicated by (+) or (-).

Variables by sex	Run timing group	Least squares mean ± SEM	N	Run timing group P	Julian date P
Gross somatic energy † ($MJ \cdot kg^{-1}$)	Early Summer ♀	n/a	n/a	0.127	n/a
	Summer ♀	10.6 ± 0.28	5		
	Late ♀	10.0 ± 0.18	12		
	Early Summer ♂	9.1 ± 0.27 [a]	4	0.018*	n/a
	Summer ♂	9.8 ± 0.38 [b]	3		
	Late ♂	10.2 ± 0.17 [b]	10		
Nose to fork length (cm)	Early Summer ♀	57.10 ± 0.67	10	0.866	0.985
	Summer ♀	57.00 ± 0.60	13		
	Late ♀	57.34 ± 0.35	35		
	Early Summer ♂	59.58 ± 0.72	13	0.586	0.551
	Summer ♂	58.99 ± 0.50	27		
	Late ♂	58.78 ± 0.31	73		
Plasma glucose ($mmol \cdot L^{-1}$)	Early Summer	7.21 ± 0.26	24	0.060	0.324
	Summer	7.82 ± 0.19	40		
	Late	7.34 ± 0.11	110		
Plasma lactate ($mmol \cdot L^{-1}$)	Early Summer	10.97 ± 1.01	24	0.699	0.312
	Summer	11.13 ± 0.73	40		
	Late	11.17 ± 0.44	110		
Plasma Na^+ ($mmol \cdot L^{-1}$)	Early Summer	179.31 ± 2.33	24	0.421	0.780
	Summer	180.88 ± 1.70	40		
	Late	182.35 ± 1.02	110		
Plasma Cl^- ($mmol \cdot L^{-1}$)	Early Summer	151.77 ± 1.53 [a]	24	0.030*	0.316
	Summer	153.37 ± 1.11 [a,b]	40		
	Late	155.60 ± 0.67 [b]	110		
Plasma osmolality ($mOsm \cdot kg^{-1}$)	Early Summer	380.96 ± 4.33	24	0.407	0.200
	Summer	382.15 ± 3.16	40		
	Late	386.04 ± 1.90	110		
Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	Early Summer	3.19 ± 0.43	24	0.279	**<0.001 (-)**
	Summer	3.32 ± 0.33	40		
	Late	3.79 ± 0.19	110		
Plasma cortisol ($pg \cdot ml^{-1}$)	Early Summer	369.67 ± 15.37	24	0.966	0.201
	Summer	373.88 ± 11.54	40		
	Late	370.68 ± 6.94	110		

Variables by sex	Run timing group	Least squares mean ± SEM	N	Run timing group P	Julian date P
Plasma testosterone ($pg \cdot ml^{-1}$)	Early Summer ♀	121.00 ± 28.25	10	0.483	**<0.001 (-)**
	Summer ♀	86.78 ± 13.26	13		
	Late ♀	78.51 ± 23.67	35		
	Early Summer ♂	89.01 ± 13.12 [a]	13	**0.003**	**<0.001 (-)**
	Summer ♂	46.33 ± 9.09 [b]	27		
	Late ♂	38.69 ± 5.61 [b]	73		
Plasma 17β-estradiol ($pg \cdot ml^{-1}$)	Early Summer ♀	4.96 ± 1.52	10	0.215	0.112
	Summer ♀	4.45 ± 1.38	13		
	Late ♀	3.59 ± 0.80	35		

All variables were $\log_{10}$ transformed prior to analysis. Values marked with an asterisk (*) indicates significance at α=0.05 and bold faced values indicate significance at Bonferroni corrected α-values: 0.005 for females, 0.006 for males.

† Due to technical difficulties, I could not measure somatic energy in the biopsied fish. Energy data were therefore collected from sockeye captured on a single date in the middle of the sampling period and were sacrificed for more extensive physiological examinations.

Table 3.2. Least squares means (+/- SEM), sample sizes, and P values for ANCOVAs of physiological differences, with Julian date as the covariate, between fate groups of homing Fraser River sockeye salmon (*Oncorhynchus nerka*) captured in Rennell Sound, Queen Charlotte Islands in 2006. Data were pooled except where gender symbols are indicated. Fish are classified as 'detected' on acoustic receivers at northwestern Vancouver Island versus those that were not 'detected'. Only male fish were detected. The direction of the relationship between Julian date and a given physiological variable, when significant, is indicated by (+) or (-).

Variables by sex	Fate	Least squares mean ± SEM	N	Fate P	Julian date P
Gross somatic energy ($MJ \cdot kg^{-1}$)	Detected ♀	n/a	n/a	n/a	n/a
	Not detected ♀	n/a	n/a		
	Detected ♂				
	Not detected ♂				
Nose to fork length (cm)	Detected ♀	n/a	n/a	n/a	n/a
	Not detected ♀	n/a	n/a		
	Detected ♂	60.14 ± 0.81	13	0.111	0.304
	Not detected ♂	58.77 ± 0.25			
Plasma glucose ($mmol \cdot L^{-1}$)	Detected	7.64 ± 0.39	13	0.518	0.328
	Not detected	7.43 ± 0.10	174		
Plasma lactate ($mmol \cdot L^{-1}$)	Detected	9.56 ± 1.50	13	0.241	0.542
	Not detected	11.24 ± 0.36	174		
Plasma Na^+ ($mmol \cdot L^{-1}$)	Detected	176.31 ± 3.47	13	0.106	0.689
	Not detected	182.08 ± 0.84	174		
Plasma Cl^- ($mmol \cdot L^{-1}$)	Detected	150.99 ± 2.33	13	0.099	0.170
	Not detected	154.79 ± 0.56	174		
Plasma osmolality ($mOsm \cdot kg^{-1}$)	Detected	377.95 ± 6.50	13	0.288	0.193
	Not detected	384.94 ± 1.57	174		
Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	Detected	4.15 ± 0.71	13	0.536	**<0.001 (-)**
	Not detected	3.68 ± 0.17	174		
Plasma cortisol ($pg \cdot ml^{-1}$)	Detected	338.05 ± 23.35	13	0.769	0.021* (-)
	Not detected	375.34 ± 5.64	174		
Plasma testosterone ($pg \cdot ml^{-1}$)	Detected ♀	n/a	n/a	n/a	n/a
	Not detected ♀	n/a	n/a		
	Detected ♂	37.53 ± 15.29	13	0.568	**<0.001 (-)**
	Not detected ♂	47.33 ± 4.81			
Plasma 17β-estradiol ($pg \cdot ml^{-1}$)	Detected ♀	n/a	n/a	n/a	n/a
	Not detected ♀	n/a	n/a		

All variables were $\log_{10}$ transformed prior to analysis. Values marked with an asterisk (*) indicates significance at α=0.05 and bold faced values indicate significance at Bonferroni corrected α-values: 0.005 for females, 0.006 for males.

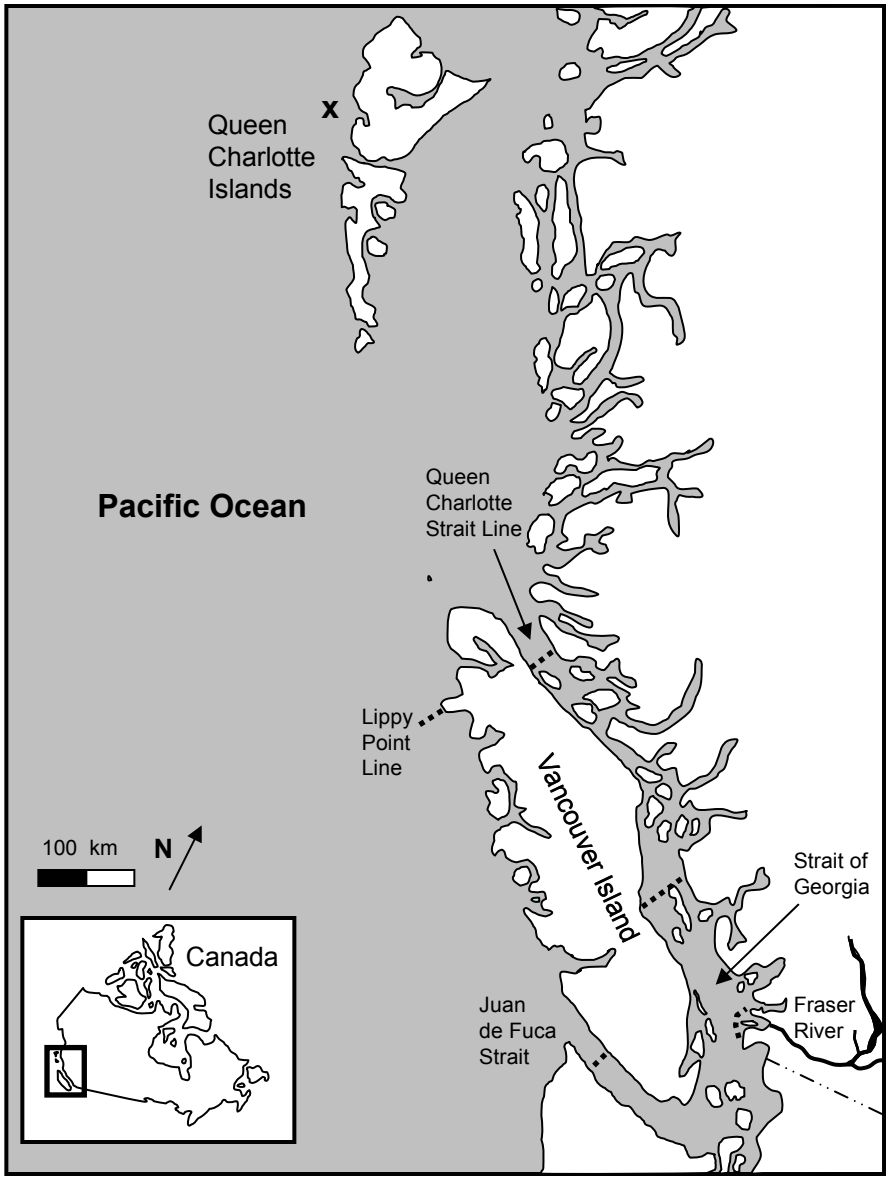

Fig. 3.1: Map of the northeast Pacific Ocean and coastal British Columbia, with an inset of Canada, showing the location in Rennell Sound, Queen Charlotte Islands (marked with 'x'), where homing sockeye salmon were captured by purse seine, biopsied, tagged, hormonally implanted, and released. Also shown are the locations of acoustic receiver arrays (dashed lines) where a homing salmon could be detected whilst homing toward and into the Fraser River.

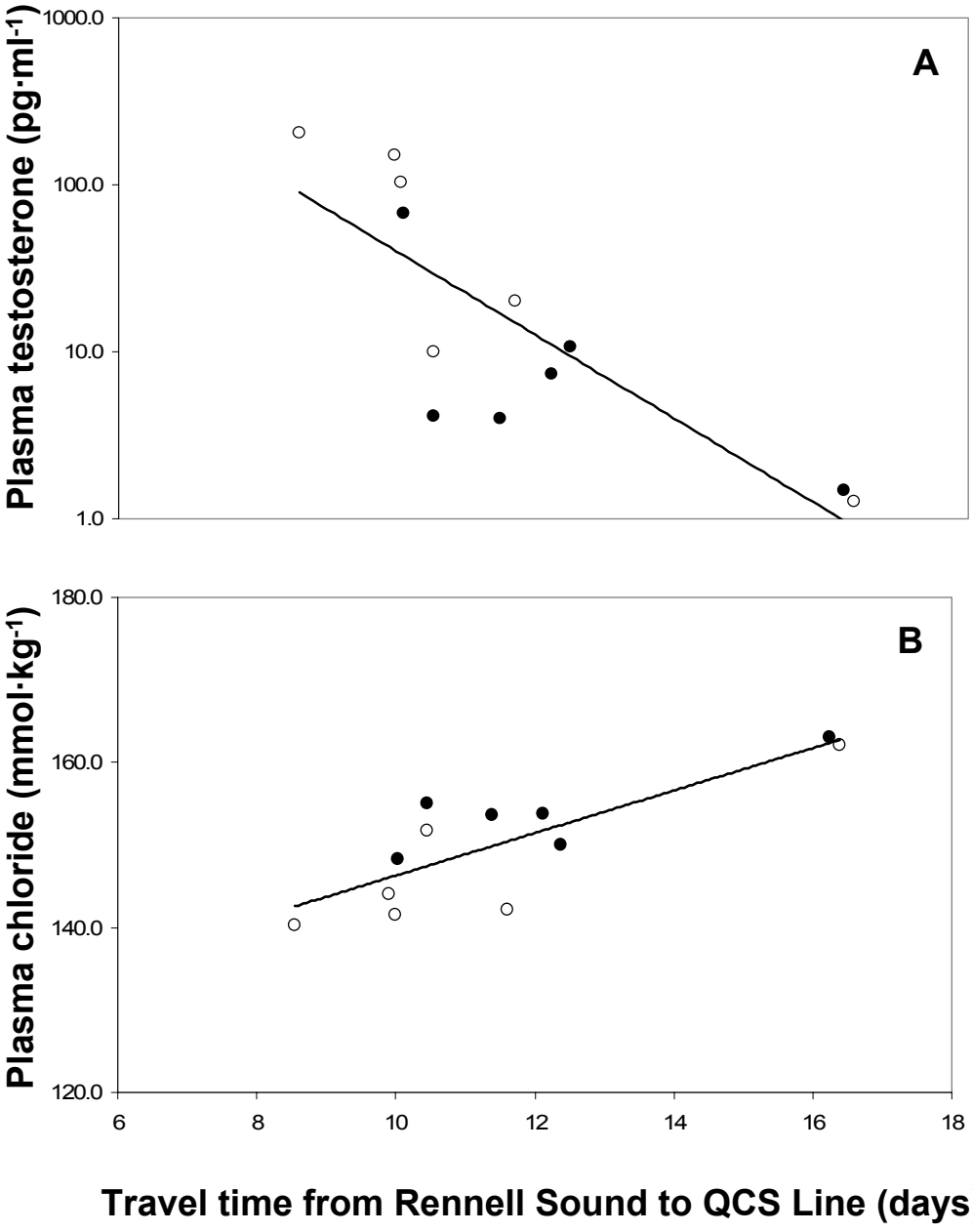

Fig. 3.2: Relationships of plasma testosterone (panel A) and chloride (panel B) with travel times of sockeye salmon after being biopsied, tagged, and hormonally implanted in Rennell Sound, Queen Charlotte Islands. Black points are hormonally treated sockeye and open points are control and sham-treated. The acoustic receiver line at Queen Charlotte Strait (QCS line) was ~480 km from the release locale in Rennell Sound. Due to small samples size, run-timing groups and hormonal treatments were pooled for the analysis. Separate ANCOVAs revealed no significant differences between travel times to QCS by run-timing or hormonal treatment (see Results section).

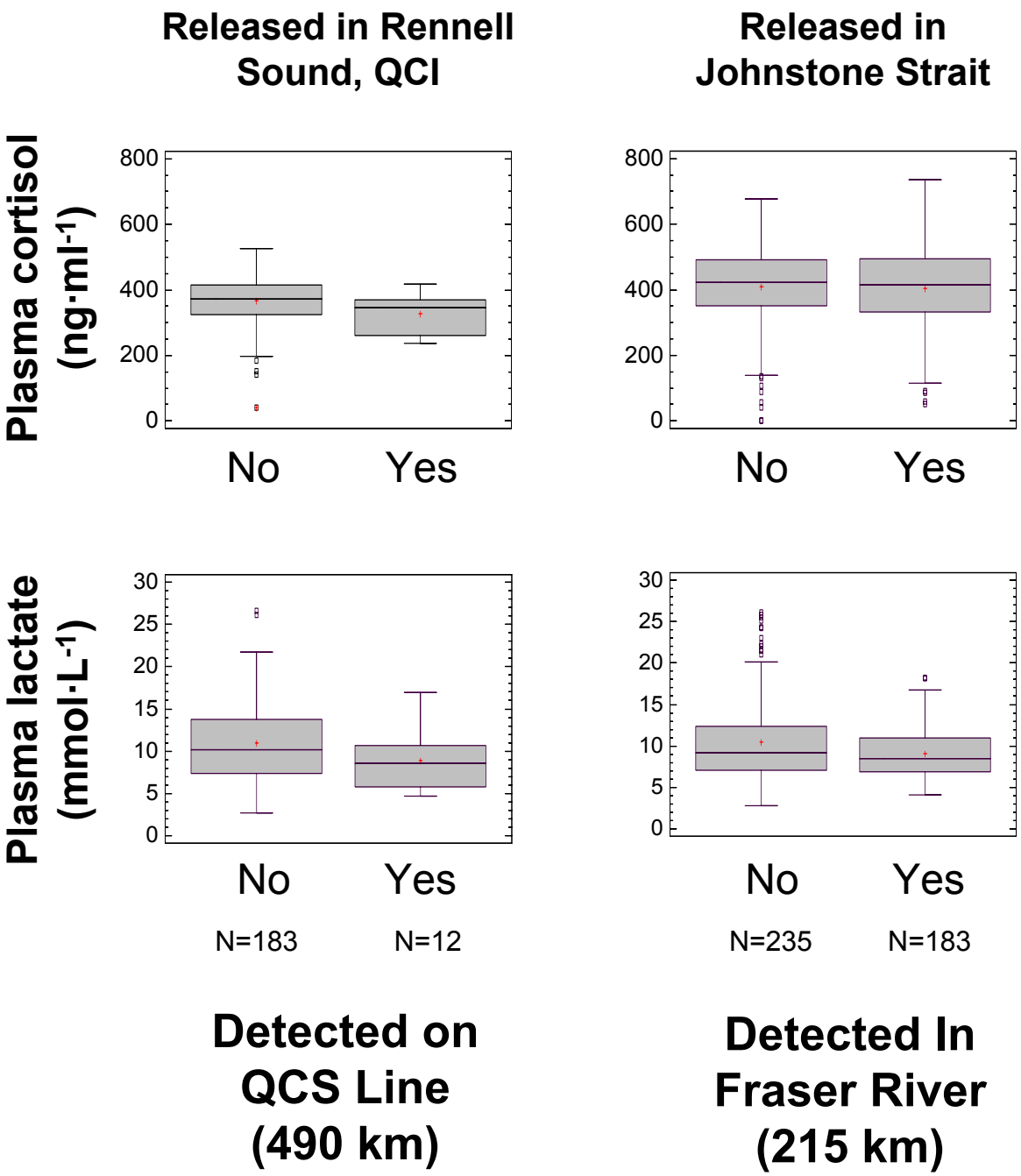

Fig. 3.3: Box and whisker plots showing the median (horizontal line in grey box), the 25th and 75th percentiles (upper and lower lines of grey box), the 5th and 95th percentiles (upper and lower error bars) and extreme data points outside of those ranges, of plasma cortisol and lactate concentrations in sockeye salmon captured in Rennell Sound (left) and Johnstone Strait (right, data from Chapter 5), implanted with acoustic transmitters, and detected on acoustic receiver lines long distances from their release locales. 'No' indicates that fish were not detected on that acoustic line and 'Yes' indicates fish were detected.

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CHAPTER 4

An experimental evaluation of the effects of GnRH and testosterone enhancement on the reproductive and osmoregulatory physiology of sub-adult pink salmon.[3]

Introduction

The initiation of reproductive migrations has been correlated in many animals with changing environmental photoperiods, an increased secretion of hypothalamic gonadotropin-releasing hormone (GnRH), and the initiation of gonadal development (birds- Dawson et al. 2001; Pacific salmon [*Oncorhynchus* spp.]- Ueda and Yamauchi 1995; Hinch et al. 2006; general review- Dingle 1996). Though the precise physiologic pathways that regulate the production and release of GnRH and subsequent activation of the hypothalamo-pituitary-gonadal axis (HPG axis) have yet to be fully elucidated, evidence points to the presence of photo-sensitive circannual oscillators in the pineal gland, other brain regions, and retinal cells that entrain photoperiodic cues to regulate the nocturnal secretions of melatonin (Lincoln et al. 2003; Falcón et al. 2007). By so doing, the relative concentrations of melatonin secreted at different times of year provide animals with an endogenous, hormonal means with which to measure day-length, or more precisely, night-length. At the onset of migrations, when days grow longer and nights shorter, the declining secretion of nocturnal melatonin stimulates GnRH cell bodies into reproductive modes, and a cascade of hormonal messengers spill through the HPG axis leading to the production of testosterone and its derivatives, and ultimately to the production of gametes. However, steroidal feedback to the central nervous system and brain also occurs, eliciting pleiotropic effects on behaviour which can motivate animals into migratory modes (Dingle 1996).

The integration of environmental and endogenous systems allows animals to adapt their physiology and behaviour to seasonally changing selective pressures. In birds, seasonal increases in photoperiod have been correlated with increased testosterone secretion and *zugenrhue*- the state of nocturnal restlessness that precedes vernal migration (Wingfield et al. 1990; Dingle 1996). Testosterone has also been correlated with other seasonal behaviours

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related peripherally to reproduction (aggression and song; Wikelski et al. 1999; Wingfield et al. 2003). Tests of hormonal effects on migratory behaviour involved the experimental implantation of GnRH in lacustrine sockeye salmon (i.e. kokanee salmon, the potamodromous sub-species of anadromous *Oncorhynchus nerka*). This resulted in an increase in circulating testosterone concentrations and a premature migration from the open lake environment to a natal inlet stream for spawning (Sato et al. 1997; Kitahashi et al. 1998). Furthermore, descriptive studies with homing sockeye salmon document an effect of increasing seasonal testosterone concentrations in the regulation of migration timing into freshwater (Cooke et al. 2006a, 2008; Chapter 2- Crossin et al. 2007; Chapter 5- Crossin et al. in review-A).

With few exceptions, the HPG axis is usually inhibited by stress (Sapolsky 2002). During stress, the activation of the hypothalamo-pituitary-adrenal (HPA) axis (or in fish, the homologous hypothalamo-pituitary-interrenal [HPI] axis) leads to the secretion of the corticosteroids, which mobilize stored energy to facilitate the appropriate responses to stress but which also have strongly anti-gonadotrophic effects. In times of stress, it is perhaps adaptive to bring costly anabolic processes to a temporary halt and direct resources to glycolytic, lipolytic, and gluconeogenic metabolism to affect immediate survival (e.g. locomotion). In salmon, cortisol is the principle stress hormone, and during spawning migrations it is secreted in massive quantities (upwards of ~650 $ng \cdot ml^{-1}$; Carruth et al. 2000; Cooke et al. 2006a,b; Hinch et al. 2006), which for most animals would be more than sufficient to completely inhibit HPG axis functionality (Sapolsky 2002). Yet in homing salmon, cortisol does not generally inhibit reproduction but seems an integral part of both reproductive and osmoregulatory development (Makino et al. 2007) and their programmed death after spawning which so characterizes their life-history (Wingfield and Sapolsky 2003; but see Pankhurst and Van Der Kraak 2000).

Recent studies suggests that an HPG/HPI cross-axis stimulation occurs during reproductive development in salmon via sustained cortisol release which, in conjunction with prolactin and thyroid hormones, enables a suite of osmoregulatory changes that are necessary for the salt-to-freshwater transition during homeward migration (Norris and Hobbs 2006). Cortisol can provide positive feedback to the HPG axis by enhancing the sensitivity of gonadal receptors to gonadotropin binding (Hirano et al. 1990; Uchida et al. 1997; Seidelin et al. 1997). Cortisol also affects gill Na^+,K^+-ATPase expression and activity- the principle osmoregulatory enzyme that must be down-regulated when traversing through declining salinity gradients (Norris and Hobbs 2006). The resultant changes in gill membrane permeability and ion-uptake capacity that allow salmon to retain ions to counter the diluting effects of freshwater residence occur in

parallel with reproductive development. Thus, rather than working in opposition, the two axes appear to work collectively in salmon to ensure a timely migration and successful spawning. Cortisol still has a role to play in the stress response, but during maturation the responsiveness of the HPA axis likely receives a new, higher set-point to limit the negative feedback effects of cortisol secretion (Pottinger et al. 1995; Carruth et al. 2000; Wingfield and Sapolsky 2003). This is presumably adaptive in an animal that makes exceptionally long migrations and has only one life-time opportunity to reproduce (i.e. semelparity; Burgner 1991). Only at times of acute stress does cortisol appear to be anti-gonadotrophic (Pankhurst and Van Der Kraak 2000). For example, the stress associated with passage through a stretch of exceedingly turbulent rapids in the Fraser River canyon of British Columbia (aptly named Hell's Gate) temporarily depressed the secretion of testosterone (T) and 17β estradiol (E_2) in homing sockeye (Hinch et al. 2006).

Previous studies examining the reproductive and stress physiologies and behaviour of homing sockeye salmon have uncovered links between reproductive maturity, osmoregulatory capacity and migratory success (Cooke et al. 2008; Chapter 5- Crossin et al. in review-A). These studies point to a coordination of HPG and HPI functionalities. In order to test hypotheses about hormonal regulation of migratory behaviour, I implanted homing sockeye with GnRH either alone or in combination with T and compared migratory behaviour to that of sham and control animals (Chapter 3- Crossin et al. in review-B). Marine travel times were monitored with acoustic telemetry as fish migrated from the capture site in the North Pacific Ocean to the mouth of a natal river over 800 km away. Interpreting the telemetry data however may be complicated by potential cross-axis stimulation in response to GnRH implantation (as described above). For example, if hormonally implanted salmon show a change in migratory behaviour relative to shams and controls, was it due to a GnRH mediated stimulation of the HPG or HPI axis, or both? And was the change in behaviour initiated by stimulation of sex steroid production, or enhanced osmoregulatory ability as fish moved toward freshwater?

The present experiment was conducted to further evaluate the temporal pattern of reproductive and osmoregulatory physiology in maturing Pacific salmon, held in saltwater, in response to GnRH and testosterone implantation. These data will help provide a means with which to interpret my parallel study of GnRH and T treatment on migratory behaviour (Chapter 3- Crossin et al. in review-B). More generally though, few studies have investigated the functional interaction of GnRH and steroidal co-treatment on reproductive and osmoregulatory physiology (but see Ando et al. 2004), despite numerous studies investigating their individual effects on either or both processes. A possible interaction between GnRH and the sex steroids

can be inferred from studies showing a link between the responsiveness of gonadotropins to GnRH and circulating steroid levels (Ando et al. 2004). GnRH and steroidal interactions should thus be examined to fully understand the neuroendocrine control of reproductive development and other processes. Particular attention will be paid to the stage of maturity at which pink salmon will be implanted, for the response to hormonal treatment depends very much on this variable (Dickey and Swanson 1998; Antonopoulou et al. 1999).

My working hypothesis was that GnRH and T would collectively elicit a reproductive and osmoregulatory response. Using sub-adult salmon held in saltwater during the 5 months preceding natural spawning dates, I predicted (1) that both males and females co-treated with GnRH and T would have higher plasma T concentrations relative to controls at the end of the experiment, (2) that females would have higher E_2 concentrations relative to controls, and (3) that larger livers and ovaries would develop as a result of E_2 mediated vitellogenesis. I also predicted (4) that GnRH+T treatment would stimulate plasma cortisol secretion due not from stress, but from a programmed cross-axis stimulation of the HPI axis as fish mature and prepare for freshwater entry - an essential component of the reproductive cycle. As such, I predicted (5) that GnRH+T treated fish would have down-regulated gill Na^+,K^+-ATPase activities. I did not predict other osmoregulatory indicators like plasma ions or osmolality to differ between hormonally treated and control fish as I expected all salmon to maintain homeostasis irrespective of treatment.

Methods and Materials

Study Animals and Experimental Design

My study animals were pink salmon (*O. gorbuscha*), an anadromous, semelparous species whose two-year life history begins when adults spawn in freshwater streams in autumn, then fertilized eggs incubate in redds overwinter and fry emerge in spring at which time they migrate immediately to the ocean. The time from egg fertilization to smolt out-migration takes about 6 months. The next 16-18 months are spent rearing and maturing on the high seas, then adults return to natal streams to spawn (Heard 1991). In November, 2004, fertilized eggs were collected at the Quinsam Hatchery on Vancouver Island, located near Campbell River, British Columbia. Fertilized eggs were collected and transferred to the Canadian Department of Fisheries and Oceans' Pacific Biological Station in Nanaimo, British Columbia. They were incubated in freshwater Heath trays until hatch after which they were transferred to large tanks containing ambient seawater pumped from the adjacent Strait of Georgia. Salinity and

temperature varied seasonally (~26-32‰ , ~6.7-18.3 ºC). Though housed indoors, the facility had windows to allow natural photoperiods. Facility lights were shut off at dusk each day.

When the pink salmon were 15 months old, 236 of them were transferred to the a Fisheries and Oceans Canada Laboratory in West Vancouver, British Columbia where I conducted my experiments. Fish were divided randomly among four outdoor tanks that were supplied with continuous, ambient seawater (Strait of Georgia, ~26-32‰, 8.9-12.3ºC). The tanks were large enough to provide room for swimming, and the circular flow allowed fish to hold station at 1.5 $BL \cdot sec^{-1}$ at the periphery, or less if fish moved toward the centre standpipe. Tanks were covered with netting to prevent avian predation, and were partially shaded to reduce the intensity of direct sunlight, but they were effectively exposed to full, natural photoperiods. Fish were, and had been previously, fed daily to satiety with a commercial fish feed (Skretting Canada, 1370 East Kent Ave., Vancouver, BC, V5X 2Y2). Transport permits and veterinary inspections were obtained from Fisheries and Oceans Canada.

Experimental design and hormonal delivery system

Pink salmon were acclimated for 3 months (February through April, 2006), then the experiment began and ran for 5 months. Thus, the salmon were ~23 months of age at the end of the experiment and were within a few weeks of their natural spawning dates. At the start of the experiment on April 28th, fish were individually and randomly removed from tanks by dipnet and transferred to a bucket with aerated, ambient seawater (~27‰, 9 ºC), and anesthetized in a dilute bath (20 $mg \cdot L^{-1}$) of tricane methane sulfinate (i.e. MS-222). Fish were then implanted with a PIT tag each bearing a unique identification number. Nose-to-fork length (FL), post-orbital-to-hypural length (POH), and body depth were measured to the nearest millimetre (mm), and body weight was recorded to the nearest 0.1 g. To provide a sustained, 8-week delivery of hormones to salmon, biodegradable microspheres were prepared according to a modified solvent-evaporation method (Mylonas et al. 1995; Mylonas and Zohar 2001, Taranger et al. 2003). Individual fish received an injection of one of three hormonal treatments or they received a sham injection (saline). Injections were delivered via injection to the dorsal sinus between the epaxial musculature, posterior to the dorsal fin. Treatments were: (1) 150 $\mu g \cdot kg^{-1}$ GnRH, (2) 150 $\mu g \cdot kg^{-1}$ GnRH and 4 $mg \cdot kg^{-1}$ T, (3) 4 $mg \cdot kg^{-1}$ T, or (4) sham injection (saline). Fish were then placed into four identical tanks according to treatment. The treatment groups were maintained separately because there is evidence in the literature that social signals can influence the HPG

axis, and that individuals treated with androgens, or naturally secreting high levels, can exert a disproportional influence on GnRH regulation in subordinate individuals (Soma et al. 1996).

Time-series biological sampling

At each sampling date, 10 fish were removed from each treatment tank and killed by concussion, and their identity was determined with a PIT tag reader. Nose-to-fork length (FL), post-orbital-to-hypural length (POH), and body depth (BD) were all measured to the nearest millimeter. Body weight was measured to the nearest 0.1 g. For assessing plasma biochemistry, a 1.5 ml blood sample was then taken from the caudal vein using a 1.5", 23 gauge heparinized [lithium] vacutainer syringe (Houston 1990). For assessing gill Na^+,K^+-ATPase activity, six to eight gill filament tips (~0.03 g) were clipped from the first gill arch (McCormick 1993). Blood samples were immediately centrifuged for 10 minutes at 3000 rpm to separate plasma from red blood cells and plasma fractions were pipetted into separate, numbered cryo-vials. Gill tissues were also placed in numbered cryovials. Gill and plasma samples were then stored on dry ice for several hours until transfer to a –80 ˚C freezer. Ovaries and testes were then dissected from the carcass and weighed to the nearest 0.1 g. The sex of each fish was recorded at this time. Livers were also dissected and weighed to the nearest 0.1 g. GSI and HIS were calculated as: gonad or liver mass/total mass*100. Condition factor (K) was calculated for each fish as $K=M \cdot 100/FL^3$, where M=mass of the fish.

Laboratory assays

Concentrations of plasma T and E_2 were measured by radioimmunoassay (McMaster et al. 1992). Plasma ions (Na^+, Cl^-), glucose, lactate, osmolality, and cortisol were quantified by procedures described in Farrell et al. (2001). Gill tissue Na^+,K^+-ATPase activity was determined by kinetic assay (McCormick 1993).

Ovarian histology

On each sampling date, dissected ovaries fixed in Bouin's fixative for 6 h then transferred to 70% ethanol for at least a week. Samples were then embedded in wax, sectioned (3µm), and stained with heamatoxylin and eosin stain. Stained section were then mounted onto slides and morphology examined with a dissecting scope. Classification of the developing oocytes was guided by methods outlined in Tyler and Sumpter (1996).

Statistical analyses

All biological data were examined for normality and variables were transformed when necessary to reduce heteroscedasticity. I used MANCOVA to explore physiological differences between the sexes whilst accounting for allometric variation related to size (POH). Variables examined were: plasma $[Na^+]$ and $[Cl^-]$, plasma osmolality, plasma lactate and glucose, and gill Na^+,K^+-ATPase activities. Sex specific variables were excluded from this analysis: FL, [T], and $[E_2]$ (females only). If the sexes did not differ, variables were pooled in subsequent analyses. I then examined multivariate differences by treatment group, incorporating into the MANOVA model plasma $[Na^+]$ and $[Cl^-]$, osmolality, lactate, glucose, and gill Na^+,K^+-ATPase activities. Because I expected, *a priori*, POH, [T] and $[E_2]$ to vary by sex, these variables were left out of the model and treatment differences by sex were explored with univariate models.

All analyses were conducted using JMP 4.0 (SAS Institute, Cary, NC, USA). Because of multiple comparisons, I conducted Bonferroni corrections to minimize the potential for Type II errors. I designated statistical significance at $\alpha=0.05$ and made Bonferroni corrections to minimize the possibility of false positives. However, due to the high conservatism of Bonferroni corrections (Cabin and Mitchell 2000), I also indicate significance at $\alpha=0.05$ and $\alpha=0.01$, thus allowing readers to define for themselves the levels that are most biologically meaningful (Cabin and Mitchell 2000).

Results

Survival during experiment

Of the 236 experimental fish, 108 (46%) were males and 128 females (54%). Barring the removal of fish for sampling, survival over the course of the experiment was high at 97%; very little mortality occurred (N=5 males, 2 females), and in the few cases when it did occur, it was from fish jumping out of the tanks. Visual examinations did not reveal any overt signs of infection or disease. There was no clear treatment bias to mortality (N=3 GnRH, 2 GnRH+T, 0 T, 2 controls).

Somatic growth

After accounting for allometric differences in body length, male and female pink salmon grew at similar rates independent of treatment (condition factor, K: two-way ANCOVA, sex $P=0.294$, treatment $P=0.127$, body length covariate $P<0.001$, $N=227$) (Figure 4.1). Little growth occurred between April and June, but began increasing between July and September. Final K

values were significantly higher in both sexes relative to baseline values (ANCOVA, both sexes $P<0.001$).

Physiological and reproductive responses to hormonal treatment

Relative to baseline, pre-treatment samples, plasma [T], [E_2], HIS, and GSI varied little in the GnRH treated and control females during the 5-month long experiment (final September pooled means, [T]=135.2 ng·ml^{-1} ± 46.4 SEM, [E_2]=13.1 ng·ml^{-1} ± 46.4, HSI=1.25 ± 0.09 SEM, GSI=2.07 ± 1.14 SEM) (Figure 4.2a-d). In contrast, GnRH+T and T treated females showed significant increases in [T] from April to June, but T treated females did not show significant increases in [E_2], HSI or GSI at the end of the experiment in September (Figure 4.2a-d). Despite the similar patterns of HSI and GSI among all treatment groups to late June, the GnRH+T treated females showed a significant departure from baseline levels to a final value of 9.88 by late September (ANCOVA, treatment $P<0.001$) (Figure 4.2c,d).

In males, [T] increased from baseline values in the GnRH+T and T treated groups early in the experiment (April to early June) (Figure 4.2e). Though both were significantly higher than baseline concentrations by September (ANCOVA, both $P<0.05$), GnRH+T treated fish had significantly higher concentrations than the T treated group (ANCOVA, $P<0.001$). Final [T] among all treatment groups was, in descending order: GnRH+T=496.6 ± 82.2 ng·ml^{-1}, Control=352.3 ng·ml^{-1} ± 82.2 SEM, GnRH=204.7 ng·ml^{-1} ± 116.2 SEM, and T=95.6 ng·ml^{-1} ± 82.0 SEM) (Figure 4.2e). Male GSI showed little variation in and among groups between baseline levels in April (12.0 g ± 4.2 SEM) to mid July (Figure 4.2f). By September, all groups had increased testes mass significantly from baseline levels ($P<0.001$), irrespective of treatment (ANCOVA, $P=0.362$, pooled mean=28.6 g ± 2.4 SEM), but male GSI did not increase significantly (Figure 4.2f).

Osmoregulatory and stress physiology

Mean plasma [Na^+], [Cl^-], and osmolality did not differ among treatment groups (all $P>0.05$) at the end of the experiment or from baseline values ($P=0.142$), suggesting that all fish were maintaining homeostasis fairly equally (Figure 4.3a-c). Final pooled treatment values: [Na^+]=162.8 mmol·L^{-1} ± 2.0 SEM, N=35; [Cl^-]=137.6 mmol·L^{-1} ± 1.5 SEM, N=35; osmolality=320.8 mOsm·kg^{-1} ± 5.0 SEM, N=35).

Plasma lactate, glucose and cortisol also did not differ among treatment groups at the end of the experiment (pooled values: glucose=4.2 ± 0.12 mmol·L^{-1}, $P=0.198$, N=35; lactate=4.7 ± 0.43 mmol·L^{-1}, $P=0.640$, N=35; cortisol=109.9 ng·ml^{-1} ± 13.4 SEM, $P=0.858$, N=35) (Figure

4.3d-f). However, both lactate and cortisol concentrations increased significantly from baseline levels (both $P<0.01$) whereas glucose concentrations did not increase (Figure 4.3d-f). Final glucose concentrations in the GnRH and GnRH+T treated fish declined from baseline levels ($P=0.012$), but the absolute decline was relatively small (Figure 4.3e). I could not assess gill Na^+,K^+-ATPase activity because gill samples were damaged by accidental thawing.

Ovarian histology

Mean egg diameters did not differ among treatment at any sampling period. Pooled mean diameter on the July 18 sampling date was 4.40 mm ± 0.30 SEM. Histological preparations from ovarian samples from salmon sampled on 18 July 2006 show the response of control (A), GnRH-treated (B), GnRH+T-treated (C), and T-treated females (Figure 4.4a-d). As indicated by the position of the germinal vesicle (GV) and the presence of lipid drops (LD), control and GnRH-treated oocytes are in the early stages of vitellogenesis. The additional presence of vitelline plaques show that GnRH+T oocytes (C) were in a more advanced stage of vitellogenesis. In contrast, T-treated oocytes (D) were enucleated and in advanced atresia (Figure 4.4a-d).

Discussion

By the end of the experiment, I observed a direct, significant effect of GnRH and T co-treatment on circulating T and E_2 concentrations, which led to hepatic hypertrophy and ultimately to increased ovarian production (e.g. GSI) in female pink salmon. GnRH and T co-treatment also led to a significant increase in circulating [T] in male pink salmon. These results support my first three predictions. However, despite significant increases in [T], final male GSI did not differ significantly from baseline levels nor did it differ among treatment groups. Nevertheless, male GSI throughout the experiment was at levels commonly observed in mature adult salmon (McBride et al. 1986), and many were spermiating when sampled on the final date. These observations suggest that, irrespective of hormonal treatment, all male groups were capable of spawning whereas only the GnRH+T females were spurred toward full sexual maturity.

The hormonal microsphere delivery system used in this study was designed to provide a sustained release of hormones over an 8-week period (Mylonas and Zohar 2001). The microspheres would thus have been depleted by or near the summer solstice on 21 June. Interestingly though, the responsiveness of the ovary appeared to begin on the first sampling date

after the solstice in July, and was strongly evident by the final sampling period two months later in September. After the initial treatment date in April, circulating concentrations of T and E_2 increased in the GnRH and GnRH+T treatment females, but concentrations fell again in July to near, but significantly higher than, baseline levels, which is consistent with the waning delivery of hormones from the microspheres. It is likely that sometime after the solstice, when daily photoperiods were decreasing, a synergistic interaction of GnRH and T led to enhanced ovarian production in advance of November spawning dates. Histological analysis of the ovaries show qualitatively that vitellogenesis was active in the developing oocytes of GnRH+T females, suggesting that the high levels of E_2 circulation was due to a synergistic priming of FSH production and secretion. In contrast, GnRH administered without T appeared no different from control oocytes, though both appeared in early vitellogenesis but at a less advanced stage than the GnRH+T oocytes. The oocytes of T-treated females however showed distinct signs of atresia, suggesting strong, negative feedback at this stage of maturity. However, some studies have documented positive feedback to T-treatment in juvenile female fishes (Crim and Evans 1982; Tiwary et al. 2002), so the atresia that I observed might have been due to overdose.

My results are consistent with a study examining GnRH and T co-treatment on juvenile and pre-vitellogenic white sturgeon (*Acipenser transmontanus*) in which long-term T treatment stimulated the accumulation of pituitary gonadotropins (GTHs) but did not affect basal GnRH-induced secretions of GTH until more than a year later (Pavlick and Moberg 1997). Though I did not measure the GTHs in this study, the ovarian response that I measured at the end of the experiment in advance of natural spawning dates suggests a GnRH+T induced accumulation of pituitary FSH, but release presumably occurred only when other cues (presumably photoperiodic) where perceived by the fish and transduced into the HPG axis. Steroidal treatment in juvenile fishes usually stimulates FSH production and accumulation in the pituitary but not release to the bloodstream (Atlantic salmon *Salmo salar*, Crim and Peters 1978; rainbow trout *O. mykiss*, Crim and Evans 1978, Crim et al. 1981, Magri et al. 1985; European eel *Anguilla anguilla*, Dufour et al. 1983). But when T was administered with leutinizing hormone releasing-hormone (LHRH- now known as GnRH) in juvenile rainbow trout, both pituitary and plasma GTH concentrations rose (Crim and Evans 1983). A similar response has been observed in post-spawning goldfish (*Carassius auratus*) in which GTH production and secretion occurred in response to T and E_2 co-treatment with GnRH, which in effect reversed gonadal regression that usually follows spawning (Trudeau et al. 1991).

Despite initial differences in female [T], [E_2], HSI and GSI in the GnRH, T, and control groups, final values did not differ among groups, nor were they different from pre-treatment baselines. The lack of response in the GnRH-treated fish is consistent with studies showing a low receptivity to GnRH stimulation in juvenile and maturing salmon (Ando et al. 1999), and the lack of response by T alone is consistent with the known inhibitory effects of steroids on FSH secretion, despite the fact that T-treatment can stimulate FSH production (reviewed by Yaron et al. 2003). That I could not find an effect, particularly in the control group, of T or GnRH-treatment suggests that these fish would not have likely spawned in November, which is when the population would normally do so in the wild. I had expected that pink salmon, with their generally fixed 2-year life-span (Heard 1990), would have been nearly fully mature by the completion of my experiments. There is evidence that rates of maturation in captive salmonids can become uncoupled from natural cycles (Gross 1998). Several studies have indicated that critical size and energy thresholds must be attained by specific times of year to induce maturation (Simpson 1992; Thorpe 1994; Hopkins and Unwin 1997). British Columbia pink salmon that were accidentally introduced to the Great Lakes in 1956 have evolved a 3 rather than 2 year life history. It is possible that the relatively low primary production of the lakes, coupled with the impossibility of anadromy, have protracted their life-span presumably to attain effective size-at-maturity.

Nonetheless, GnRH and T co-treatment induced maturation in this study whereas GnRH and T independently did not, which is consistent with other studies in juvenile and sub-adult fishes. The case is somewhat different in males though for, despite their small sizes, all groups had final testes masses that were significantly higher than pre-treatment values and all were spermiating in September. Testes masses and GSIs did not differ among treatment groups which suggests that all were prepared for spawning irrespective of hormonal treatment. Qualitatively, nearly all males were spermiating and had morphed into spawning forms and colourations.

Throughout the experiment, treated and control salmon grew at similar rates, and condition factors did not differ significantly until the end of the experiment, when GnRH+T treated females were heavier at a given length than the other treatment groups. This greater mass-at-length was likely due to the heavier ovaries in GnRH+T treated females. In males, there were ultimately no differences in condition factor among treatment groups.

Regarding osmoregulatory physiology, GnRH and T treatments, either alone or in combination, appeared to have little influence on cortisol secretion, which by September, had increased significantly from baseline levels though it did not differ among treatment groups. I

had predicted gill Na^+,K^+-ATPase activities to show a seasonal down-regulation in the GnRH+T treated fish as a result of potential HPA axis cross-stimulation and as part of the maturation process (maturing fish are also preparing for eventual re-entry to freshwater). Unfortunately, I could not assess this, but given cortisol's direct role in gill Na^+,K^+-ATPase expression (see Norris and Hobbs 1997), and that all fish experienced the same salinities, it seems unlikely that gill ATPase activities would have differed among treatment groups. Nevertheless, plasma ions and osmolality did not differ among sexes nor treatment groups at any stage of the experiment, indicating a remarkable ability of salmon to maintain homeostasis despite different hormonal treatments and suggesting that gill Na^+,K^+-ATPase expressions were similar in all groups.

In conclusion, GnRH in combination with testosterone induced maturation in captive female pink salmon held in saltwater, but the reproductive response occurred many months after the initial treatment. Presumably, GnRH+T led to the accumulation of FSH and LH transcripts in the pituitary gland, which were released only when daily photoperiods became shorter in the weeks/months preceding natural spawning dates, leading to hepatic hypertrophy and increased vitellogenic deposition to oocytes. Male pink salmon did not show a clear response to hormonal treatment, and hormonally treated and control fish alike had GSIs throughout the experiment (mean=7.1 ± 0.8) that were comparable to those measured in mature adult pink salmon upon river entry during spawning migrations (~5.5; McBride et al. 1986). In nature, male salmon are more likely to mature precocially than females (Foote and Larkin 1988; Foote et al. 1997). Thus, despite tank confinement and their small stature, all were ready to spawn whereas only the GnRH+T females were ready to do so. During the last six months of ocean residency, fish make 'decisions' whether to migrate via the integration of exogenous (photoperiod, temperature) and endogenous (gross somatic energy, circannual rhythms) cues (Hinch et al. 2006). That females appeared to require hormonal intervention to mature, whilst males did not, suggests that females are more risk averse and perhaps at greater susceptibility to a confinement/tank effect. Ultimately GnRH and T co-treatment spurred reproductive physiology in males and females, thus supporting predictions 1-3, but I found little support for the idea that GnRH provides cross axis stimulation to HPA cortisol increased (prediction 4). I could not address the subsequent stimulation of gill Na^+,K^+-ATPase activity (prediction 5) due the degradation of samples, but plasma [Na^+], [Cl^-] and osmolalities did not differ among treatment.

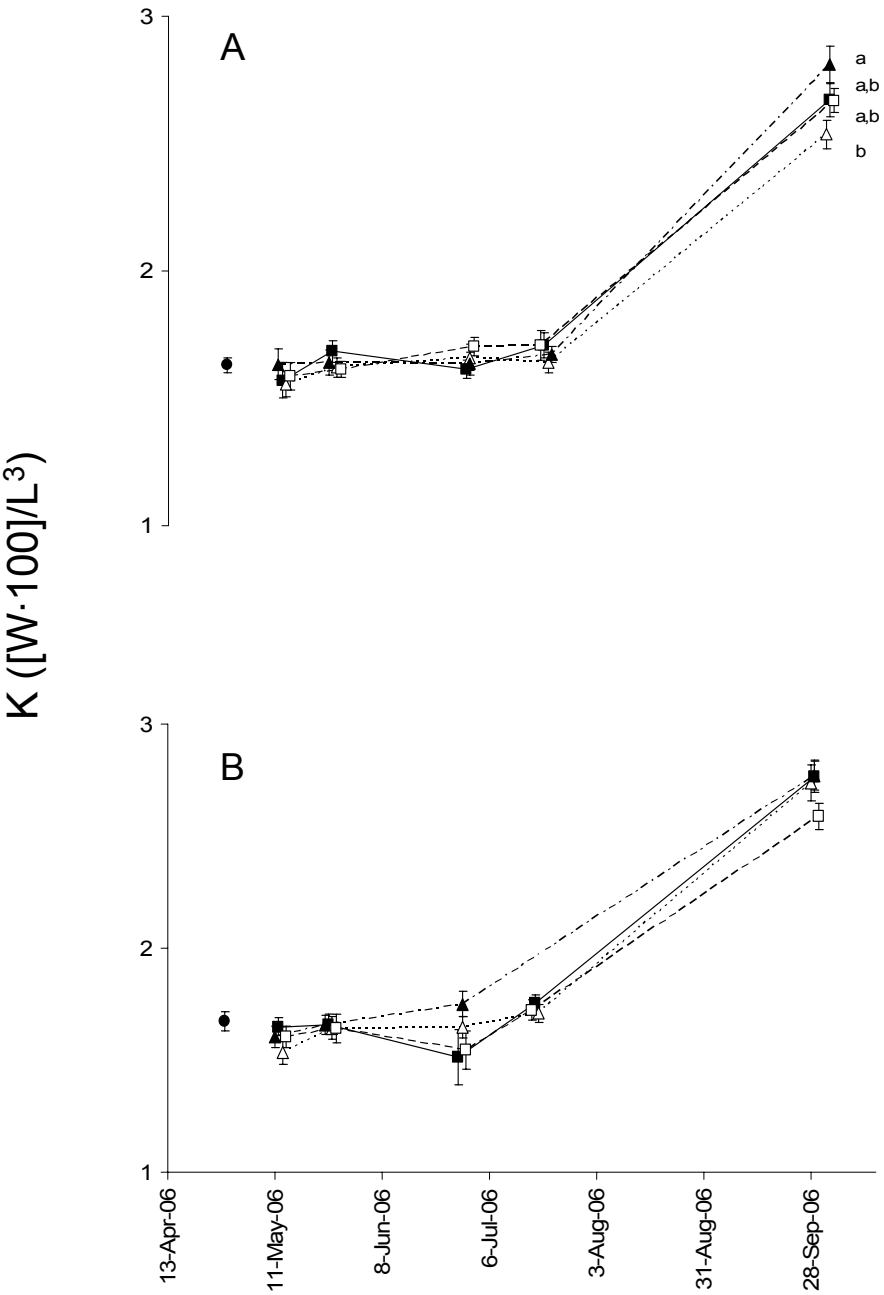

Fig. 4.1: Temporal response of condition factor in female (A) and male (B) pink salmon to experimental hormonal manipulation. Black circles signify pre-treatment baseline values. Black boxes signify controls, white triangles are GnRHa, black triangles are GnRHa+T, white boxes are T. Values are least squares means +/- SEM. N=5-8 for each point.

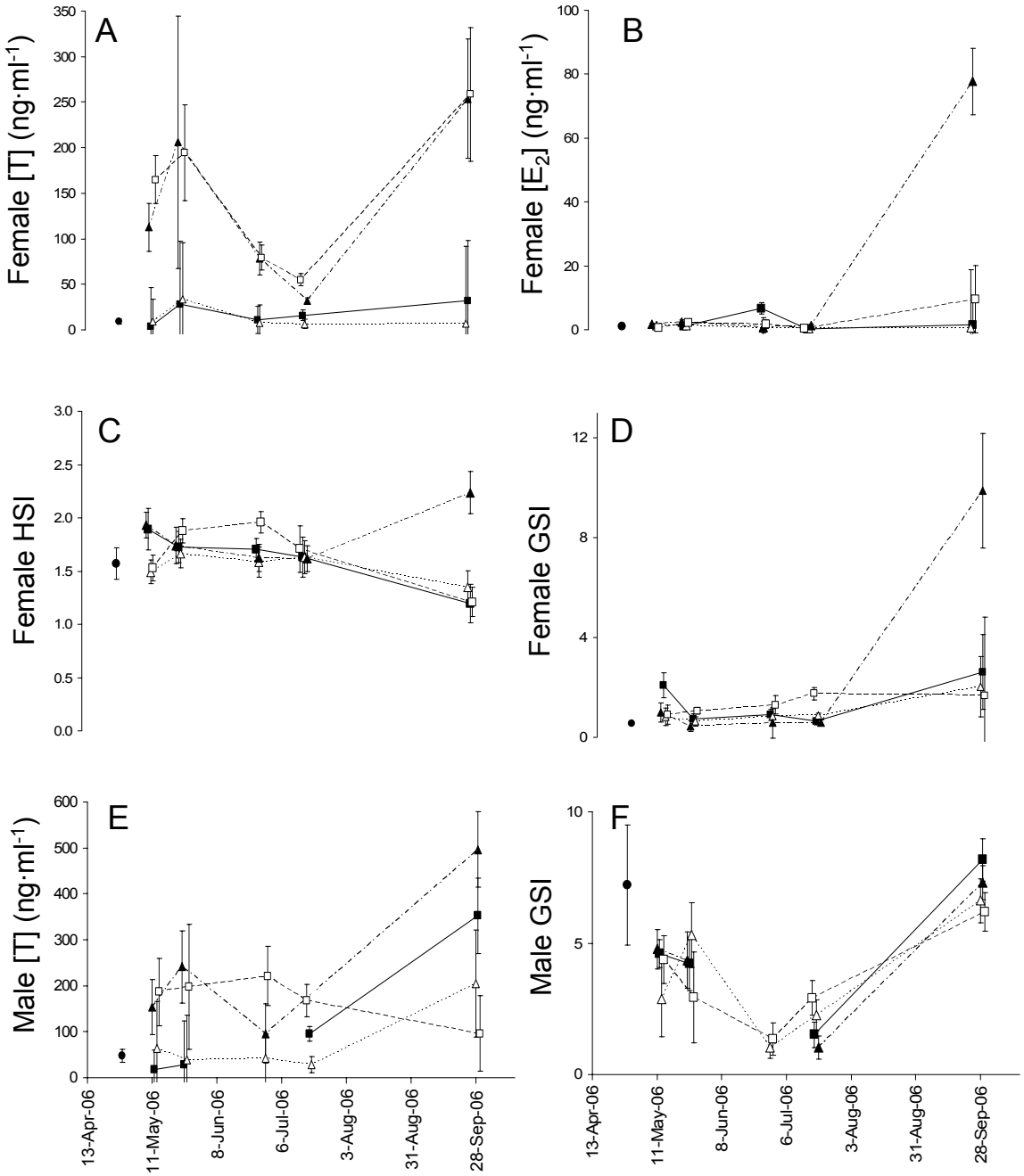

Fig. 4.2: Temporal response of female pink salmon testosterone (A), 17β estradiol (B), hepato-somatic index (C), gonado-somatic index (D), and of male testosterone (E) and gonado-somatic index (F) to experimental hormonal manipulations. Black circles signify pre-treatment baseline values. Black boxes are controls, white triangles are GnRHa, black triangles are GnRHa+T, and white boxes are T. Values are least squares means +/- SEM. N~5-8 for each point.

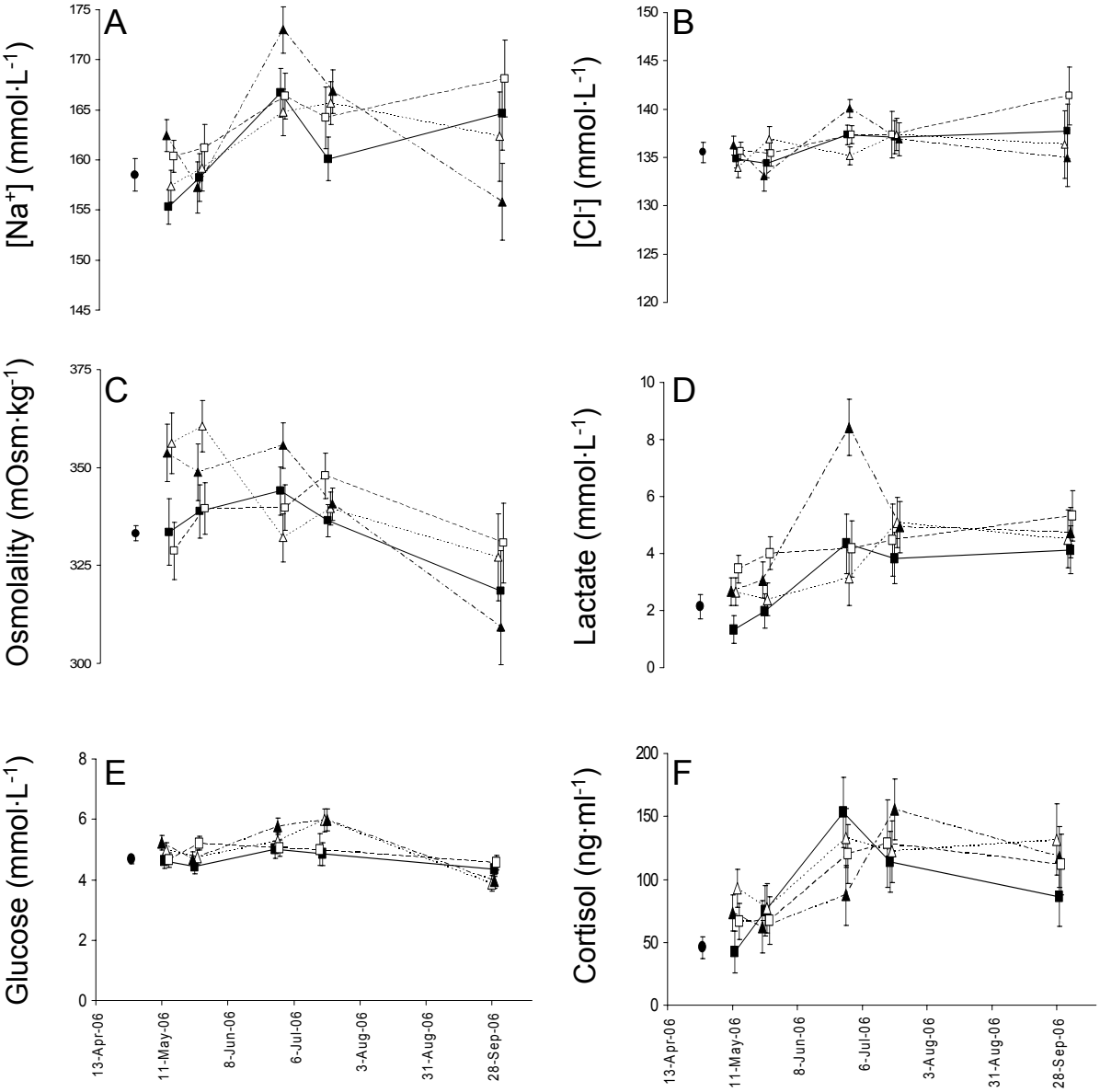

Fig. 4.3: Temporal response of plasma sodium (A), chloride (B), glucose (C), lactate (D), osmolality (E), and cortisol (F) to hormonal treatment in maturing pink salmon. Sexes were pooled. Black circles signify pre-treatment baseline values. Black boxes are controls, white triangles are GnRHa, black triangles are GnRHa+T, and white boxes are T. Values are least squares means +/- SEM. N~5-8 for each point.

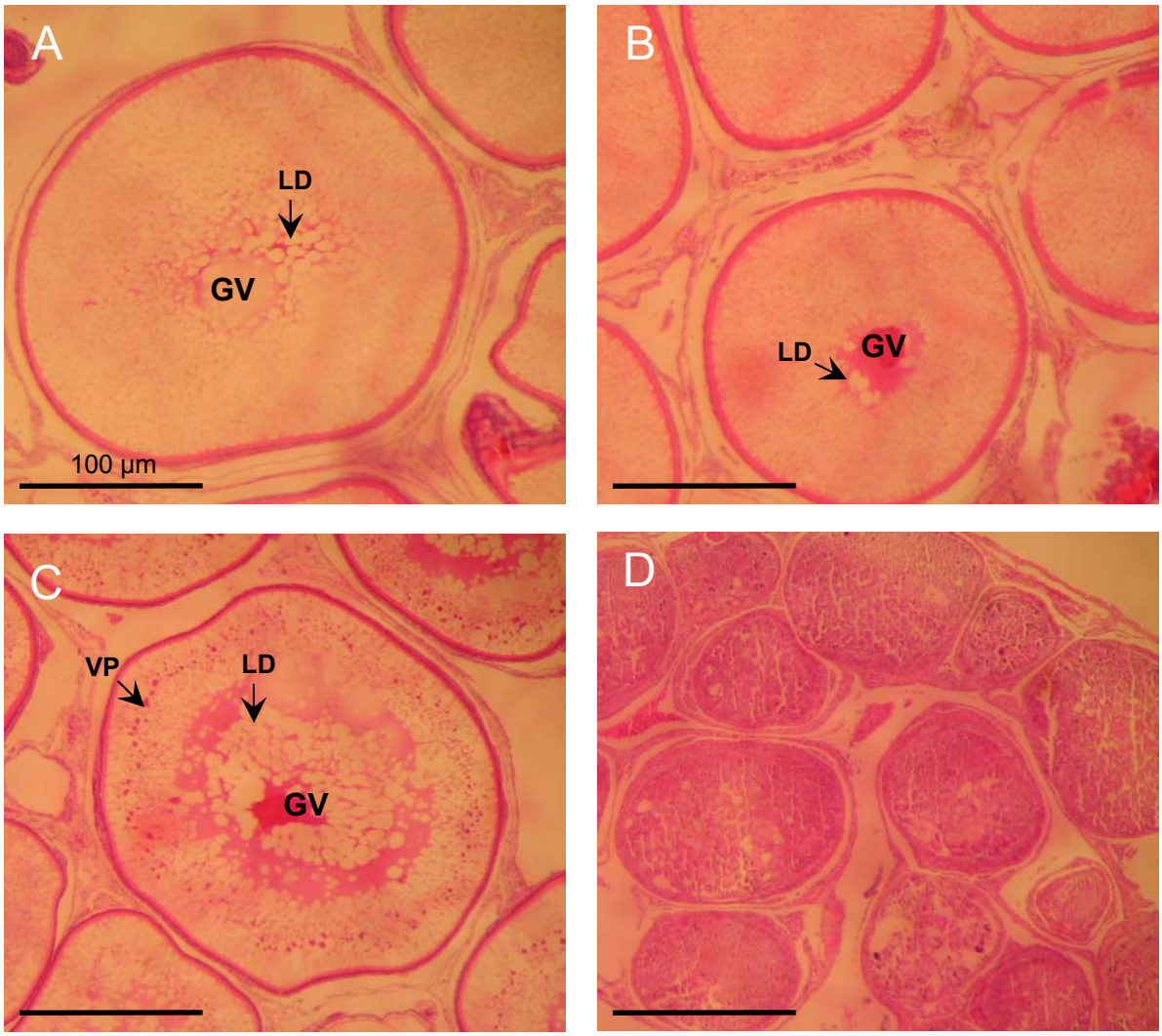

Fig. 4.4: Representative histological sections from the developing ovaries of maturing pink salmon. Samples were collected on the same date (18 July 2006) and the response of control (A), GnRH-treated (B), GnRH+T-treated (C), and T-treated females are shown. As indicated by the position of the germinal vesicle (GV) and the presence of lipid drops (LD), control and GnRH-treated oocytes are in the early stages of vitellogenesis. The additional presence of vitelline plaques show that GnRH+T oocytes (C) are in a more advanced stage of vitellogenesis. In contrast, T-treated oocytes (D) have been enucleated and are in advanced atresia. Scale bar in panel A is the same for all panels.

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CHAPTER 5

Factors affecting the timing and survival of sockeye salmon spawning migrations.[4]

Introduction

Animal migrations are characterized by the directed, predictable movement of large numbers of individuals among habitats. From an evolutionary point of view, migrations usually accompany key life-history transitions and present opportunities for individuals to maximize lifetime fitness, and act as strong selective agents in the evolution of life-history variation (Stearns 1992). During reproductive migrations, the demands of homeostatic metabolism, growth, and reproduction must all be satisfied (Calow 1985), but with limited energy to parse among these varied processes, trade-offs evolve in the form of physiological and behavioural constraint (Reznick 1992; Rose and Bradley 1998; Zera and Harshman 2001). For capital breeding animals like Pacific salmon (*Oncorhynchus* spp.) that fuel migrations exclusively through endogenous energy reserves (i.e. lipid and protein catabolism), migration timing is an adaptive life-history trait (Burgner 1991; Hodgson and Quinn 2002) that commonly lies within a narrow phenological window when environmental conditions for spawning are appropriate (Dingle 1996; Allerstam and Lindström 1990; Hodgson and Quinn 2002; Prop et al. 2003). For example, upon their return from the high seas, Late-summer populations of sockeye salmon (*O. nerka*) that spawn in the Fraser River of British Columbia, Canada will hold in the estuary at the river mouth for 2-6 weeks before initiating upriver migrations to natal spawning areas. This holding tactic is associated with high river migration success (>80%) (Cooke et al. 2004) and has presumably evolved to minimize exposure to peak river temperatures in mid- to late summer. A key factor influencing timing is the seasonal, photoperiodic release of gonadotropin-releasing hormone and subsequent production of sex steroid hormones (Dingle 1996). A modulating influence is endogenous energy supply, which, for homing salmon, becomes finite shortly before river entry when salmon stop feeding and then declines throughout the spawning migration (Burgner 1991).

Many studies have described the nature and pattern of animal migrations, most notably in

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birds, insects and fish (see Dingle and Drake 2007), and the influence of endogenous energy levels and reproductive hormones on migration timing are well known from descriptive studies (reviewed by Dingle 1996). Until recently however, it has been difficult to test the proximate physiological mechanisms of migratory behaviour. Recent studies with Pacific salmon have coupled biopsy with individual positional bio-telemetry to identify links between migration behaviour and underlying physiology (Cooke et al. 2005; Cooke et al. 2006a,b; Young et al. 2006; Chapter 2- Crossin et al. 2007; Chapter 6- Crossin et al. 2008). Upon entering the river, successful migrants are characterized by relatively high gross somatic energy densities and relatively low concentrations of circulating reproductive hormones (Cooke et al. 2006a,b). Conversely, "Late-run" sockeye that do not hold in the estuary and initiate upriver migration directly have lower migration success (<50%) (Cooke et al. 2004) and relatively lower somatic energy densities and higher reproductive hormones (Cooke et al. 2008). Given that migration timing is adaptive, the higher mortality observed as a result of the change in migration timing is perhaps predictable but most certainly has negative effects on fitness. These results suggest that somatic energy levels and the degree of reproductive maturity are key factors governing the timing and success of upriver migration long before salmon enter their natal river.

Therefore, I examined the hypothesis that behaviour and survivorship during coastal and river migrations are indeed influenced by energetic and reproductive states of coastal migrating sockeye. I accomplished this by assessing migration rates and survivorship at three spatio-temporal scales with bio-telemetry, and establishing functional relationships with physiological measures obtained at time of capture. First, I examined the baseline physiology of two populations of sockeye (Adams and Chilko) over a three week period of arrival in coastal areas approximately 250 km from the mouth of a natal river (i.e. the Fraser River). Both are large populations that co-migrate coastally but Chilko migrate directly up-river (i.e. a summer run population) whereas Adams usually hold in the estuary for several weeks before entering (i.e. a Late-summer run population) though segments of this latter population have recently forgone estuarine holding (Cooke et al. 2004) thus providing a contrast within a population for river entry behaviour. Second, using acoustic and radio bio-telemetry I examined river entry timing and the physiologic correlates of travel rates and survival as salmon migrated an approximately 200 km stretch of the southern British Columbia coast and nearly 500 km up the Fraser River to spawning grounds. Lastly, I contrasted the physiology of salmon that held in the estuary for three weeks before entering the river and subsequently survived migration to natal spawning

versus those that entered after holding for only one week upon estuarine arrival and subsequently died in river.

Drawing from the fact that energy levels and reproductive hormones levels tend to work in opposition and are fundamentally important to the initiation of migration in many animals (Woodhead 1975; Ueda and Yamauchi, 1995), and especially in salmon (Cooke et al. 2006a, 2008; Young et al. 2006; Chapter 2- Crossin et al. 2007), I predicted that (1) sockeye forgoing the adaptive estuarine holding tactic, favoring instead to begin upriver migration without estuarine delay, and subsequently dying *en route* to spawning areas, would have lower energy densities than individuals that delay in the estuary and survive to spawning areas. I also predicted that (2) sockeye forgoing estuarine delay and dying *en route* would have correspondingly higher circulating reproductive hormone concentrations. Because homing salmon rely principally on catabolized body fat to fuel their metabolism, early migrations have been associated with waning somatic energy reserves (Cooke et al. 2006a,b). My final prediction was that (3) sockeye entering the river early and dying would have higher gill Na^+,K^+-ATPase activities. As this enzyme is the principle agent involved in ionoregulatory function, fish moving from the hyperosmotic marine environment into the hypoosmotic river must down-regulate its activity in order to survive (Clarke and Hirano 1995; Shrimpton et al. 2005).

Methods and Materials

Capture, physiological sampling, and radio and acoustic tagging

In the summer of 2006, a purse seining vessel was chartered to intercept homing Fraser River sockeye salmon in two marine areas adjacent to Vancouver Island, British Columbia: at the western end of Juan de Fuca Strait (JDF) and in the southern end of Johnstone Strait (JS) (Figure 5.1). Fraser River sockeye return from the open ocean predominantly through JDF though in recent years most fish have migrated via the northern passage through JS (J. Cave, Pacific Salmon Commission, Vancouver, BC, pers. comm.) – in a given year, and for a given population, the proportion migrating through JS and JDF can change. Sampling began in JDF on August 6-10 when the earliest segment of Adams sockeye and co-migrating Chilko sockeye were detected by in-season test-fisheries administered by the Pacific Salmon Commission. Test-fisheries then indicated that the majority of salmon began favouring the northern passage through JS shortly after that, so capture operations were shifted to JS on August 11-12. Sampling continued in that location from August 16-19 and August 24-27 (see Robichaud and English 2007 for more details).

In each sampling area, the seine vessel served as the platform for fish biopsy, transmitter implantation, and fish release. Upon completion of each seine set, the purse seine was brought along the starboard rail of the vessel, and while still in the water, individual salmon were dip-netted and transferred to a large flow-through holding tank on the boat's deck. Ten to 20 sockeye were taken from each seine set, and once onboard, salmon were individually processed and released as quickly as possible. Post-biopsy and transmitter insertion, sockeye were held in recovery tanks for a maximum of 15 minutes (mean 2 minutes) until they regained equilibrium and then returned over the side of the boat. Most fish were on board for less than 20 minutes. In the collection of physiological samples, I used protocols for the non-lethal, un-anaesthetized sampling of sockeye salmon (see Cooke et al. 2005; English et al. 2005) which were approved by the University of British Columbia Animal Care Committee in accordance with the Canadian Council of Animal Care.

Details of the handling, biopsy, and tagging procedures used in this study can be found in Cooke et al. (2005). Briefly, biopsy protocols were as follows: individual salmon were removed from the holding tank and placed in a padded V-shaped trough provided with a continuous supply of ambient seawater from a tube near the salmon's mouth. I then measured and collected: nose-to-fork length (FL, cm), scale samples (N=10) for stable isotope and age analysis, a 0.5 g clip of adipose fin for DNA stock identification, a 1.5 ml blood sample for assessing plasma biochemistry, a 1 cm deep by 3 mm diameter plug of muscle for gene array analyses (not discussed in this paper), and a < 4 mm clip of gill filament tips (~0.03 g) (McCormick 1993) for assessment of gill Na^+,K^+-ATPase activity. Tissues were packaged in cryovials and stored on dry ice until transfer to a –80 ˚C freezer. Gross somatic energy (GSE, $MJ \cdot kg^{-1}$) was determined with a hand-held micro-wave energy meter (Crossin and Hinch 2005). A spaghetti tag was secured through the dorsal musculature adjacent to the dorsal fin. Either a radio or acoustic transmitter was then inserted into the fish intragastrically (Cooke et al 2004, 2006a for details on transmitters). To assess the effects of capture and handling on fish survival, relationships between capture-to-release time (i.e. total time on board the vessel, or simply handling time), stress measures, and survival to Mission, BC were examined with ANOVA and regression models. These analyses focused on Adams sockeye which had sufficient numbers to provide strong statistical power. To further assess the effects of handling on the survival of sockeye, analyses of seine set durations and abundances were examined relative to the subsequent movement of individuals once released.

Radio and Acoustic receiver arrays

For salmon bearing acoustic tags, first possible detection of JS released fish was ~70 km at the northern Strait of Georgia (NSOG) acoustic receiver line, and for JDF released fish ~50 km at the JDF line (Figure 5.1). Salmon from both release sites were next detected at the southern Strait of Georgia line (SSOG) extending in an arc around the river mouth (Figure 5.1). River entry was assessed in this study as detection ~85 km upriver at Mission, BC, which lies at the tidal boundary. Acoustic receivers were then placed at intervals throughout the Fraser River watershed but the greatest concentration was along the migration path of Adams River sockeye, which were the dominant run of 2006. Detailed information on the position of acoustic receivers in 2006 are provided in KRC (2007).

For salmon bearing radio tags, first detection was at Mission, BC, ~300 km from both the JS and JDF release sites (Figure 5.1). Several additional receivers were positioned throughout the watershed and details are provided in Robichaud and English (2007).

There are several potential reasons why a fish might not be detected at an acoustic or radio receiver station after its release. In my experience with gastrically inserted transmitters, tag expulsion is rarely observed or reported (Cooke et al. 2005), thus I assumed tag retention to be 100%. Regarding detections, it should be noted that marine detection of radio tags is not possible as radio signals attenuate quickly in salt-water. Acoustic receiver lines and stations were evaluated for their performance in both marine and freshwater, and detection efficiency was ~100% (KRC 2007). Therefore, fish that were undetected at an acoustic line or receiver were likely mortalities. Mortality can then be attributed to either fisheries exploitation, handling stress, natural factors (e.g. predation, environmental stressors), or potentially some combination of these (e.g. handling stress increased the likelihood of fish being harvested or preyed upon). I attempt to ascribe mortality levels to the first three factors, though I accept that there are several unknowns in this endeavor.

Laboratory assays

Population identity for individual sockeye was determined using DNA analyses (Beacham et al. 1995, 2004). True identity was later confirmed in fish successfully reaching spawning areas as determined via telemetry data. DNA assignments in Fraser River sockeye have a 96% accuracy. Plasma testosterone (T) and 17β-estradiol (E_2) levels were measured by radioimmuno-assay (McMaster et al. 1992) and used to assign fish sex as sexual dimorphism was not yet fully expressed at that point of migration. Plasma ions (Na^+, Cl^-), glucose, lactate,

osmolality, and cortisol were quantified by procedures described by Farrell et al. (2001a). Gill tissue Na^+,K^+-ATPase activity was determined by kinetic assay (McCormick 1993).

Statistical analyses

All physiological data were $\log_{10}$ transformed to reduce heteroscedasticity. Individuals from 16 Fraser River sockeye populations were biopsied and telemetered, but I limited my analyses and discussion to the Chilko and Adams populations as these were the only two with sufficient numbers of individuals to permit rigorous statistical analyses. I analyzed individuals collected in JS and JDF separately because previous studies have shown that salmon sampled in these areas differ physiologically (Miller et al. 2007). Thus, within each capture location, I used MANOVA to explore physiological differences at time of capture between the sexes. Variables examined were: plasma concentrations of Na^+ and Cl^-, plasma osmolality, plasma lactate and glucose, and gill Na^+,K^+-ATPase activities. Sex-specific variables were excluded from this analysis: gross somatic energy (GSE), nose-to-fork length (FL), testosterone (T), and 17β estradiol (E_2, females only). I next examined multivariate differences by population, incorporating into the MANOVA model: plasma Na^+ and Cl^-, osmolality, lactate, glucose, and gill Na^+,K^+-ATPase activities. Population differences in GSE, FL, T and E_2 were run for each sex separately (MANOVA).

To identify the relative importance of individual variables underlying the differences revealed by MANOVA models, I conducted a series of analyses of covariance (ANCOVA). When analyzing the sexually monomorphic variables as stated above, population was the main effect, and because sampling of salmon occurred over an approximately three week period, during which time fish are running against a biological time-clock, Julian date of capture was used as a covariate and temporally corrected least squares means were generated for each variable. Population differences were assessed in each capture area separately. When analyzing the sexually specific variables, population differences were also determined for each sex separately interactions between population and Julian date were examined in all models. When interaction terms were non-significant, they were removed and the model was re-run.

To describe population- and sex-specific differences in travel times from each release location (JS and JDF) to the various receiver locales, analysis of covariance (ANCOVA) was used to generate length-corrected least squares means (FL as the model covariate). To assess population- and sex-specific relationships between travel times from each release location to other locations and physiological variables, I conducted a series of correlation analyses. If significant correlations were found, I used linear regression to describe the relationship between

the physiological factor (the independent variable) and the travel time of individuals (the dependent variable).

Key to my analysis of migration timing was the calculation of estuarine holding time before river entry. I defined this as the time lapse between release in JS/JDF to first detection at Mission, BC, ~85 km up the Fraser River (Figure 5.1). I chose Mission as the definitive point of river entry because it represents the tidal boundary and because it is the first location on the migratory route where both acoustic- and radio-tagged fish could be jointly detected (radio-tag transmissions attenuate in salt-water). To differentiate between holding and non-holding tactics, I divided holding times into the 25th and 75th percentiles in order to contrast extreme differences. The 25th percentile represented salmon that entered the Fraser River in <10 days which were classified as non-holding, and those entering >20 days were classified as holding. I focused my analysis on Adams sockeye released in JS simply because (a) the number of fish in the 25th and 75th percentiles were sufficient for analysis, (b) because it allows us to compare results with those of Cooke et al. (2008) who used a similar approach, and (c) there were too few fish released in JDF to enable this analysis. The physiology underlying the different behaviour/fate differences were compared with ANCOVA and Julian date was used as a covariate.

All analyses were conduced using JMP 4.0 (SAS Institute, Cary, NC, USA). Because of multiple comparisons, I conducted Bonferroni corrections to minimize the potential for Type II errors (Rice 1989). I designated statistical significance at α=0.05 and made Bonferroni corrections to minimize the possibility of false positives. Due to the high conservatism of Bonferroni corrections (Cabin and Mitchell 2000), I also indicate significance at α=0.05 and α=0.01, thus allowing readers to define for themselves the levels that are most biologically meaningful (Cabin and Mitchell 2000).

Results

Tagging summary, baseline physiology of salmon intercepted in the marine environment, and handling effects

In total, 797 sockeye salmon from 16 Fraser River populations were captured, biopsied and tagged (see Hinch et al. 2007 for complete tagging summary). For the present study, 409 Adams and 96 Chilko sockeye were analyzed to provide contrasts between the most abundant Late-run and Summer-run timing populations. Table 5.1 summarizes information about the numbers of salmon captured in each marine capture location, the numbers and types of transmitters deployed, and an abridged detection summary at receivers positioned throughout the

study area (Figure 5.1). A detailed summary of all fish captured in 2006 is presented in Hinch et al. (2007).

Analysis of the physiological variables (i.e. GSE, Na^+, Cl^-, glucose, lactate, osmolality, cortisol, and gill Na^+,K^+-ATPase activity) revealed a significant difference between males and females in each marine capture location (MANOVA: JS, $P<0.001$, N=400; JDF, $P<0.005$, N=105). Models examining sex differences with Julian date of capture as a covariate indicated that the variables driving the significant MANOVA were gross somatic energy (GSE, ANCOVA: JS, $P<0.001$, N=389; JDF, $P<0.026$, N=103) and plasma glucose (ANCOVA: JS, $P<0.001$, N=390; JDF, $P<0.001$, N=105). I thus removed GSE and glucose from the model and reclassified them as sex-specific variables. When the MANOVA was re-run, no differences between sexes were found in plasma Na^+, Cl^-, osmolality, lactate, cortisol, and gill Na^+,K^+-ATPase activities (JS, $P=0.588$, N=400; JDF, $P=0.312$, N=105). Males and females were thus pooled for these variables when univariate analyses were run.

MANOVA models examining population differences in physiology (i.e. lactate, Na^+, Cl^-, osmolality, gill Na^+,K^+-ATPase) at capture were significant in JS ($P<0.003$, N=400), but not in JDF ($P=0.079$, N=105). ANCOVA using Julian date of capture as a covariate revealed that the population differences driving the significant MANOVA model in JS were lactate ($P<0.002$) and Cl^- ($P=0.013$) (see Table 5.2). When the sex-specific variables were analyzed, the MANOVA model was significant for both males and females (JS: both $P<0.001$, male N=195, female N=202; JDF: both $P<0.001$, male N=48, female N=58). ANCOVA showed that the population level variables that were significantly different were male FL ($P<0.001$) and female testosterone ($P=0.022$) (Table 5.2).

Handling effects that lead to fish mortality are expected to occur within 2 days after release (English et al. 2005). Analyses of handling effects are presented in Hinch et al. (2007), but to summarize, no relationships with survival were found with set duration and abundance, or the time onboard the vessel. Of those acoustic-tagged Adams sockeye released in JS, 23% were undetected on the NSOG line approximately 2 days swim from JS, but all Chilko sockeye were detected. Likewise, all Adams and Chilko sockeye released in JDF were detected on the JDF line 1-2 days away from the release site. After accounting for variation in handling time before biopsy (i.e. capture and pre-biopsy holding times), there was no significant difference in plasma cortisol concentrations between JS released Adams sockeye that successfully entered the river and those that did not (survivor cortisol=404.8 $ng \cdot ml^{-1}$ ± 9.7 SEM, mortality cortisol=407.7 $ng \cdot ml^{-1}$ ± 8.9 SEM, ANCOVA, fate $P=0.825$, pre-biopsy time $P<0.001$, N=366). However, there

was a significant difference in lactate concentrations between groups (survivor lactate = 9.17 ± 0.28 mmol·L^{-1}, mortality lactate = 10.50 mmol·L^{-1} ± 0.25 SEM, ANCOVA, fate P<0.001, pre-biopsy time P<0.001, N=417) (Figure 5.2).

Physiological correlates of survival to river entry

In salmon released in JS, comparisons between Adams sockeye that failed to reach Mission, BC with those that succeeded showed significant differences between plasma glucose (ANCOVA, P=0.032), lactate (P=0.009), Na^+ (P=0.022), Cl^- (P<0.001), and osmolality (P=0.003) (Table 3). No physiological differences were found between JS-released Chilko sockeye (Table 5.3). In JDF-released sockeye, the only significant difference between failed and successful fish was in the FL of male Adams sockeye: failed fish were significantly smaller than successful fish (P=0.021).

Marine migration rates

Rates of migration through the marine environment could only be assessed in fish bearing acoustic transmitters. Within population, length-adjusted travel times by males and females to each marine location did not differ (ANCOVA, all P>0.05), so the sexes were pooled. From JS, Adams sockeye took 2.6 days ± 0.1 SEM (~24.2 km·day^{-1}) to reach the NSOG line, approximately 63 km away, and Chilko sockeye took 1.4 days ± 0.4 SEM (~45.0 km·day^{-1}) (Figure 5.2a). These differences were statistically significant (ANCOVA, P=0.002, N=53). To reach the SSOG line approximately 183 kms from the capture site, Adams sockeye took 5.2 days ± 0.2 SEM (~35.2 km·day^{-1}) and Chilko took 3.4 days ± 0.4 SEM (~53.8 km·day^{-1}) (ANOVA, P<0.001, N=53). Thus, upon release in JS, Chilko sockeye swam at nearly twice the rate of Adams sockeye through the Strait of Georgia.

From JDF, Adams sockeye (N=11) took 3.1 days ± 0.4 SEM (~16.1 km·day^{-1}) to reach the JDF acoustic receiver line approximately 50 kms away, while Chilko sockeye (N=5) took 2.6 days ± 0.5 SEM (~19.2 km·day^{-1}) (Figure 5.2b). These population-specific rates were not significantly different (ANCOVA, P=0.534, N=16). Travel rates from JDF to the southern Strait of Georgia (SSOG) receiver line positioned approximately 200 kms away at the Fraser River mouth were 7.0 days ± 0.5 SEM (~28.6 km·day^{-1}) for Adams sockeye (N=7) and 7.3 days ± 0.9 SEM (~27.4 km·day^{-1}) for Chilko sockeye (N=3). These rates were not significantly different (ANCOVA, P=0.770, N=9).

Within population and release area, travel times to Mission did not differ between radio and acoustically tagged fish: Chilko P>0.05; Adams P>0.05). However, between populations, rates of travel differed from both areas: from JS, Adams sockeye took 12.9 days ± 0.3 SEM to

reach Mission (~21.5 km·day^{-1}) and Chilko took 7.0 days ± 0.9 SEM (~39.6 km·day^{-1}) (ANCOVA, P<0.001, N=185), and from JDF, Adams took 14.9 days ± 1.0 SEM (~19.0 km·day^{-1}) and Chilko took 9.5 days ± 1.5 SEM (~29.8 km·day^{-1}) (P<0.005, N=43) (Figure 5.3). Thus, despite the similar speeds at which Chilko and Adams fish migrated through JDF to the river mouth, Adams fish entered the river holding for ~6 days while Chilko fish entered after a day (travel from SSOG to Mission is ~1 day).

River migration success in Adams sockeye as a function of estuarine holding tactic, fate and physiology

I was able to ascribe a river-migration fate to the Adams fish by examining detection data at in-river and terminal area receiver stations (see Figure 5.1). Fish were thus classified into 4 groups based on estuarine holding tactic and fate: i) held in estuary and failed to reach spawning areas (hold/fail, N=0), ii) held in estuary before entering river and successfully reached terminal spawning areas (hold/succeed, N=13), iii) did not hold in estuary and failed to reach spawning areas (no-hold/fail, N=24), or iv) did not hold in estuary and successfully reached spawning areas (no-hold/succeed, N=13). A test of the null hypothesis that estuarine delay or hold tactic is not associated with migratory success or failure was significant (Chi square, P<0.001) and must therefore be rejected.

For each of the four behaviour/fate categories, I calculated upriver travel rates between Mission and the Thompson River confluence. The hold/succeed group were the slowest swimmers, taking 11.3 days ± 0.84 SEM (~15.5 km·day^{-1}) to swim the 172 km stretch of river to the Thompson confluence. The no-hold/fail and the no-hold/succeed salmon were significantly faster swimmers, taking 7.8 ± 0.71 and 8.9 ± 0.80 days respectively (~22.6 and ~19.2 km·day^{-1}) to cover the same distance (Figure 5.4).

Physiological correlates of estuarine holding tactic and river migration fate

Physiological comparisons were made between the four hold-tactic-fate groups homing from JS (Table 5.4). River entry behaviours occurred ~5-8 days after biopsies were taken, and fate was assessed upwards of 20 days after biopsy. Female sockeye that did not hold and subsequently failed to reach spawning areas had significantly lower GSE (8.4 ± 0.13 MJ·kg^{-1}) than holding fish that were both successful and unsuccessful (8.9 ± 0.17, and 8.8 ± 0.14 MJ·kg^{-1} respectively) (ANCOVA, P=0.009, N=22). Additionally, female sockeye that held and were successful had significantly lower circulating testosterone concentrations (17.5 ± 11.28 ng·ml^{-1}) than both non-holding groups (60.3 ± 8.62 and 61.2 ± 9.73 ng·ml^{-1}) (ANCOVA, P=0.010, N=22). Gill Na^+,K^+-ATPase activities were significantly lower in male and female sockeye that

held and were successful (2.2 ± 0.37 μmol ADP·mg^{-1} protein·h^{-1}) than in the non-holding groups (3.8 ± 0.27 and 3.9 ± 0.35 μmol ADP·mg^{-1} protein·h^{-1}) (Table 5.4). Thus, female salmon that delayed river entry by holding in the estuary and were successful upriver migrants were less mature, had a higher GSE, and were more prepared for freshwater entry than salmon that did not delay, independent of their river migration fate. Salmon that did not hold and eventually died in river were more reproductively advanced and had lower GSE and testosterone concentrations. In male salmon, GSE and testosterone concentrations were not significantly related to river entry tactic and fate, but the trend was similar to that observed in females (Table 5.4).

Correlations were examined between the time it took individual Adams sockeye salmon to enter the Fraser River and their physiological profile at time of release, independent of holding tactic. Correlations were drawn for two groups of salmon: those that died in the Fraser River *en route* to spawning areas (N=35) and those that successfully reached spawning areas (N= 114) (Table 5.5). In successful migrants, gill Na^+,K^+-ATPase activities in both sexes ($r=-0.316$, $P<0.001$) and plasma testosterone concentrations in females ($r=-0.340$, $P=0.008$) were negatively correlated with travel times to Mission (Table 5.5). None of the physiological variables were significantly correlated with travel times to Mission in fish that subsequently died in river (Table 5.5). Mean travel times to Mission, corrected for variation in FL, clearly show that the successful fish were those that held in the estuary (14.1 days ± 0.39 SEM), while those that entered the Fraser more quickly with little estuarine holding (11.4 days ± 0.64 SEM; ANCOVA, $P<0.001$, N=178) died in river.

Discussion

In this study, the migratory behaviour of 505 sockeye salmon was monitored with acoustic and radio telemetry as salmon traversed two different coastal approaches to the mouth of a natal river and then onward to spawning areas, a total distance of over 650 km. I am aware of no other study that has contrasted salmon behaviour and physiology relative to the direction of their marine approach to a natal river- in this case from either the northern or southern passages around Vancouver Island *en route* to the Fraser River. Through both inter- and intra-population analyses, I found support for the hypothesis that the timing and success of spawning migrations are influenced jointly by contrasting energetic and reproductive processes, a finding that is consistent with previous studies (Cooke et al. 2006a,b, 2008; Chapter 2- Crossin et al. 2007). I found for example that advanced reproductive development, as indicated by high concentrations of circulating testosterone, and correspondingly low somatic energy concentrations were

significantly related to early river entry timing and failed migration to spawning areas (i.e. *en route* mortality). A novel discovery was that somatic energy, in addition to its importance to migration timing, was vitally important for buffering the negative effects of senescence once upriver migrations had begun. Individuals with relatively higher somatic energy levels were more likely to survive migration to spawning areas. Novel too was that only in females were these processes significantly detectable, a finding that lends support to earlier ideas that male sockeye salmon initiate upriver migrations in response to the schedules of females (i.e. protandrous migrations, Chapter 2- Crossin et al. 2007), and that their own energetic and reproductive processes affect entry timing to a lesser degree. Finally, I also discovered a key role of the gill Na^+,K^+-ATPase enzyme in mediating river entry timing *and* upriver migration success in both sexes, something that has long been hypothesized (Cooke et al. 2004, 2006a,b) but lacked empirical support. Critical to establishing these linkages was my statistical accounting for variation in Julian date (fish were captured over an ~3 week period), which is something that had not been done in earlier studies.

Initial physiology, behavioural tactics, and survival to spawning grounds

A model of migration behaviour and survivorship was put forth by Cooke et al. (2006), in which the survival probability of Late-summer runs of Fraser River sockeye was best in salmon that held at the river mouth before entering the river, and poorest in those that entered directly without holding. My results are consistent with this model. However, when I contrasted the different migratory behaviours and fates of Late-run Adams sockeye, I observed nearly equal numbers of males and females in each behaviour/fate category, but it was only in females that I found significant associations between physiology and behaviour/fate. Consistent with my first prediction, high somatic energy and low circulating reproductive hormone concentrations were found in female salmon that held before entering the river and subsequently survived to spawning areas. In contrast, females with low somatic energy and high hormone concentrations entered the river quickly but died before reaching spawning areas, a tactic bestowing zero life-time fitness to these semelparous animals. Not surprisingly, it is the former behavioural tactic that has been most commonly observed in Adams sockeye and is believed to be an adaptive component of their life-history (Burgner 1991). In male sockeye, the relationships between energy, reproductive hormone concentrations, and behaviour/fate were not significant, but nevertheless trended as observed in females. Thus, energetics and reproductive development are important determinants of migration timing in both sexes, but in males the influence on timing is weaker. This suggests that males have more flexibility around entry timing, and perhaps because

they invest comparatively less energy to gamete production during upriver migration than females (and more for locomotion and other processes peripheral to reproduction), they may take their entry cues from females who are under greater developmental constraint (Morbey and Ydenberg 2001; Chapter 2- Crossin et al. 2007). This idea remains to be tested.

Notwithstanding the overall relationship between early migration and fate, some individuals entered the river early without estuarine holding and survived to reach spawning grounds. These fish had higher somatic energy concentrations than those that entered early and failed (significantly so in females, trending in males). In fact, somatic energy in these 'no-hold and surviving' fish was nearly equal to that in those that held and survived (i.e. the adaptive tactic). Thus, in salmon straddling the line between advanced maturity and senescence and choosing to migrate directly into the river, somatic energy may provide an important buffer against migration failure but is not necessarily a trigger of upriver migration. However, it is important to note that I defined migratory success in this study as a fish's positive detection at spawning areas, but I did not actually monitor spawning (reproduction), which for a semelparous animal is the true measure of success. It should be noted however that previous studies suggest that most no-holding sockeye reaching spawning areas ultimately fail to spawn (Wagner et al. 2006). Thus, while high somatic energy may buffer early, no-holding migrants against failure during up-river migration, they will likely not reproduce (i.e. pre-spawning mortality) (Cooke et al. 2004; Wagner et al. 2006).

Survival to spawning areas was significantly related to processes tied to biological, circannual clocks, specifically maturation (Fostier and Jalabert 1986; Kobayashi et al. 1997; Norberg et al. 2004) and osmoregulation (Arendt 1998; Kulczykowska 2002). As mentioned earlier, those salmon that held in the estuary and successfully reached spawning areas, river entry timing was significantly and negatively correlated with circulating testosterone (in females) and gill Na^+,K^+-ATPase activities (in both sexes), and values for both variables were significantly higher in salmon that did not hold and failed during river migration. Thus, a synchronicity of maturational and osmoregulatory processes at the salt-to-fresh water interface are vitally important to migratory survival by homing sockeye, observations that support my second and third predictions. Osmoregulatory physiology is mediated in part by cortisol secretion (McCormick 1995), and there is some evidence of seasonally rhythmic secretion in salmonids but results are equivocal, varying by species and study (see Shrimpton et al. 2000). I did not see an effect of Julian date on cortisol concentrations, but the stress of purse-seine capture may have masked my ability to detect this. Nevertheless, cortisol's direct role in Na^+,K^+-ATPase

regulation is well established (McCormick 1995), and the down-regulation of gill ATPase was influenced by Julian date.

Rates of migration

Whilst homing from JS to the Fraser River estuary, Chilko sockeye swam at nearly twice the rate of Adams sockeye. However, this was not the case in sockeye homing from JDF, where the populations swam at similar rates. I do not have an explanation for this difference, nor do I have an explanation for the fact that both populations swam at similar rates in JDF. Adams sockeye had higher stress levels in JS relative to JDF, but the slower migration speeds observed in JS are what I would normally anticipate and is in fact consistent with anecdotal evidence from fisheries managers that Adams and other Late-run populations swim at slower rates and take longer to enter the river (see Crossin et al. 2004). Thus I would not attribute the slower speeds of Adams sockeye from JS to the effects of stress, nor would I attribute their similar speeds to Chilko in JDF to the lack of stress.

Once in river, I examined rates of travel among Adams sockeye from each of the various timing-and-fate combinations. The estuarine holding/successful fish swam upriver at slower-rates (~15.5 $km \cdot day^{-1}$) through an approximately 172 km stretch of the lower Fraser River than fish from the no-hold/successful and no-hold/failing groups (~19.2 and 22.6 $km \cdot day^{-1}$ respectively). River temperatures and discharge rates have well documented effects on fish swim speeds (Lee et al. 2003; MacNutt et al. 2006; Hanson et al. 2008). Salmon that did not hold in the estuary and entered the river quickly would have encountered higher temperatures and flows than those that held and entered later (Patterson et al. 2007). I might thus have expected the early, no-hold fish to swim at slower speeds but this is not what I observed. This was likely due to the higher circulating concentrations of testosterone in salmon that did not hold before entering, which has known stimulatory effects on migratory behaviour (Munakata et al. 2001; Cooke et al. 2006a,b, 2008; Chapter 2- Crossin et al. 2007). A recent study, however, found that the physiology of sockeye sampled in JS had little bearing on swim speeds once sockeye entered the Fraser River (Hanson et al. 2008), but this study only examined successful migrants from summer-run populations, whereas my analysis focused on both successful and failing fish from a Late-summer run population. Summer-run populations (e.g. Chilko) have not been exhibiting the radical shift in migration timing that has been afflicting Late-run sockeye for the past decade (Cooke et al. 2004; Chapter 6- Crossin et al. 2008). The range of entry timing behaviours observed in Adams sockeye in this study extended from ones that are likely adaptive

to ones that are less so. As such, I predicted that there would be physiological differences underpinning behaviour and fate.

Despite evidence of circannual biological rhythms in many animals (most extensively studied in birds; for review, see Wingfield et al. 1990; Ramenofsky and Wingfield 2007) and the synchronizing effect of seasonally predictable events like changing photoperiods, there have been few studies examining the role of hormones in the regulation of migratory behaviour (reviewed by Ramenofsky and Wingfield 2007). This present study and recent others point to a consistent role of testosterone and its derivatives in the coordination of river entry timing by migratory Pacific salmon (Munakata et al. 2001; Cooke et al. 2006a,b, 2008; Young et al. 2006; Chapter 2- Crossin et al. 2007). However, experimental tests of hormonal regulation of migration timing at this and other scales are needed.

During homeward migration through coastal areas, a salmon encountering areas of low salinity presumably has lower gill Na^+,K^+-ATPase activities in anticipation of river entry (Hinch et al. 2006). Consistent with this idea, I observed lower gill ATPase activities in JS than in JDF, where surface salinities were approximately ~10 to <30‰ in the former and >30‰ in the latter (Institute for Ocean Sciences, Fisheries and Oceans Canada, Sydney, BC, online data). However, when I accounted for variation in Julian date, geographic differences in ATPase activity vanished, which suggests that gill ATPase activity is expressed along a fixed temporal trajectory during migration. This is consistent with studies showing links between the seasonal and photoperiodicly induced secretions of prolactin and cortisol in homing salmon, hormonal processes that directly influence gill Na^+,K^+-ATPase expression and total osmoregulatory capacity (Hirano et al. 1990; McCormick 2001; Manzon 2003).

As with gill ATPase activity, circulating testosterone concentrations did not differ between capture locales when Julian date was accounted for, suggesting that reproductive development also advances along a fixed temporal trajectory, an observation supported by a vast body of literature on seasonality in birds, mammals, reptiles, amphibians, and other fish (Dawson et al. 2001; review in Dingle 1996). Interestingly, 17β estradiol (E_2) concentrations in Adams females were higher in JDF relative to JS, independent of sampling date. Chilko females followed a similar trend. These differences may be indicative of an acute physiological stress as plasma lactate, glucose, Na^+, and Cl^- were all higher in JS than JDF (McDonald and Milligan 1997). Stress is known to have inhibitory effects on the hypothalmo-pituitary-gonadal (HPG) axis and the synthesis of testosterone and E_2 (for reviews see Sapolsky 2002; Wingfield and Sapolsky 2003; for sockeye, see Hinch et al. 2006). The lower E_2 concentration in JS raises the

possibility of a stress inhibition of aromatase activity in ovarian follicles. Inhibition of E_2 has been documented in the brains of chronically stressed mammals and birds (reviewed by Balthazart and Ball 1998), but whether this occurs in regions other than the brain, and whether this response occurs in chronically stressed salmonids, remains to be tested.

The influence of stress during homeward migration

Plasma lactate, Na^+, Cl^- and osmolality were all significantly higher in Adams salmon that failed to enter the river whilst homing from JS, suggesting a greater susceptibility to stress and ionoregulatory imbalance. This stress might make it more difficult for some individuals to overcome the ionic gradient at the salt-to-freshwater interface, thus river entry may be a strong selective agent, though certainly other selective agents are at work prior to river entry (e.g. predation). In Chilko sockeye, however, there were no physiological associations with survival to river entry, nor were there any for Adams and Chilko fish migrating through JDF. This finding suggests that there is something inherently more stressful to Adams sockeye when migrating through though JS. The path that homing sockeye choose around Vancouver Island and links to their physiology is an interesting topic about which little is known (but see Blackbourn 1987; McKinnell et al. 1999).

Two stress-related issues are generally unavoidable when studying wild, homing salmon: fisheries harvest and fish handling. Fortunately, in this study commercial harvest in the northern and southern Strait of Georgia was virtually zero (M. Lapointe, Pacific Salmon Commission, Vancouver, BC, pers. comm.), but somewhat higher in JDF at ~30% per day. Despite the possibility of a cash reward (indicated in print on the transmitters), very few of the transmitters were returned which suggests that fisheries harvest was indeed low or that tags were overlooked when fish were captured and processed.

If salmon succumbed to the stress of handling, either directly through a systems-related failure or indirectly by an increased susceptibility to predation, I would have expected to see it within the first 1-2 days after release (as per Robichaud and English 2007), which corresponds to the NSOG line for JS released salmon, and the JDF line for JDF released salmon. Only for Adams sockeye released in JS was there indication of this possibility (23% loss before NSOG line). But for Adams sockeye in JDF, and Chilko sockeye in both JS and JDF, there was no loss before the first acoustic lines.

However, studies have shown that high lactate levels following exhaustive exercise in fish can lead to delayed mortality. The cumulative time of capture and tank holding prior to biopsy had significant associations with both lactate concentration and survival to the Fraser

River. I must make clear however that the biopsies do not include the stress of biopsy itself, which is likely additive. Upon release from the boat, I do not know whether individual lactate concentrations increased further or began falling. Whatever the case, my data suggest that in order to survive to the river, a threshold concentration of ~18-20 $mmol \cdot L^{-1}$ must not be breached. Furthermore, the likelihood of further increases appears to diminish after ~40-60 min. Studies with salmon and trout have shown that metabolic recovery after exhaustive exercise (a type of stress) is prolonged by post-exercise inactivity (Milligan et al. 2000), and that high post-exercise lactate concentration (i.e. lactacidosis) is related to delayed mortality (Farrell et al. 2001a,b). The key to reducing lactacidosis after exercise, or in this case after the stress of capture, holding and biopsy, and to prevent further increases in cortisol, is exposure to a light, aerobic exercise regime before release (~0.9 $body\text{-}lengths \cdot sec^{-1}$; Milligan et al. 2000; Farrell et al. 2001a,b). The aerobic increases in cardiac output and ventilation rate from such a regime promotes the oxidation of plasma lactate and clearance of glycolytically produced CO_2, and the return of blood pH to normal levels. In this study, fish were held from 2-15 min in a large fish box. Though provided with an adequate supply of well oxygenated water, the design of this box was not optimal for promoting aerobic exercise. In future studies, a Fraser Box (which aids the recovery of exhausted fish; Farrell et al. 2001a) or an equivalent design should be used for recovery to reduce the risk of post-release mortality.

In conclusion, this study has provided new insights to the behaviour and fate of a migratory fish species. I have contributed to our understanding of the physiological determinants of migration timing and survivorship by describing relationships between somatic energy concentrations and sexual maturity, and how these jointly influence migration timing and survivorship in sockeye (Young et al. 2006; Cook et al. 2006a,b, 2008; Chapter 2- Crossin et al. 2007). Also, I have also strengthened previously observed relationships of behavioural physiology by accounting for the temporal variation inherent to seasonal patterns of somatic energy, reproductive maturity, and osmoregulatory ability. By so doing, I found that gill Na^+,K^+-ATPase activities were vitally important for successful migration. My results have advanced our collective, general understanding of migration and raised some interesting comparative questions. For example, I found that both reproductive and osmoregulatory systems develop in concert in homing salmon, processes that are mediated by two different endocrine axes, the HPG and HPA (hypothalamo-pituitary-adrenal, or in fish the hypothalamo-pituitary-interrenal homologue [HPI]), which in most birds, mammals and other animals usually work in opposition (i.e. activation of the HPA/HPI axis usually has anti-gonadotropic effects on the HPG

axis [Sapolsky 2002]), but this does not appear to be the case in salmon (Donaldson 1970; Wingfield and Sapolsky 2003). How are salmon able to produce gametes and breed when adreno/interrenal-cortical functions (leading to high cortisol expression and gill ATPase activity) are so active? Testing the effects of sexual maturation and osmoregulation as proximate triggers of migration timing are required to expand our knowledge of migration beyond simple correlative associations. And testing the combined effects could yield interesting insights to cross-axis stimulations and/or inhibitions and their influence as drivers of migration behaviour.

Table 5.1. Numbers of acoustic and radio tagged sockeye salmon (*Oncorhynchus nerka*) released in JS and JDF, and the numbers that were subsequently detected on the first marine acoustic line after the point of release (i.e. the Northern Strait of Georgia acoustic receiver line (NSOG) for JS released fish, and the Juan de Fuca line for those released in JDF. Also listed are the numbers of fish detected at the Fraser River mouth on the Southern Strait of Georgia acoustic line (SSOG), and those detected in river at Mission, BC (see Figure 5.1).

Release Area	Population	Transmitter Type	Total Released	Detected on First Line	Detected on First and SSOG Lines	Detected in Fraser River (Mission, BC)	Detected at spawning areas †
Johnstone Strait	Adams	Acoustic	68	52	45	36 (52%)	21 (31%)
		Radio	275	n/a	n/a	122 (44%)	98 (36%)
	Chilko	Acoustic	7	7	7	6 (75%)	n/a
		Radio	41	n/a	n/a	15 (37%)	n/a
Juan de Fuca Strait	Adams	Acoustic	11	11	7	4 (36%)	2 (18%)
		Radio	55	n/a	n/a	25 (46%)	13 (24%)
	Chilko	Acoustic	5	5	2	1 (20%)	n/a
		Radio	43	n/a	n/a	18 (42%)	n/a

† Detection of Chilko sockeye salmon at terminal areas could not be assessed with confidence as the position of terminal receivers was upstream of where some sockeye are know to spawn. Thus, fish may have been successful in reaching spawning areas but may not have been detected. Cells marked with n/a indicate the inability radio-tags to be detected in salt-water.

Table 5.2. Comparison of the biological attributes of two populations of sockeye salmon (*Oncorhynchus nerka*) intercepted and sampled in Johnstone and Juan de Fuca Straits

		Marine area					
Variables by sex	Population by Sex	Johnstone Strait	N	Juan de Fuca Strait	N	Marine Area P	Julian day (covariate) P
Gross somatic energy ($MJ \cdot kg^{-1}$)	Adams ♀	8.8 ± 0.03	182	8.7 ± 0.10	35	0.632	**0.001**
	Adams ♂	8.6 ± 0.05	160	8.6 ± 0.16	27	0.465	**<0.001**
	Chilko ♀	9.1 ± 0.16	25	9.1 ± 0.15	31	0.933	0.045*
	Chilko ♂	8.8 ± 0.17	24	8.7 ± 0.20	19	0.619	0.118
Nose to fork length (cm)	Adams ♀	58.6 ± 0.19	182	58.7 ± 0.59	35	0.894	0.627
	Adams ♂	60.6 ± 0.21	160	61.4 ± 0.63	27	0.299	0.506
	Chilko ♀	57.6 ± 0.68	25	58.2 ± 0.66	31	0.644	0.911
	Chilko ♂	58.9 ± 0.88	24	60.4 ± 1.05	19	0.386	0.996
Plasma glucose ($mmol \cdot L^{-1}$)	Adams ♀	6.5 ± 0.08	182	4.9 ± 0.25	35	**<0.001**	**<0.001**
	Adams ♂	7.2 ± 0.08	160	5.6 ± 0.24	27	**<0.001**	**<0.001**
	Chilko ♀	7.1 ± 0.31	25	5.2 ± 0.30	31	**0.002**	**0.004**
	Chilko ♂	7.6 ± 0.23	24	5.8 ± 0.28	19	**<0.001**	0.065
Plasma lactate ($mmol \cdot L^{-1}$)	Adams pooled	10.4 ± 0.23	342	7.1 ± 0.68	62	**<0.001**	0.089
	Chilko pooled	9.4 ± 0.71	49	8.2 ± 0.75	50	0.251	0.425
Plasma Na^+ ($mmol \cdot L^{-1}$)	Adams pooled	179.5 ± 0.64	342	172.9 ± 1.86	62	**<0.001**	**<0.001**
	Chilko pooled	178.2 ± 1.97	49	175.5 ± 2.10	50	0.424	0.703
Plasma Cl^- ($mmol \cdot L^{-1}$)	Adams pooled	153.5 ± 0.29	342	151.2 ± 0.83	62	0.012*	0.295
	Chilko pooled	151.4 ± 0.94	49	150.0 ± 1.00	50	0.406	0.558
Plasma osmolality ($mOsm \cdot kg^{-1}$)	Adams pooled	373.3 ± 0.98	342	370.5 ± 2.83	62	0.412	0.425
	Chilko pooled	370.7 ± 2.98	49	370.7 ± 3.17	50	0.999	0.993
Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	Adams pooled	3.4 ± 0.10	342	3.7 ± 0.29	62	0.861	**<0.001**
	Chilko pooled	4.1 ± 0.40	49	4.1 ± 0.42	50	0.905	0.045*

Variables by sex	Population by Sex	*Marine area* Johnstone Strait	N	Juan de Fuca Strait	N	Marine Area P	Julian day (covariate) P
Plasma cortisol	Adams pooled	400.7 ± 7.8	342	430.0 ± 25.2	62	0.297	0.059
($ng \cdot ml^{-1}$)	Chilko pooled	414.9 ± 18.6	49	432.8 ± 22.5	50	0.608	0.996
Plasma testosterone	Adams ♀	38.5 ± 1.99	182	40.8 ± 6.21	35	0.683	**<0.001**
($ng \cdot ml^{-1}$)	Adams ♂	25.8 ± 1.42	160	26.4 ± 4.32	27	0.151	**<0.001**
	Chilko ♀	31.3 ± 6.53	25	42.2 ± 6.34	31	0.828	**0.004**
	Chilko ♂	17.3 ± 3.97	24	25.3 ± 4.71	19	0.416	0.006**
Plasma 17β-estradiol	Adams ♀	7.9 ± 0.49	182	13.6 ± 1.44	35	**<0.001**	**<0.001**
($ng \cdot ml^{-1}$)	Chilko ♀	4.5 ± 1.74	25	11.6 ± 1.60	31	0.074	0.009**

Sockeye known to be captured in fisheries were removed from the analysis. When necessary, means were adjusted to account for covariation with Julian day of sampling (ANCOVA). All variables were $\log_{10}$ transformed prior to analysis. Values marked with an asterisk (*) indicates significance at $\alpha < 0.05$, a double asterisk (**) indicates $\alpha < 0.01$, and bold faced values indicate significance at Bonferroni corrected α-values: 0.005 for females, 0.006 for males.

Table 5.3. Comparison of the biological attributes of sockeye salmon (*Oncorhynchus nerka*) that disappeared *en route* to the Fraser River whilst homing from Johnstone and Juan de Fuca Straits with those that survived to enter the river.

		Marine Fate					
Variables by Release Area	Population and Sex	Disappeared before entering river	N	Successfully entered river	N	Fate (within population) P	Julian day (covariate) P
Johnstone Strait:							
Gross somatic energy	♀ Adams	8.8 ± 0.04	103	8.8 ± 0.05	79	0.697	**0.002**
($MJ \cdot kg^{-1}$)	♀ Chilko	8.8 ± 0.23	17	8.9 ± 0.23	8	0.883	0.083
	♂ Adams	8.4 ± 0.07	88	8.5 ± 0.08	72	0.342	**<0.001**
	♂ Chilko	8.6 ± 0.11	16	8.8 ± 0.15	8	0.223	**0.004**
Nose to fork length	♀ Adams	58.3 ± 0.24	103	58.9 ± 0.27	79	0.082	0.435
(cm)	♀ Chilko	57.3 ± 0.40	17	58.4 ± 0.61	8	0.171	0.721
	♂ Adams	60.5 ± 0.26	88	61.1 ± 0.29	72	0.119	0.516
	♂ Chilko	58.5 ± 0.89	16	58.9 ± 1.27	8	0.786	0.990
Plasma glucose	♀ Adams	6.3 ± 0.10	103	6.6 ± 0.12	79	0.032*	**<0.001**
($mmol \cdot L^{-1}$)	♀ Chilko	6.2 ± 0.32	17	6.7 ± 0.48	8	0.531	0.013*
	♂ Adams	7.1 ± 0.10	88	7.0 ± 0.11	72	0.559	**<0.001**
	♂ Chilko	7.2 ± 0.20	16	7.1 ± 0.28	8	0.684	0.014*
Plasma lactate	Adams	10.8 ± 0.30	191	9.4 ± 0.33	151	0.009**	0.189
($mmol \cdot L^{-1}$)	Chilko	9.4 ± 0.60	33	7.4 ± 0.90	16	0.089	0.505
Plasma Na^+	Adams	180.2 ± 0.81	191	177.4 ± 0.91	151	0.022*	0.164
($mmol \cdot L^{-1}$)	Chilko	180.2 ± 1.71	33	175.7 ± 2.54	16	0.163	0.392
Plasma Cl^-	Adams	154.2 ± 0.34	191	152.4 ± 0.38	151	**<0.001**	0.392
($mmol \cdot L^{-1}$)	Chilko	152.1 ± 0.82	33	151.0 ± 1.22	16	0.489	0.545
Plasma osmolality	Adams	375.4 ± 1.20	191	369.9 ± 1.35	151	**0.003**	0.815
($mOsm \cdot kg^{-1}$)	Chilko	371.8 ± 2.57	33	368.6 ± 3.82	16	0.498	0.615

Variables by Release Area	Population and Sex	*Marine Fate* Disappeared before entering river	N	Successfully entered river	N	Fate (within population) P	Julian day (covariate) P
Gill Na^+,K^+-ATPase	Adams	3.3 ± 0.11	191	3.3 ± 0.13	151	0.846	**<0.001**
(μmol ADP·mg^{-1} protein·h^{-1})	Chilko	3.4 ± 0.32	33	3.7 ± 0.47	16	0.583	0.035*
Plasma cortisol	Adams	415.4 ± 10.4	37	394.7 ± 11.2	29	0.179	0.035*
(ng·ml^{-1})	Chilko	419.0 ± 23.5	35	409.9 ± 26.4	12	0.804	0.851
Plasma testosterone	♀ Adams	43.3 ± 2.67	103	42.5 ± 3.06	79	0.919	**<0.001**
(ng·ml^{-1})	♀ Chilko	52.6 ± 7.58	17	47.7 ± 11.38	8	0.805	**0.002**
	♂ Adams	29.2 ± 1.92	88	27.9 ± 2.14	72	0.679	**<0.001**
	♂ Chilko	23.7 ± 4.49	16	30.4 ± 6.41	8	0.440	<0.009**
Plasma 17β-estradiol	♀ Adams	9.7 ± 0.63	103	8.6 ± 0.72	79	0.126	**<0.001**
(ng·ml^{-1})	♀ Chilko	9.8 ± 1.57	17	8.2 ± 2.36	8	0.487	0.015*
Juan de Fuca Strait:							
Gross somatic energy	♀ Adams	9.0 ± 0.09	21	9.0 ± 0.12	14	0.918	0.303
(MJ·kg^{-1})	♀ Chilko	9.4 ± 0.11	21	9.0 ± 0.20	6	0.101	0.809
	♂ Adams	9.0 ± 0.11	16	9.2 ± 0.12	15	0.277	0.486
	♂ Chilko	8.9 ± 0.26	13	8.9 ± 0.39	6	0.838	0.432
Nose to fork length	♀ Adams	58.5 ± 0.53	21	59.5 ± 0.70	14	0.295	0.747
(cm)	♀ Chilko	58.1 ± 0.67	21	58.0 ± 1.23	6	0.775	0.785
	♂ Adams	60.2 ± 0.54	16	62.1 ± 0.60	15	0.024*	0.673
	♂ Chilko	59.8 ± 1.13	13	61.4 ± 1.69	6	0.468	0.936
Plasma glucose	♀ Adams	5.6 ± 0.18	21	5.5 ± 0.23	14	0.586	0.014*
(mmol·L^{-1})	♀ Chilko	5.9 ± 0.18	21	6.0 ± 0.32	6	0.724	0.552
	♂ Adams	6.4 ± 0.28	16	6.5 ± 0.32	15	0.872	0.248
	♂ Chilko	6.3 ± 0.33	13	6.1 ± 0.50	6	0.975	0.753

Variables by Release Area	Population and Sex	*Marine Fate* Disappeared before entering river	N	Successfully entered river	N	Fate (within population) P	Julian day (covariate) P
Plasma lactate	Adams	8.3 ± 0.57	37	7.6 ± 0.66	29	0.591	0.082
($mmol \cdot L^{-1}$)	Chilko	8.4 ± 0.64	35	10.4 ± 1.19	12	0.221	0.607
Plasma Na^+	Adams Chilko	176.7 ± 1.64	37	174.8 ± 1.88	29	0.564	0.044*
($mmol \cdot L^{-1}$)		174.1 ± 1.77	35	177.7 ± 3.31	12	0.384	0.942
Plasma Cl^-	Adams	151.9 ± 0.98	37	151.5 ± 1.12	29	0.669	0.092
($mmol \cdot L^{-1}$)	Chilko	149.4 ± 0.85	35	150.1 ± 1.60	12	0.571	0.349
Plasma osmolality	Adams	373.9 ± 2.70	37	369.9 ± 3.10	29	0.446	0.078
($mOsm \cdot kg^{-1}$)	Chilko	368.7 ± 2.67	35	378.0 ± 5.00	12	0.236	0.356
Gill Na^+,K^+-ATPase	Adams	4.9 ± 0.37	37	4.5 ± 0.42	29	0.260	0.668
($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	Chilko	4.4 ± 0.38	35	5.8 ± 0.71	12	0.097	0.432
Plasma cortisol	Adams	391.9 ± 20.2	37	404.8 ± 22.4	29	0.672	0.484
($ng \cdot ml^{-1}$)	Chilko	427.0 ± 16.4	35	440.6 ± 19.5	12	0.599	0.441
Plasma testosterone	♀ Adams	13.2 ± 1.85	21	15.1 ± 2.44	14	0.418	0.007**
($ng \cdot ml^{-1}$)	♀ Chilko	25.3 ± 2.80	21	16.5 ± 5.14	6	0.115	0.750
	♂ Adams	11.4 ± 2.43	16	8.4 ± 2.72	15	0.996	0.280
	♂ Chilko	14.7 ± 3.04	13	12.6 ± 4.52	6	0.617	0.412
Plasma 17β-estradiol	♀ Adams	6.7 ± 1.12	21	6.0 ± 1.37	14	0.473	0.059
($ng \cdot ml^{-1}$)	♀ Chilko	7.0 ± 1.23	21	8.9 ± 2.36	6	0.876	0.441

Sockeye known to be captured in fisheries were removed from the analysis. When necessary, means were adjusted to account for covariation with Julian day of sampling (ANCOVA). All variables were $\log_{10}$ transformed prior to analysis. Values marked with an asterisk (*) indicates significance at $\alpha < 0.05$, a single asterisk (**) indicates $\alpha < 0.01$, and bold faced values indicate significance at Bonferroni corrected α-values: 0.005 for females, 0.006 for males.

Table 5.4. Comparison of the biological attributes of Adams sockeye salmon (*Oncorhynchus nerka*) homing from Johnstone Strait that held in the estuary and survived to spawning areas versus those that did not hold in the estuary and disappeared in river.

		Migratory tactic and fate									
Variables	Sex	Held and disappeared	N	Held and survived	N	Did not hold and disappeared	N	Did not hold and survived	N	Migratory tactic & fate P	Julian day (covariate) P
Gross somatic energy ($MJ \cdot kg^{-1}$)	♀	n/a	0	8.9 ± 0.17	6	8.4 ± 0.13	9	8.8 ± 0.14	7	0.009**	0.186
	♂	n/a	0	9.1 ± 0.47	7	8.0 ± 0.30	15	8.7 ± 0.46	6	0.208	0.026*
Nose to fork length (cm)	♀	n/a	0	57.6 ± 0.95	6	58.8 ± 0.72	9	59.2 ± 0.82	7	0.483	0.196
	♂	n/a	0	61.5 ± 0.98	7	62.3 ± 0.61	15	61.6 ± 0.95	6	0.980	0.211
Plasma glucose ($mmol \cdot L^{-1}$)	♀	n/a	0	6.8 ± 0.48	6	6.9 ± 0.37	9	6.8 ± 0.42	7	0.996	0.017*
	♂	n/a	0	6.9 ± 0.26	7	7.0 ± 0.16	15	7.2 ± 0.25	6	0.764	**<0.001**
Plasma lactate ($mmol \cdot L^{-1}$)	Pooled	n/a	0	9.6 ± 1.00	13	11.2 ± 0.75	24	8.5 ± 1.0	13	0.097	0.891
Plasma Na^+ ($mmol \cdot L^{-1}$)	Pooled	n/a	0	178.2 ± 4.68	13	176.8 ± 3.51	24	178.2 ± 4.50	13	0.735	0.116
Plasma Cl^- ($mmol \cdot L^{-1}$)	Pooled	n/a	0	151.7 ± 1.23	13	152.6 ± 0.92	24	151.5 ± 1.19	13	0.731	0.580
Plasma osmolality ($mOsm \cdot kg^{-1}$)	Pooled	n/a	0	373.0 ± 4.72	13	374.6 ± 3.54	24	368.3 ± 4.53	13	0.549	0.455
Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}$ protein$\cdot h^{-1}$)	Pooled	n/a	0	2.2 ± 0.37	13	3.8 ± 0.27	24	3.9 ± 0.35	13	**<0.001**	0.045*
Plasma cortisol ($ng \cdot ml^{-1}$)	Pooled	n/a	0	436.0 ± 26.1	13	368.5 ± 24.3	24	392.4 ± 27.6	13	0.172	0.959
Plasma testosterone ($ng \cdot ml^{-1}$)	♀	n/a	0	17.5 ± 11.28	6	60.3 ± 8.62	9	61.2 ± 9.73	7	0.010**	**<0.001 (-)**
	♂	n/a	0	21.5 ± 6.64	7	31.8 ± 4.15	15	36.1 ± 6.48	6	0.216	**0.005**

		Migratory tactic and fate									
Variables	Sex	Held and disappeared	N	Held and survived	N	Did not hold and disappeared	N	Did not hold and survived	N	Migratory tactic & fate P	Julian day (covariate) P
Plasma 17β-estradiol ($ng \cdot ml^{-1}$)	♀	n/a	0	10.3 ± 3.19	6	12.5 ± 2.44	9	7.65 ± 2.75	7	0.208	0.007** (-)
	♂	n/a	0	n/a		n/a		n/a		n/a	n/a

Sockeye known to be captured in fisheries were removed from the analysis. Means were adjusted to account for covariation with Julian day of sampling (ANCOVA). All variables were $\log_{10}$ transformed prior to analysis. Values marked with a single asterisk (*) indicates $\alpha < 0.05$, a double asterisk (**) indicates $\alpha < 0.01$, and bold faced values indicate significance at Bonferroni corrected α-values: 0.005 for females, 0.006 for males.

Table 5.5: Correlation coefficients and p-values relating the time it took individual Adams sockeye salmon to enter the Fraser River with their physiological profile at time of release. Correlations are drawn for fish that disappeared in the Fraser River *en route* to spawning areas and for fish that successfully reached spawning areas.

		Travel time from Johnstone Strait to Mission			
Variables	Sex	Disappeared in river	N	Survived to spawning areas	N
Gross somatic energy ($MJ \cdot kg^{-1}$)	♀	0.115 (0.660)	17	0.104 (0.424)	61
	♂	-0.210 (0.404)	18	0.133 (0.341)	53
Nose to fork length (cm)	♀	0.114 (0.581)	17	-0.147 (0.257)	61
	♂	-0.346 (0.159)	18	0.129 (0.351)	54
Plasma glucose ($mmol \cdot L^{-1}$)	♀	-0.267 (0.300)	17	-0.028 (0.829)	60
	♂	-0.046 (0.856)	18	0.019 (0.894)	54
Plasma lactate ($mmol \cdot L^{-1}$)	Pooled	-0.108 (0.538)	35	0.118 (0.210)	114
Plasma Na^+ ($mmol \cdot L^{-1}$)	Pooled	-0.125 (0.488)	35	0.090 (0.343)	114
Plasma Cl^- ($mmol \cdot L^{-1}$)	Pooled	0.075 (0.667)	35	0.040 (0.675)	114
Plasma osmolality ($mOsm \cdot kg^{-1}$)	Pooled	0.015 (0.932)	35	0.110 (0.244)	114
Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	Pooled	-0.126 (0.472)	35	-0.316 (**<0.001**)	114
Plasma cortisol ($ng \cdot ml^{-1}$)	Pooled	0.035 (0.941)	7	0.129 (0.126)	114
Plasma testosterone ($ng \cdot ml^{-1}$)	♀	-0.115 (0.662)	17	-0.340 (0.008**)	60
	♂	0.156 (0.537)	18	-0.184 (0.186)	53
Plasma 17β-estradiol ($ng \cdot ml^{-1}$)	♀	0.95 (0.716)	17	0.17 (0.896)	61
	♂	n/a		n/a	

Sockeye known to be captured in fisheries were removed from the analysis. All variables were $\log_{10}$ transformed prior to analysis. Values marked with a double asterisk (**) indicates significance at $\alpha < 0.01$, and bold faced values indicate significance at Bonferroni corrected α-values: 0.005 for females, 0.006 for males.

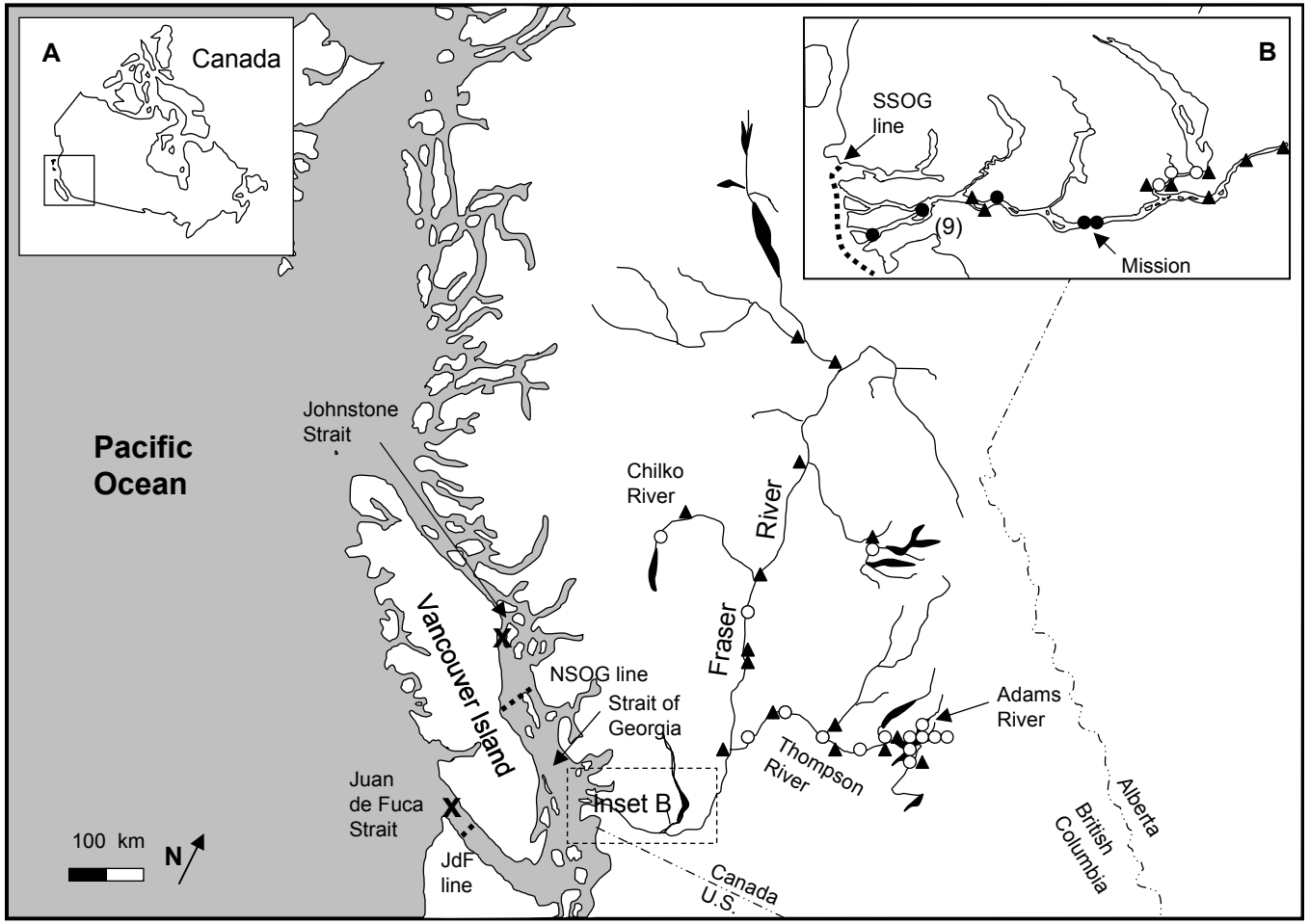

Fig. 5.1: Map of coastal British Columbia and the Fraser River watershed indicating the locations of capture and sampling efforts on sockeye salmon (*Oncorhynchus nerka*), and the location of acoustic (circles) and radio (dark triangles) receivers. Acoustic telemetry arrays positioned in Johnstone and Juan de Fuca Straits and at the Fraser River mouth are indicated with dotted lines. Insets include a map of Canada highlighting the study location, and a blow-up of the mouth and lower reaches of the Fraser River.

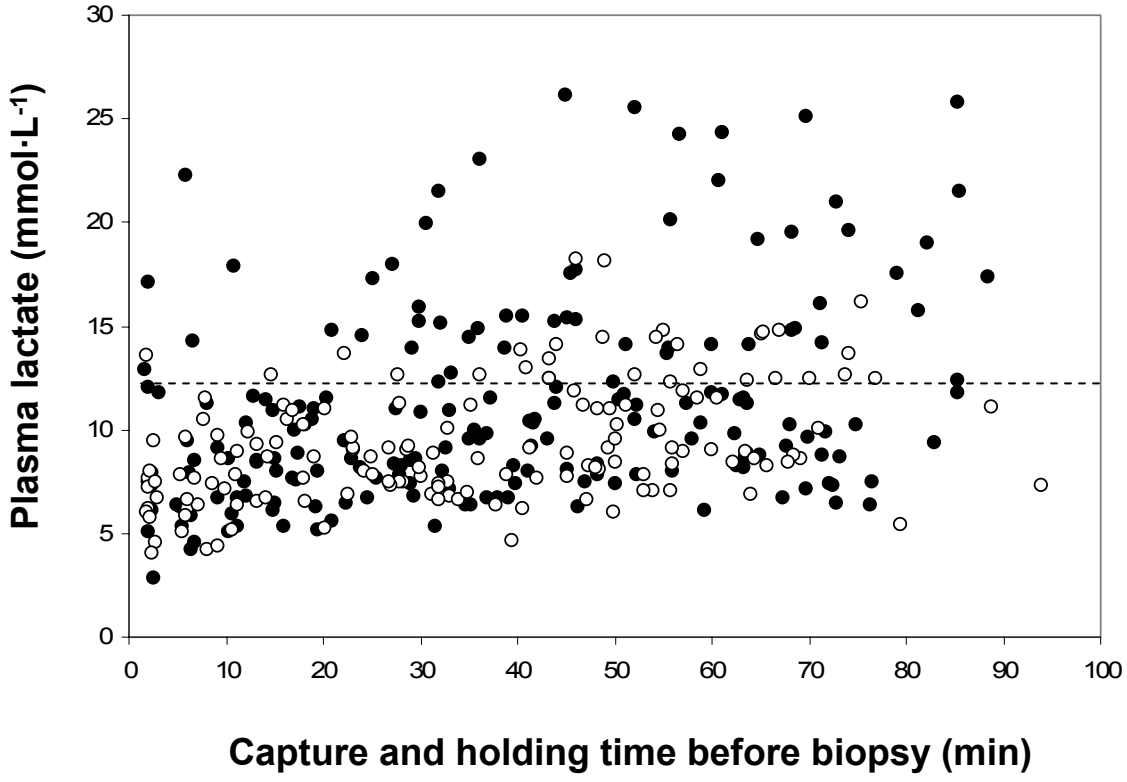

Fig. 5.2: Relationship between plasma lactate concentration and cumulative capture and pre-biopsy holding times in sockeye captured and released in Johnstone Strait. Black points are sockeye that failed to enter the Fraser River, white points are those that did so successfully. Dashed line indicates the threshold above which salmon have difficulty recovering from anaerobic stress (~12 mmol·L^{-1}, Jain and Farrell 2003). For failed sockeye, 28% are above and 72.% are below the dashed line. For successful sockeye, 18% above and 82% below.

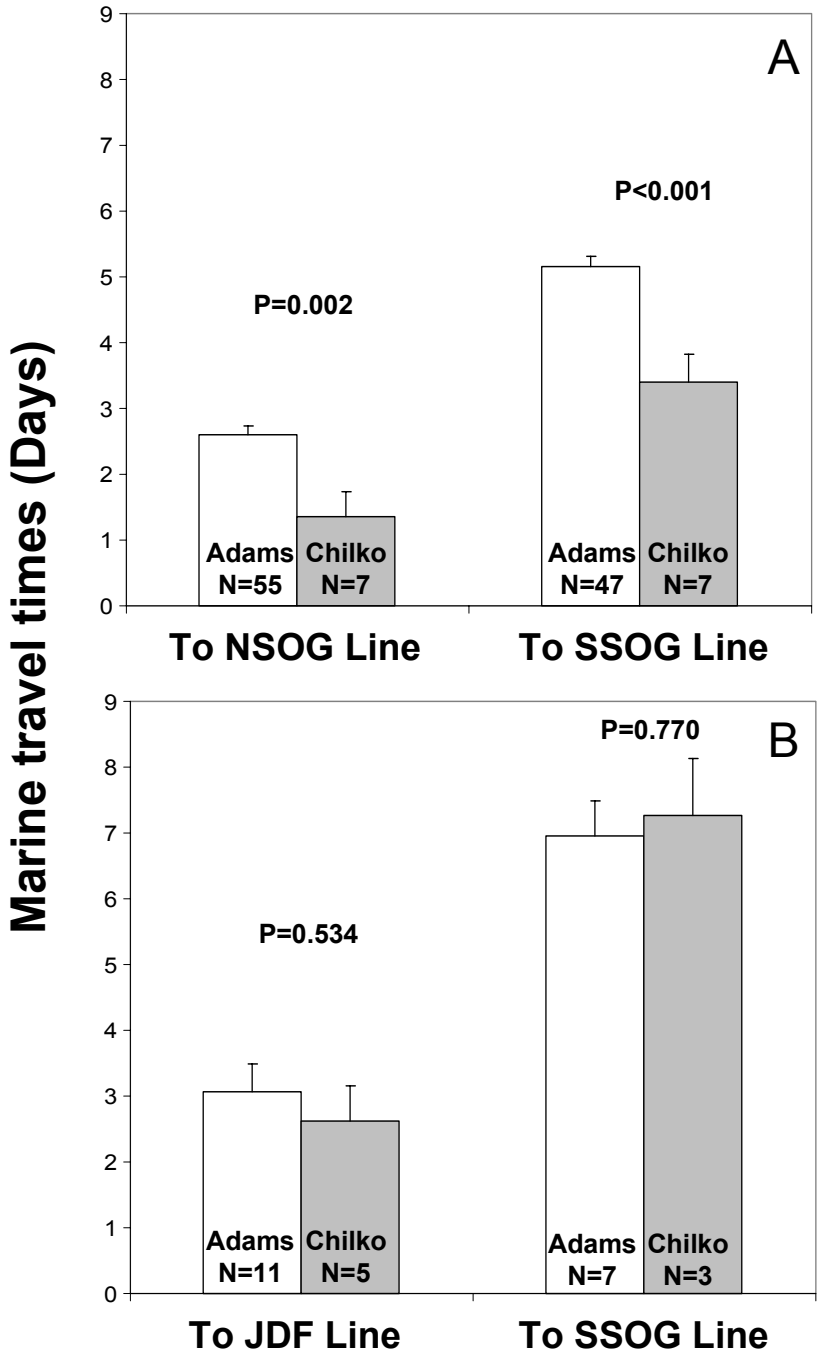

Fig. 5.3: Mean travel times by Adams and Chilko sockeye salmon from Johnstone Strait (Panel A) and Juan de Fuca Strait (Panel B) to acoustic receiver lines positioned in the marine environment *en route* to the Fraser River. The distances from point of release to each receiver line in Panel A are ~64 and 183 kms respectively, and in Panel B are ~50 and 200 kms. Fewer sockeye were captured and released in JDF as capture operations were moved to JS after one week due to higher diversion through JS as the season progressed (see Methods and Materials). Sexes were pooled. Travel times were corrected to account for variation in body length. Error bars are +1 SEM.

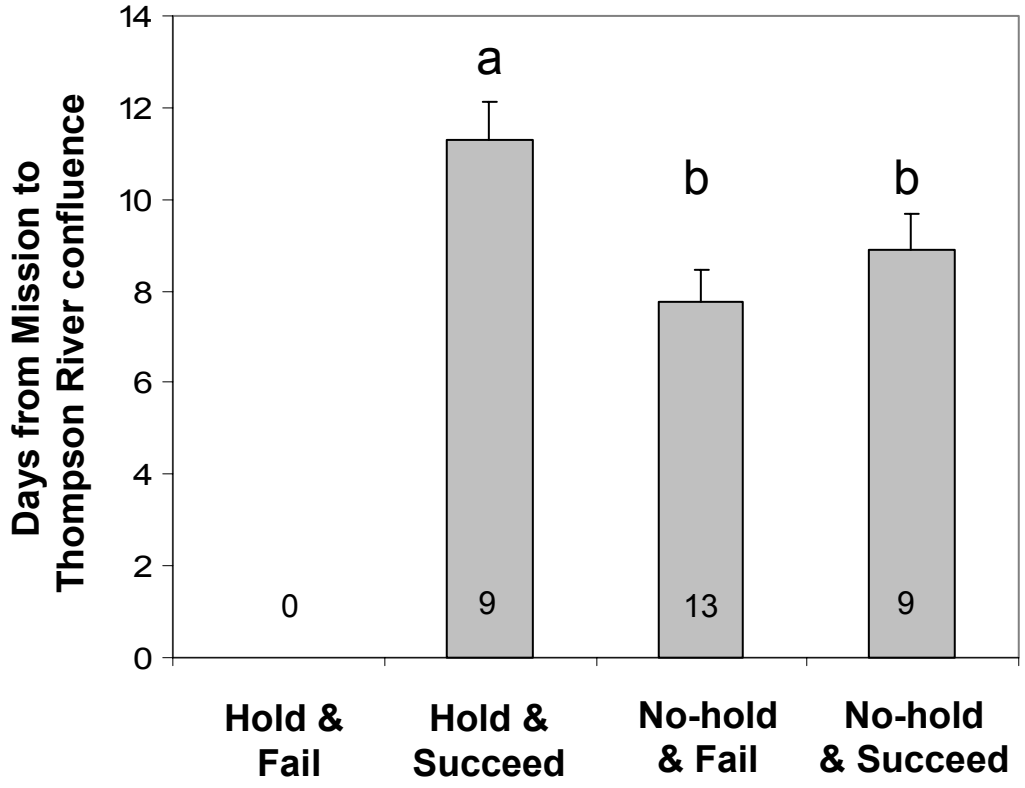

Fig. 5.4: Travel times by Adams sockeye salmon, release in Johnstone Strait, over an ~172 km stretch of the Fraser River between Mission and the Thompson River confluence. Travel times are presented as a function of estuarine holding behaviour and fate: fish either held at the river mouth and subsequently survived river migration to spawning areas, entered the river directly without holding and died in river, or entered without holding and survived. No fish that held died in river. Travel times were corrected to account for variation in variation in Julian date which would include variation in temperature and flow regimes. Error bars are +1 SEM.

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CHAPTER 6

Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migration.[5]

Introduction

Each summer, homing sockeye salmon (*Oncorhynchus nerka*) begin their spawning migrations through the Fraser River of British Columbia, and for a given population, the timing of river entry rarely deviates by more than a week inter-annually (Woodey 1987), though timing can vary appreciably among populations (Hodgson and Quinn 2002). Upon return from the high seas, the late summer run of homing Fraser River sockeye (forthwith called "Late-run" sockeye) represents a group of populations that are characterized not only by their river entry date but also by a holding behaviour in the Strait of Georgia near the Fraser River estuary that generally lasts 2-6 weeks before fish finally enter the river and migrate to natal streams. Since 1995 however, variable but large segments of Late-run sockeye populations have abandoned this holding behavior (Cooke et al. 2004), and have advanced freshwater entry by 2-8 weeks. Because spawning dates for these fish have not changed, Late-run salmon entering prematurely spend several weeks longer in freshwater relative to historic norms (Lapointe et al. 2003). The reason for this change in behaviour by Late-run sockeye is not known, but here I examine the consequences of this behaviour.

Given the highly adaptive nature of migration timing, not only in salmon but in many organisms (Brown and Brown 2000; Drent et al. 2003; Prop et al. 2003; Bêty et al. 2004), serious fitness consequences are expected when migration dates deviate significantly from historic averages. Early river entry by Late-run sockeye has been associated with high levels of mortality (60-90%), exceeding 4 million fish since this was first noted in 1996 (M. F. Lapointe, Pacific Salmon Commission, Vancouver, BC, Canada, unpublished data). Recent studies have shown that these early entering fish are reproductively more advanced than those entering at normal times (Cooke et al. 2006; Chapter 2- Crossin et al. 2007). In addition, premature entry

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means that Late-run sockeye leave cool coastal waters (10-13 °C) and enter the Fraser River in mid-summer when river temperatures are ≥ 18 °C rather than 10-14 °C in late summer and autumn which is what they would normally experience after estuarine holding (Patterson et al. 2007). This seems anomalous as no sockeye populations anywhere have been known to initiate spawning migrations into rivers at times when average seasonal temperatures are higher than 19 °C (Hodgson and Quinn 2002). Compounding matters is an approximately 1.5 °C warming of peak summer temperatures in the main stem of the Fraser River over the past 60 years due to climate change (Patterson et al. 2007). The year that the present study was conducted (2004), the earliest migrants of the Weaver Creek population of Late-run sockeye salmon experienced river temperatures in excess of 21 °C, which greatly exceeds the optimum temperature for metabolic aerobic scope for this population (Lee et al. 2003) and approaches the 5-day lethal temperature of 22 °C for adult sockeye (Servizi and Jensen 1977).

The high routine metabolic costs associated with exposure to high water temperature likely results in the rapid depletion of somatic energy stores in salmon that have ceased feeding. This may put salmon at risk of exhaustion prior to spawning because somatic energy reserves are vital for the successful completion of migration and spawning. Most sockeye have little reserve energy after spawning (Brett 1995; Hendry and Berg 1999; Crossin et al. 2004; Hinch et al. 2006) and die, in part, from exhaustion. Exposure to high temperature may also promote disease in salmon (Fagerlund et al. 1995), like the myxosporean kidney parasite *Parvicapsula minibicornis* which has been implicated in the mortality of early migrating Fraser sockeye. Despite the potential consequences of exposure to high river temperatures, no study has directly investigated whether the temperature experienced by homing sockeye affects migration success, and, because they have only one life-time reproductive opportunity, fitness.

I tested the hypothesis that exposure of sockeye to warm temperatures at and above 18 °C reduces migration success compared to salmon exposed to cooler temperatures (10 °C). Late-run salmon from the Weaver Creek population were caught *en route* to spawning grounds and were experimentally exposed to temperatures that have commonly been encountered by early-timed migrants (18 °C), and to temperatures historically encountered by normal-timed fish (10 °C; Patterson et al. 2007). These temperatures also bracket the optimal temperature for swimming performance (14-15 °C) for this population (Lee et al. 2003). The holding temperatures also spanned a disease threshold for *P. minibicornis* infection (Wagner et al. 2005). Fish were biopsied to assess aspects of their physiology, and were then implanted with acoustic transmitters and returned to the river where migration behaviour was observed by underwater

acoustic receivers as fish swam to the spawning stream. Biopsy was used to examine plasma ions, metabolites, reproductive hormones, heat shock proteins, and somatic energy levels, and to assess whether handling and holding affected behaviour and survival of fish. Examination of moribund fish during the holding component of the study was used to assess *P. minibicornis* disease development. I predicted that warm-treated sockeye would have less somatic energy, elevated stress measures (e.g. high plasma cortisol, lactate and glucose, high heat shock proteins levels), and higher levels of parasitic infection relative to cool-treated sockeye. After release, I predicted that warm-treated sockeye would exhibit higher rates of migration mortality.

Methods and Materials

Study animals and capture technique

Sockeye salmon were captured by beach seine 10 km downstream from the spawning grounds at Weaver Creek (Figure 6.1) on September 13 and 14, 2004. Weaver sockeye historically migrate into the Fraser River from the Pacific Ocean between mid-September and mid-October, but premature entry begins as early as mid-August (Lapointe et al. 2003; Cooke et al. 2004). I sampled fish at the start of the historic migration window when the river temperature was ~15 °C. Fish were then transferred by dip-net into a holding pen anchored on the river bank, then floy tagged and biopsied. Fish were subsequently held at the Fisheries and Oceans Canada Chehalis Hatchery (located ~5 km from capture site) and scale analyses were conducted to determine population of origin (Gable and Cox-Rogers 1993). Weaver sockeye were transported to experimental holding tanks at Cultus Lake the next day while salmon identified as belonging to co-migrating populations were returned to the capture site and released. There was no mortality during holding at the hatchery or prior to release. Population identity of Weaver sockeye was later verified by DNA analyses (Beacham et al. 1995, 2004). All procedures were approved by the Animal Care Committees of the University of British Columbia and Fisheries and Oceans Canada. Capture, transplant, and release permits were issued by Fisheries and Oceans Canada.

Experimental design

Weaver sockeye (N=100) were transported in large aerated transport tanks from the capture site 50 km to the Fisheries and Oceans Canada Cultus Lake Research Laboratory (Figure 6.1). Salmon were randomly assigned to the two tanks (3.7 m diameter; 1.5 m deep) and maintained at 15 °C for the first two days. Over the subsequent two days, water temperature in one tank was raised to 18 °C (N=50 fish), and lowered to 10 °C in the other (N=50 fish). Fish

were held for 24 days at these temperatures. A unidirectional flow was maintained in both tanks around the periphery at 0.35-0.50 m•sec^{-1} (measured at the mid-water level, see Patterson et al. 2004). By assuming a 6.5 day migration at 15 °C in the Fraser River prior to reaching the capture site (English et al. 2005), the cumulative freshwater temperature experienced by the fish (degree days, or DD) prior to release from the holding tanks was 510 DD for the warm treated fish and 325 DD for the cool-treated fish. This range brackets the cumulative temperature threshold (~400 DD) for the full expression of the kidney parasite (*Parvicapsula minibicornis*) (Cooke et al. 2004; Wagner et al. 2005). During holding, moribund salmon (<2 h dead) were removed and histological examinations of the kidneys were conducted to determine the severity of *P. minibicornis* infection. Biopsy of additional tissues or plasma was not performed on dead or moribund fish as these samples are time sensitive and most dead fish were recovered up to several hours after death. Assay and biopsy details are provided below.

On October 7th, fish were biopsied again, gastrically implanted with acoustic transmitters (details below), and transported to the release location at Derby Reach, Fraser River, ~85 km downstream of the initial capture site on the Harrison River (Figure 6.1), Temperature in the Fraser River at release and in the Harrison River when migrants re-ascended were similar (~12-14 °C) Acoustic receivers positioned at sites along the migration route (details below; Figure 1) allowed calculation of migration rates and survival estimates for individual fish. Radiotagging studies show that Weaver sockeye can swim from Derby Reach to spawning areas in 3 to 7 days. Peak spawning for this population occurred in mid October (10th-15th).

Experimental controls

To control for the effects of capture, handling and biopsy on post-release rates of migration and survival of the thermally treated fish, a set of additional salmon were captured 5 km upriver from the site where the thermally treated fish were captured on the Harrison River and had numbered floy tags inserted through the dorsal musculature anterior to the dorsal fin. These fish were captured on October 6th when, like the experimental fish, they were migrating during the normal migration window for this population. The experimental fish were thus at the leading edge of the normal migration window, and the control fish toward the back half. Neither were early migrants. Control fish were held overnight while scale analyses were conducted for population identification. Once Weaver sockeye were identified (N=13, 8 males, 5 females), they were removed and biopsied, telemetry tagged, and transported to the same release location at Derby Reach on October 7th, and collectively the treated fish and control fish were released.

These control fish were handled identically as the experimental fish with the exception of thermal treatment.

Biopsy, tagging and biotelemetry

The biopsy procedures followed those described by Cooke et al. (2005). Fish were individually dip-netted and placed in a V-shaped, foam lined trough with a continuous flow of fresh ambient water directed into the mouth and across the gills. A cinch tag printed with a unique identifying number was inserted through the dorsal musculature of each fish, anterior to the dorsal fin. Biopsies collected: a) a small section of adipose fin (0.5 g) for DNA confirmation of population identity (collected at capture only), (b) a single scale for determining fish age (collected at capture only), (c) 3 ml of blood collected from the caudal vasculature with an heparinized syringe (1.5", 21 gauge; Houston 1990) for assessing plasma chemistry, and (d) <4 mm of gill tissue from the tips of 6 to 8 filaments (<0.3 g) for assessing gill Na^+,K^+-ATPase activity (McCormick 1993). From the 3 ml sample of blood, a small volume (<0.01 ml) was removed by capillary tube and centrifuged for 3 minutes to measure hematocrit (Hct = percentage packed red blood cells). Blood samples were then centrifuged for 7 min to separate plasma from red blood cells, both of which were pipetted into separate cryo-vials and placed immediately on dry ice until they could be transferred to a -86 °C freezer. Gill tissues were stored similarly. Fish nose-to-fork length was measured. Gross somatic energy (GSE, $MJ \cdot kg^{-1}$) was assessed using a micro-wave energy meter (Distell Fish Fatmeter model 692, Distell Inc, West Lothian, Scotland, UK), with readings taken from the left side of the fish in two locations (see Crossin and Hinch 2005). Equipment malfunctions meant that GSE was not measured in any of the salmon at initial capture, though all were measured prior to release. As a result, this variable was not included in the multivariate analysis of variance (MANOVA) model that examined for physiological differences pre-holding. However, GSE at capture was measured in a separate group of Weaver fish that were collected at the same time and sacrificed for more extensive physiological analyses not reported here, giving us some indication of mean GSE at capture. Fish sex was determined either visually whenever possible (sexual dimorphism is expressed in sockeye salmon approaching the spawning grounds) or by the proportion of plasma 17β-estradiol to testosterone which is higher in maturing female salmon (Fostier et al. 1983).

Acoustic transmitters (16 mm diameter; 56 mm long; ~25.0 g in air; V16-3H-R04K coded pingers, Vemco Inc., Shad Bay, NS, Canada) were inserted into the stomach with a smooth plastic applicator (see Cooke et al. 2005). After tagging, fish were placed into a transport tank containing aerated 15 °C water and moved to the release site where they were

released individually. The total time to process a fish including biopsy, transmitter insertion and length assessment, was 2-3 minutes. The biopsy and transmitter insertion procedures were done on fish that were not anesthetized and this technique has been carefully evaluated in previous studies to ensure that fish handled in this manner do not suffer any deleterious effects to migratory behaviour and survival (Cooke et al. 2005, 2006).

Fourteen acoustic receivers (VR-2s, Vemco Inc., Shad Bay, NS, Canada) were positioned at sites along the migratory route and near the spawning grounds (i.e. Morris Lake) of Weaver Creek sockeye (Figure 6.1): 1) Pitt River confluence, downstream of release site, 2) Albion, BC, 3) Mission, BC, 4) Harrison and Fraser Rivers confluence, 5) Kilby Park, 6) Chehalis Nation, 7) Morris Lake and Weaver Creek, 8) Upper Harrison River, 9-11) Harrison Lake. Paired receivers were mounted at sites 1-3 and single receivers at all other sites. The time of first detection at each receiver was recorded for each tagged fish and travel times were calculated relative to the time of release. Detection at site 1 was used to assess fish fallback after release, and to confirm mortalities. I monitored fish by foot and via boat surveys throughout the study area with a hand-held receiver (VR-60, Vemco Inc., Shad Bay, NS, Canada).

Laboratory Assays

Plasma testosterone (T), and 17β-estradiol (E_2) levels were measured by radioimmunoassay (McMaster et al. 1992). Plasma osmolality and concentrations of lactate, glucose, and cortisol were determined with protocols described by Farrell et al. (2001), and gill tissue Na^+,K^+-ATPase activity was determined with a kinetic assay (McCormick 1993). Pre- and post-treatment red-blood cell samples were analyzed for heat shock 70 protein (Hsp70) levels. Hsp70 levels were determined by SDS-PAGE (Forsyth et al. 1997), using rabbit polyclonal anti-Chinook salmon Hsp70 primary antibody (Stressgen, Victoria, BC, Canada). Two 5-μm sections of kidney from the moribund or recently dead salmon were fixed in Davidson's solution, mounted on glass slides, and stained with haematoxylin and eosin. The number of spores per 25 glomeruli was used as a measure of infection level, with 25 spores per 25 glomeruli representing maximal (100%) infection (Jones et al. 2003; Wagner et al. 2005).

Statistical Analyses

All analyses were conduced with JMP 4.0 (SAS Institute, Raleigh, NC, USA). All data were log (10) transformed to reduce heteroscedasticity but non-transformed values were used in tables and figures. All physiological, reproductive, and energetic variables were then analyzed for normality. MANOVA on log (10) transformed data (McGarigal et al. 2000) was used to

assess physiological differences in fish upon their initial capture on the Harrison River. The model effect for this analysis was treatment designation (warm or cool), and genders were analyzed separately. After experimental temperature treatment, individual differences between initial capture and post-treatment physiological differences were analyzed with paired *t*-tests. Pearson's correlations were used to explore relationships between post-treatment migratory rates physiological state. Chi square tests were used to compare survival of fish numbers from the point of release to various upriver locales. All analyses were assessed for statistical significance at α=0.05.

Because multiple comparisons among the physiological variables were made in the MANOVA models, Bonferroni corrections were applied depending on the number of variables included in a given statistical model: 6 variables α=0.008, 7 variables α=0.007, 8 and 9 variables α=0.006, 10 and 11 variables α=0.005. However, due to the highly conservative nature of Bonferroni corrections, I indicate when variables were significant at the α=0.05 and at the specific Bonferroni-corrected levels listed above (See Tables 1 and 2), so that readers can define for themselves which levels are most biologically meaningful, as suggested by Cabin and Mitchell (2000).

Results

At capture, there were no significant differences in any of the physiological variables (i.e. pre-treatment plasma osmolality, plasma Na^+, plasma K^+, plasma glucose, plasma lactate, plasma testosterone, hematocrit, Hsp70, and gill Na^+,K^+-ATPase) between warm and cool treated fish (MANOVA; females P=0.105, F=1.630, N=22; males P=0.725, F=1.183, N=15). Gross somatic energy could not be measured at capture, but in separate group of Weaver fish captured at the same time for a different study, GSE was 6.12 $MJ \cdot kg^{-1}$ +/- 0.2 SEM.

The temperature experienced during treatment had a significant effect on survival: 31 fish survived at 10 °C (15 females and 16 males), and 17 fish at 18 °C (8 females and 9 males). Thus, survival was much lower for warm-treated (34%), than for cool-treated sockeye (62%; ANOVA, P=0.011). Despite these differences in survival, when survival was expressed as a function of cumulative thermal experience (Figure 6.2a), the two curves followed a similar decreasing trajectory.

The expression of *P. minibicornis* in moribund fish was temperature dependent. The kidneys of 18 °C treated fish (N=14) had significantly higher levels of *P. minibicornis* spores than 10 °C-treated fish (N=13; P=0.002; Figure 6.2b). The infection scores were maximal (25)

in 7 of the 14 fish that were exposed to 18 °C and that had accrued >350 DD, whereas none of the 10 °C-treated fish showed histological evidence of infection. Qualitatively, warm treated sockeye also had more visible external fungal infections (e.g. *Saprolegnia* spp.).

After Bonferroni-correction, the only significant differences in physiological state between warm and cool treatment groups were an increase in gill Na^+,K^+-ATPase activity in cool-treated fish and a decrease in activity in warm-treated fish (Table 6.1: female P=0.003, N=21; male P=0.002, N=20). There was a trend toward a post-treatment decline in plasma glucose in warm-treated females, though this difference was not significant after Bonferroni correction (Table 6.1). Unlike other physiological variables, GSE and cortisol were only measured fish at the end of the treatment period, so I could not assess how these changed during experimental holding. There was however no significant difference in either GSE or cortisol concentrations between warm and cold treated sockeye at the end of thermal treatments prior to being returned to the river (Table 6.1).

Regarding the control sockeye, sex-steroid assays were not run due to budget constraints, but I was confident assigning sex based on secondary sexual characteristics. Comparisons with the thermally treated fish however were thus done with one-way ANOVA using treatment (10 °C, 18 °C, and control) as the only model effect. Prior to this analysis, I compared all the post-treatment physiological variables in males and females from both thermal treatment groups with two-way ANOVA, using treatment and sex as model effects. After Bonferroni correction, the analysis did not find any significant differences between the sexes, but at the α=0.05 level differences in plasma Na^+ (P=0.019), plasma Cl^- (P=0.008), gill ATPase (P=0.009), and GSE (P=0.009) were observed. Between treatments, a Bonferroni significant difference was in gill ATPase (P<0.0001), and an α=0.05 difference in glucose (P=0.007). These treatment differences are consistent with the results of paired *t*-tests presented for each sex in Table 6.1. Using one-way ANOVA to compare the physiology of the control fish (which were captured, biopsied and handled the same as the treated fish but were not held or exposed to thermal treatment) and the thermally treated fish, control fish did not differ from 10 °C treated fish (GSE, hematocrit, Na^+, Cl^-, glucose, lactate, osmolality, gill Na^+,K^+-ATPase, all P>0.05), but both the 10 °C cold-treated and the control fish differed significantly from 18 °C in plasma Na^+, glucose, and gill ATPase (see Table 6.2).

After the treatment period, forty eight thermally treated salmon were transported ~85 km downstream from the initial collection site and were released back to the Fraser River. In total, the 18 °C-treated fish (N=17) had accrued 506-510 DD and the 10 °C-treated fish (N=31) 323-

327 DD. Thirteen control fish were also transported to and released at the same site. As during the holding period, fish held at 18 °C showed elevated mortality after release into the river (Figure 6.3), and these had only 35% (1 female, 5 males) success in reaching the spawning grounds. In contrast, fish held at 10 °C (N=31) were 68% (10 females, 11 males) successful, and control fish were 62% (3 females, 5 males) successful. Fish held at 18 °C migrated to Site 4 (Fraser-Harrison confluence) significantly slower than either control or the 10 °C-held salmon (P=0.045; Figure 6.4). This was the only site where travel times were significantly different. Interestingly, travel times to spawning areas at site 7 in successful 18 °C males and the one successful 18 °C female did not differ from travel times of successful 10 °C fish (Figure 6.4). When survival to spawning areas was examined by sex, survival was significantly lower (Chi square 6.727, P=0.035) for females held at 18 °C (N=1 of 8, or 13%) compared to 10 °C-held females (N=10 of 15, or 67%) and control fish (N=3 of 6, or 50%), and lower than all of the male groupings, which did not differ from one another (Chi square 0.568, P=0.753: 10 °C males- N=11 of 16, or 69%; control males- N=5 of 7, or 71%; 18 °C males- N=5 of 9, or 56%) (Figure 6.3).

An individual's physiological state prior to release was correlated with subsequent migration behaviour. For convenience, I only present correlations to sites where significant correlations were observed: site 3 (Mission, BC), 4 (Harrison River confluence), and 7 (spawning grounds) (Table 6.3). Independent of holding temperature, plasma lactate levels in females at time of release were positively correlated with travel times to site 3 at Mission (Table 6.3; 18 °C-held salmon: lactate, r=0.872, P=0.03; 10 °C-held salmon: lactate r=0.757, P=0.0027). During migration to site 3, GSE levels in females held at 18 °C were positively correlated with travel time (r=0.791, P=0.03), while 17β-estradiol levels in females held at 10 °C were negatively correlated with travel time (r=-0.760, P=0.0036). To site 4, testosterone and glucose levels in females held at 18 °C were also negatively correlated with travel time (testosterone, r=-0.934, P=0.018; 11-keto testosterone, r=-0.884, P=0.046; glucose, r=-0.934, P=0.02). Thus, females held at 18 °C generally took longer to migrate upriver if they had high levels of somatic energy (high GSE), were stressed (high lactate and glucose), and were possibly less reproductively advanced (lower plasma testosterone and 11-keto testosterone). Females held at 10 °C took longer to migrate if they stressed (high plasma lactate, site 3) and were possibly less reproductively advanced (low plasma 17β-estradiol, site 3). Only one female held at 18 °C survived to spawning grounds at site 7. Correlations with physiology cannot be

explored with a single fish, but plasma variables were similar in this fish to 10 °C females as were travel times (see Figure 6.4).

In 18 °C males, gill Na^+,K^+-ATPase activity was negatively correlated with travel time to site 3 (r=-0.809, P=0.008). In 10 °C males, cortisol was positively correlated with travel times to site 3 (r=0.830, P=0.011), and testosterone was negatively correlated with travel time to site 7 (r=-0.813, P=0.048). Thus, 18 °C males generally took longer to swim upstream when gill ATPase activities were low, and 10 ° C males took longer if they were less reproductively advanced and stressed.

Discussion

Exposure to high but sub-lethal temperatures had a profound negative effect on sockeye survival during the exposure period, especially on females. It also had a negative effect on migratory performance and survival in both sexes after being released back to the Fraser River. Greater than two thirds of the salmon exposed to 10 °C successfully completed the upriver migration after release, whereas less than one third of those exposed to 18 °C survived. The control fish, which were handled and biopsied but were not thermally treated, migrated at similar speeds and survived at similar levels as the 10 °C fish. Thus, exposure to high temperature may be an important factor contributing to the ~60 to 95% mortality experienced by early migrants from the Weaver population, which have tended to migrate through higher than normal temperatures in recent years (Cooke et al. 2004).

The physiological and parasitological observations may provide insights to potential mechanisms by which exposure to high temperature induces mortality. Adult sockeye arrive at the Fraser River without any overt histological (spores in kidney glomeruli) or molecular (PCR analysis of posterior kidney tissue) expression of *Parvicapsula minibicornis*. However, once in the river, full expression is detectable once salmon have accrued upwards of 450 DD, but expression is absent or minimal in salmon accruing less than 350 DD (Wagner et al. 2005). Forty-four percent of the fish that died while being held at 18 °C showed signs of severe infection, and every salmon that died after accruing >350 DD was infected to some degree. In contrast, no fish in the 10 °C group accrued more than 325 DD, and none that died showed any signs of *Parvicapsula*. These findings confirm that the cumulative temperature experience of Fraser River sockeye in fresh water is central to the expression of *Parvicapsula*, and presumably to other parasites and diseases to which they are exposed when migrating to spawning areas.

The mechanisms by which *Parvicapsula* or any other infection might cause salmon mortality are unknown. Any infection certainly contributes to metabolic stress and therefore accelerated energy use, but the impacts of *Parvicapsula* on energy use and on kidney function are unclear. Controlled infections of adult sockeye with *P. minibicornis* altered plasma Na^+ and Cl^- concentrations in fish expressing advanced infections, suggesting that *Parvicapsula* infection creates an osmoregulatory challenge (S. Larsson, Umeå University, Umeå, Sweden, unpublished data). However, in the present study, plasma electrolytes did not differ between fish held at 10 °C and 18 °C. Though I was unable to measure the change in somatic energy densities in the thermally treated fish during the holding period, there was no significant difference in GSE between treatment groups at the end of the thermal treatments. If it were assumed that GSE levels in the two groups were the same at the start of the experiment, as fish were captured together en mass and divided randomly between the two treatment tanks, then any temperature driven metabolic loading was potentially minor. However, the role of temperature and infection on somatic energy trends in maturing sockeye salmon requires further study. Nevertheless, somatic energy levels did not differ between the treatment and control fish upon release to the river.

The effect of 28 days of holding did not appear to impose an energetic constraint on the experimental sockeye that would have accounted for the loss of fish *en route* to spawning areas. Energy modeling exercises using swimming speed information from migrating sockeye show that fish can become energy exhausted if swimming speeds are maintained at excessive levels (Hinch and Bratty 2000) and/or if normal swimming speeds are maintained for many more days or weeks than usual (Rand and Hinch 1998), both of which have fitness consequences to these semelparous animals. Previous energetic analysis of dying, post-spawning Fraser River sockeye indicate that death occurs when somatic energy levels fall below 4 $MJ \cdot kg^{-1}$ (Crossin et al. 2004). Despite holding fish for 28 days, energy levels in the thermally treated fish were well above this terminal level at release (5.29-5.42 $MJ \cdot kg^{-1}$ for females and 5.06-5.13 $MJ \cdot kg^{-1}$ for males), thus any mortality during holding was not likely caused by energy exhaustion. Furthermore, current speeds in the holding tanks were set so that fish were maintaining their station and swimming in place (swim speeds of 10 to 15 $cm \cdot sec^{-1}$). Underwater video studies of river migrating sockeye show that swim speeds (i.e. the speeds at which their swimming is propelling them if they were in still water) are typically 30 to 40 $cm \cdot sec^{-1}$ (Hinch and Rand 2000). Thus, swim speeds of experimental fish were ½ to ¼ slower than averages in freely migrating fish, which would have conferred a significant energy savings to the experimental fish. Energetic analyses reveal that

Weaver sockeye use ~0.12 $MJ \cdot kg^{-1} \cdot d^{-1}$ to swim and ripen gonads during migration from the ocean to spawning grounds (Crossin et al. 2004). Thus, the 18 ºC fish in this study, which after release took between 4 and 7 days to reach spawning grounds, would have expended 0.48-0.84 $MJ \cdot kg^{-1}$. A conservative estimate would see these fish arriving at spawning areas with between 4.22 and 4.58 $MJ \cdot kg^{-1}$, which is above terminal levels. Therefore, I contend that energy depletion resulting from the holding and subsequent migration was not likely a significant contributor to the high mortality of 18 ºC treated salmon in this study.

During treatment, gill Na^+,K^+-ATPase activity declined in salmon held at 18 °C but increased in male and female salmon held at 10 °C, suggesting an inverse relationship between the enzyme activity and holding temperature. However, I cannot be certain whether these significant differences reflect an ionoregulatory challenge resulting from a severe kidney parasite infection because this important enzyme is down-regulated when salmon enter fresh water and then shows a modest upregulation when fish arrive on the spawning grounds (Shrimpton et al. 2005). The difference may simply be related to different maturation trajectories resulting from the two holding temperatures. However, a similar inverse relationship between temperature and gill Na^+,K^+-ATPase activity has been observed in juvenile salmon (McCormick et al. 1996), raising the possibility that the difference was simply a temperature effect unrelated to either maturation or a kidney parasite infection. Regardless of the cause, gill Na^+,K^+-ATPase activities in both treatment groups were within the normal working range for salmon at this life history stage (Hinch et al. 2006), and were sufficient for maintaining ionic homeostasis. In fact, plasma electrolyte composition and osmolality did not differ significantly between the treatment groups. However, plasma Na^+ and Cl^- were significantly lower in treatment fish relative to control fish. This is likely because the treatment fish spent upwards of 25 days in fresh water whereas control fish were only ~3 days from the ocean.

Despite the significant differences in gill Na^+,K^+-ATPase between treatment groups, the effect on fish behaviour was weak. Only in 18 °C males was gill ATPase correlated with travel times, a negative relationship suggesting that among these already compromised individuals, those with higher activities swam more quickly upriver. This suggests further that gill Na^+,K^+-ATPase may facilitate protandrous migration, which is commonly observed in sockeye (Morbey 2000). The physiological variables that correlated most with behaviour were the reproductive hormones.

Based on the literature, I did not expect, nor did I find, reproductive hormone concentrations to differ between treatment groups at the end of the holding period. The

significant negative correlations that I observed between reproductive hormone levels and migration travel times however are consistent with a wide body of literature. Testosterone for example has a potent effect on the migratory behaviour of salmon (Munakata et al. 2001; Onuma et al. 2003; Young et al. 2006; Cooke et al. 2008; Chapter 2- Crossin et al. 2007), and is highly correlated with aggressiveness, restlessness, and overall migratory activities across taxa (reviewed by Dingle 1996). In 10 °C males and females, testosterone and 17β estradiol, respectively, concentrations were highest in the fish that arrived first at spawning areas. This trend was also observed in 11-keto testosterone concentrations in 18 °C females, the group of fish that suffered the highest mortality. Though only a single 18 °C female survived to spawning areas, the driving influence of reproductive hormones on migration time in these semelparous animals was evident at multiple locales.

I had predicted that as a reflection of stress in the broadest sense, plasma cortisol, lactate and glucose levels (Pickering et al. 1982), and Hsp70 might be elevated in salmon held at 18 °C. Previous studies of Hsp70 expression in rainbow trout (*O. mykiss*) erythrocytes show that individuals mount a heat shock response when temperatures approach acute lethal levels at 25-26 °C, but not at 20 °C (Currie and Tufts 1997). Thus, exposure of adult sockeye salmon to 18 °C may not have been stressful enough to elicit an Hsp70 response, indicating that this holding temperature did not represent an acute, but rather a chronic, temperature stress. Plasma cortisol and lactate did not differ between treatments, nor did concentrations differ from normal background levels. Plasma glucose was within normal levels of 4-6 $mmol \cdot L^{-1}$ for all but the 18 °C females which had significantly low levels but still within physiologic norms. This small change may reflect a subtle but reduced nutritional and energetic state induced by the high temperature exposure, especially since the migration success of these females was the lowest of all sex-treatment combinations at only 13%. Migration times were also slower in 18 °C females, although this was also true of 18 °C males. Interestingly though, travel times for successful 18 °C males and the one successful 18 °C female did not differ from travel times in successful 10 °C fish. This suggests that there is some individual variation in response to thermal stress.

Stress responses are known to differ between male and female salmon (Afonso et al. 2003). Perhaps because females are investing significantly more energy toward reproductive development during upriver migration (nearly 50% of their gross somatic energy stores vs. just 4% for males; Crossin et al. 2004), the metabolic stress imposed by high temperature may put females at a greater risk of mortality. The greater energetic flexibility of males may allow them to buffer their response to stress.

Implications for Salmon conservation

There is a growing body of evidence to suggest that temperature is a key determinant of mortality in sockeye salmon that migrate into freshwater earlier than historic averages (Hinch et al. 2006). My results clearly demonstrate that river temperatures only modestly above long-term averages, yet below acute thermal limits, can deleteriously affect individual migratory performance and survival, and increase the likelihood of infections. In years to come, salmon are likely to encounter temperatures well above average due to climate-change (Morrison et al. 2002; Rand et al. 2006). Therefore, managers and biologists should anticipate elevated levels of *en route* mortality in adult Fraser River sockeye each year. Determining which salmon population is particularly at risk is important information that will enable longer-term conservation plans and in-season management decisions that reflect the variable levels of temperature related risk.

The timing of freshwater entry by Pacific salmon is hypothesized to serve two fitness-related functions: 1) to expose homing individuals to river temperatures that support optimal swim performance, metabolism, and energy use (Lee et al. 2003), and 2) to synchronize reproduction, embryogenesis, and alevin emergence to high springtime levels of primary and secondary production (Brannon 1987; Webb and McLay 1996). As such, the annual migrations and reproduction of salmon tends to occur within a narrow phenological window, one influenced by the long-term average temperature regime encountered by a population *en route* to and upon spawning areas (Hodgson and Quinn 2002). Natural selection has thus shaped the run timings of Pacific salmon populations and their various phenotypes (e.g. aerobic capacity) to perform maximally at the water temperatures most commonly experienced once upriver migration has been initiated. That I observed a significant decreases in performance (i.e. travel times) and increases in *en route* mortality among homing sockeye exposed to temperatures above their population's optima has major implications not only for the fitness of these animals, but also for population level management and conservation.

During the summer months immediately preceding this study, the mainstem Fraser River temperatures exceeded the 60-y maxima. A segment of the Weaver population which migrated into the Fraser River earlier than normal encountered temperatures of 19-20 °C . None of those early migrants apparently reached the spawning grounds (Cooke and Hinch 2005). There is of course a maximum temperature that Pacific salmon can tolerate and acute temperature tolerance is consistently reported between 22-26 °C (Richter and Kolmes 2005). However, holding studies in my lab have shown that acute exposure to 20 °C , leads to 50% mortality after nine

days, and 100% after 15 days (S.G. Hinch, unpublished data), thus the chronic thermal tolerances of salmon may be better suited than acute tolerances when shaping management decisions. Ultimately, the thermal factors causing mortality in homing adult sockeye are related to chronic metabolic stresses and an inability to maintain sufficient cardiac performance to meet the demands placed on the salmon during their upriver migration (Farrell 2002).

In summary, this study demonstrates clearly that river temperatures only modestly above long-term averages can deleteriously affect migratory performance and survival in homing sockeye salmon. Physiologically, it is difficult to ascribe mechanisms to this mortality, though thermally mediated parasitic infections were certainly involved. The only clear physiological difference between treatment groups after treatment was in gill Na^+,K^+-ATPase activities, which were depressed in 18 °C males and females. Ultimately, the reproductive hormones were the only significant correlates of migration timing to spawning areas. Though analysis of the physiological data failed to reveal clear energetic or stress mechanisms for the observed mortality as per predictions 1 and 2, I nevertheless observed patterns indicative of energetic and metabolic stress in warm-treated fish. I do however document significant increases in the rate of parasitic infection with warm temperature exposure, and subsequent reductions in migratory performance and fate, supporting predictions 3, 4 and 5.

Table 6.1. Comparison of the physiological attributes of Weaver sockeye salmon (*Oncorhynchus nerka*, Walbaum in Artedi, 1792) prior to and after experimental temperature treatment. Analyses were conducted using paired *t*-tests. All variables were log-transformed prior to analysis. Significant treatment related changes within treatments and significant mean differences between treatments are indicated with asterisks: * P<0.05, and ** Bonferroni-corrected P<0.0036 (females, 14 variables), and P<0.0039 (males, 13 variables).

Sex	Variables	Treatment	Capture values	Post treatment release values	*N*	*P*-values, Within treatment	*P*-values, Between treatments
Females	Gross somatic energy ($MJ \cdot kg^{-1}$)	10 °C	n/a†	5.42 ± 0.1	15	n/a	0.479
		18 °C		5.29 ± 0.2	8		
	Hematocrit (%)	10 °C	36.7 ± 0.9	36.0 ± 1.0	15	0.434	0.879
		18 °C	37.6 ± 1.4	35.1 ± 1.4	7	0.095	
	Plasma Na^+ ($mmol \cdot L^{-1}$)	10 °C	150.6 ± 2.6	147.6 ± 1.3	15	0.322	0.670
		18 °C	156.6 ± 3.5	144.1 ± 1.9	7	0.0005*	
	Plasma K^+ ($mmol \cdot L^{-1}$)	10 °C	1.6 ± 0.3	2.4 ± 0.1	15	0.026*	0.996
		18 °C	2.2 ± 0.3	1.8 ± 0.2	7	0.292	
	Plasma Cl^- ($mmol \cdot L^{-1}$)	10 °C	131.1 ± 1.6	132.4 ± 0.8	15	0.456	0.053
		18 °C	138.9 ± 2.1	132.1 ± 1.2	7	0.030*	
	Plasma osmolality ($mOsmo \cdot kg^{-1}$)	10 °C	304.7 ± 4.9	306.3 ± 1.9	15	0.338	0.491
		18 °C	314.5 ± 6.8	298.0 ± 2.8	7	0.062	
	Plasma glucose ($mmol \cdot L^{-1}$)	10 °C	5.5 ± 0.2	5.8 ± 0.2	15	0.226	0.047*
		18 °C	5.3 ± 0.3	4.9 ± 0.3	7	0.365	
	Plasma lactate ($mmol \cdot L^{-1}$)	10 °C	5.6 ± 1.3	2.1 ± 0.2	15	0.029*	0.290
		18 °C	7.4 ± 1.8	2.4 ± 0.3	7	0.0053*	
	Plasma cortisol ($ng \cdot ml^{-1}$)	10 °C	n/a	325.4 ± 35.9	15	n/a	0.743
		18 °C		362.4 ± 52.6	7		
	Plasma testosterone ($pg \cdot ml^{-1}$)	10 °C	17,563 ± 1567	18,844 ± 2174	15	0.437	0.939
		18 °C	18,854 ± 2145	19,021 ± 3182	7	0.817	
	Plasma 11-ketotestosterone ($pg \cdot ml^{-1}$)	10 °C	n/a	2693 ± 793		n/a	0.426
		18 °C		1150 ± 1160			
	Plasma 17β-estradiol ($pg \cdot ml^{-1}$)	10 °C	13,229 ± 4222	10,886 ± 5206	12	0.261	0.937
		18 °C	5614 ± 4951	5187 ± 10,412	3	0.951	
	Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	10 °C	1.8 ± 0.1	2.2 ± 0.1	14	0.008*	0.0035**
		18 °C	1.8 ± 0.2	1.5 ± 0.1	8	0.383	
	Erythrocyte Hsp70 (Relative to reference)	10 °C	1.72 ± 0.26	1.60 ± 0.23	6	0.120	0.321
		18 °C	1.34 ± 0.26	1.25 ± 0.23	6	0.419	

Sex	Variables	Treatment	Capture values	Post treatment release values	*N*	*P*-values, Within treatment	*P*-values, Between treatments
Males	Gross somatic energy ($MJ \cdot kg^{-1}$)	10 °C	n/a†	5.13 ± 0.1	16	n/a	0.566
		18 °C		5.06 ± 0.1	8		
	Hematocrit (%)	10 °C	36.3 ± 1.2	34.6 ± 1.0	11	0.013*	0.432
		18 °C	37.5 ± 1.4	31.0 ± 1.4	8	0.040*	
	Plasma Na^+ ($mmol \cdot L^{-1}$)	10 °C	156.6 ± 2.2	150.7 ± 1.7	15	0.078	0.557
		18 °C	159.4 ± 2.9	150.2 ± 2.2	9	0.048*	
	Plasma K^+ ($mmol \cdot L^{-1}$)	10 °C	1.4 ± 0.3	2.7 ± 0.3	15	0.0004*	0.601
		18 °C	1.9 ± 0.4	1.9 ± 0.3	9	0.866	
	Plasma Cl^- ($mmol \cdot L^{-1}$)	10 °C	132.3 ± 1.7	127.2 ± 0.9	15	0.025*	0.174
		18 °C	136.2 ± 2.3	129.1 ± 1.2	8	0.004*	
	Plasma osmolality ($mOsmo \cdot kg^{-1}$)	10 °C	310.9 ± 4.26	300.0 ± 2.6	15	0.095	0.503
		18 °C	315.8 ± 5.68	300.9 ± 3.5	8	0.029*	
	Plasma glucose ($mmol \cdot L^{-1}$)	10 °C	6.4 ± 0.3	5.2 ± 0.2	15	0.0054*	0.562
		18 °C	5.9 ± 0.4	5.3 ± 0.3	9	0.032*	
	Plasma lactate ($mmol \cdot L^{-1}$)	10 °C	6.6 ± 1.2	2.5 ± 0.4	15	0.011*	0.422
		18 °C	7.0 ± 1.5	2.5 ± 0.5	7	0.002**	
	Plasma cortisol ($ng \cdot ml^{-1}$)	10 °C	n/a	108.6 ± 15.4	11	n/a	0.365
		18 °C		119.8 ± 18.1	9		
	Plasma testosterone ($pg \cdot ml^{-1}$)	10 °C	12,596 ± 1411	13,208 ± 824	7	0.860	0.162
		18 °C	11,268 ± 1411	11,529 ± 868	8	0.934	
	Plasma 11-ketotestosterone ($pg \cdot ml^{-1}$)	10 °C	n/a	10,464 ± 980		n/a	0.456
		18 °C		11,586 ± 1096			
	Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg^{-1}\ protein \cdot h^{-1}$)	10 °C	1.8 ± 0.1	2.6 ± 0.1	16	0.0001**	0.0016**
		18 °C	1.8 ± 0.1	1.6 ± 0.1	9	0.028*	
	Erythrocyte Hsp70 (Relative to reference)	10 °C	1.38 ± 0.22	1.26 ± 0.09	6	0.115	0.343
		18 °C	1.30 ± 0.22	1.13 ± 0.09	6	0.619	

† Values at capture were not measured but mean GSE for a group of N=20 co-migrating Weaver sockeye not analyzed in this study was measured at 6.12 $MJ \cdot kg^{-1}$ ± 0.2 SEM.

Table 6.2. Results of a two-way ANOVA, with treatment and sex as model effects, comparing the physiological variables in thermally treated and control sockeye salmon from the Weaver Creek population at time of release to the Fraser River. Single asterisks indicate statistical significance at α=0.05, and double asterisks indicate significance after Bonferroni correction (9 variables, P=0.0056). Differing letters show where statistical differences lie.

Physiological variables	18 ºC ± SEM	*N*	10 ºC ± SEM	*N*	Control ± SEM	*N*	Treatment P	Sex P	Treatment x Sex P
Gross somatic energy ($MJ \cdot kg^{-1}$)	5.25 ± 0.1	16	5.27 ± 0.1	31	5.36 ± 0.1	12	0.788	0.162	0.186
Hematocrit (%)	39.3 ± 1.1	15	37.6 ± 0.8	26	37.1 ± 1.4	9	0.366	0.688	0.638
Plasma Na^+ ($mmol \cdot L^{-1}$)	147.0 ± 1.5^a	16	149.9 ± 1.1^a	30	153.9 ± 2.0^b	7	0.034*	0.309	0.169
Plasma K^+ ($mmol \cdot L^{-1}$)	2.13 ± 0.1^a	16	2.50 ± 0.1^b	30	2.65 ± 0.3^b	7	0.006*	0.472	0.673
Plasma Cl^- ($mmol \cdot L^{-1}$)	130.5 ± 0.8	16	130.1 ± 0.6	30	132.4 ± 1.1	7	0.231	0.027*	0.266
Plasma osmolality ($mOsm \cdot kg^{-1}$)	299.6 ± 2.3	15	303.9 ± 1.7	30	304.4 ± 3.1	7	0.282	0.350	0.158
Plasma glucose ($mmol \cdot L^{-1}$)	4.86 ± 0.3^a	16	5.65 ± 0.2^b	30	4.24 ± 0.3^a	7	0.0013**	0.324	0.556
Plasma lactate ($mmol \cdot L^{-1}$)	2.13 ± 0.2	15	2.11 ± 0.2	30	1.90 ± 0.3	7	0.777	0.374	0.290
Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg\ protein^{-1} \cdot h^{-1}$)	1.43 ± 0.1^a	16	2.45 ± 0.1^b	30	2.33 ± 0.1^b	7	<0.0001**	0.191	0.062

Table 6.3. Pearson's correlation coefficients and p-values (in parentheses) relating gross somatic energy and blood plasma biochemistry of surviving sockeye salmon to upriver travel times as determined by acoustic telemetry. Point of release was Derby Reach in Ft. Langley, British Columbia. Units for physiological variables are presented in Table 6.1. Single asterisks indicates statistical significant a α=0.05, and double asterisks indicate significance after Bonferroni correction (females, 13 variables, P=0.0038; males, 12 variables, P=0.0042).

Treatment by sex	Variables	Travel time to Site 3 (~35 km upriver)	*N*	Travel time to Site 4 (~70 km upriver)	*N*	Travel time to Site 7 (~85 km upriver)	*N*
18 °C treated females	Gross somatic energy	0.791 (0.034)	7	0.849 (0.069)	5	n/a	1
	Hematocrit	-0.679 (0.138)	6	-0.566 (0.320)	5	n/a	1
	Plasma Na^+	0.159 (0.764)	6	-0.001 (0.998)	5	n/a	1
	Plasma K^+	-0.123 (0.817)	6	0.476 (0.418)	5	n/a	1
	Plasma Cl^-	-0.550 (0.258)	6	-0.550 (0.337)	5	n/a	1
	Plasma osmolality	-0.497 (0.316)	6	-0.405 (0.499)	5	n/a	1
	Plasma glucose	-0.787 (0.063)	6	-0.934 (0.020*)	5	n/a	1
	Plasma lactate	0.872 (0.024*)	6	0.461 (0.435)	5	n/a	1
	Plasma cortisol	0.370 (0.471)	6	0.765 (0.132)	5	n/a	1
	Plasma testosterone	-0.388 (0.447)	6	-0.939 (0.018*)	5	n/a	1
	Plasma 17β estradiol	-0.615 (0.578)	3	-0.331 (0.785)	3	n/a	1
	Plasma 11-ketotestosterone	-0.620 (0.189)	6	-0.884 (0.046*)	5	n/a	1
	Gill Na^+,K^+-ATPase	-0.532 (0.219)	7	-0.507 (0.384)	5	n/a	1
10 °C treated females	Gross somatic energy	0.094 (0.759)	13	-0.014 (0.965)	12	0.179 (0.621)	10
	Hematocrit	0.011 (0.972)	13	-0.008 (0.979)	12	0.271 (0.448)	10
	Plasma Na^+	-0.213 (0.485)	13	-0.378 (0.226)	12	-0.017 (0.964)	10
	Plasma K^+	0.286 (0.343)	13	0.218 (0.497)	12	0.605 (0.064)	10
	Plasma Cl^-	0.479 (0.098)	13	0.249 (0.435)	12	0.277 (0.438)	10
	Plasma osmolality	-0.044 (0.886)	13	-0.002 (0.995)	12	0.102 (0.780)	10
	Plasma glucose	-0.023 (0.942)	13	0.253 (0.427)	12	-0.253 (0.480)	10
	Plasma lactate	0.757 (0.0027**)	13	0.552 (0.063)	12	0.455 (0.186)	10
	Plasma cortisol	-0.360 (0.228)	13	-0.553 (0.062)	12	0.435 (0.209)	10
	Plasma testosterone	-0.251 (0.409)	13	-0.313 (0.322)	12	0.477 (0.163)	10

Treatment by sex	Variables	Travel time to Site 3 (~35 km upriver)	*N*	Travel time to Site 4 (~70 km upriver)	*N*	Travel time to Site 7 (~85 km upriver)	*N*
10 °C treated females	Plasma 17β estradiol	-0.760 (0.0036**)	11	-0.620 (0.056)	10	-0.713 (0.047*)	8
	Plasma 11-keto testosterone	-0.266 (0.379)	13	-0.338 (0.283)	12	-0.446 (0.197)	10
	Gill Na^+,K^+-ATPase	-0.379 (0.225)	12	-0.271 (0.395)	12	-0.088 (0.808)	10
18 °C treated males	Gross somatic energy	0.361 (0.340)	9	0.684 (0.062)	8	-0.086 (0.891)	5
	Hematocrit	0.084 (0.842)	8	0.599 (0.155)	7	-0.253 (0.747)	4
	Plasma Na^+	-0.590 (0.094)	9	-0.022 (0.958)	8	-0.006 (0.993)	5
	Plasma K^+	-0.161 (0.679)	9	0.352 (0.393)	8	0.065 (0.918)	5
	Plasma Cl^-	0.019 (0.965)	8	0.587 (0.166)	7	0.231 (0.709)	5
	Plasma osmolality	-0.298 (0.473)	8	0.101 (0.830)	7	-0.296 (0.629)	5
	Plasma glucose	-0.458 (0.215)	9	-0.283 (0.497)	8	0.487 (0.405)	5
	Plasma lactate	-0.434 (0.243)	9	-0.230 (0.585)	8	0.436 (0.464)	5
	Plasma cortisol	-0.542 (0.132)	9	-0.003 (0.995)	8	0.467 (0.428)	5
	Plasma testosterone	0.456 (0.217)	9	-0.162 (0.701)	8	0.672 (0.214)	5
	Plasma 11-keto testosterone	0.202 (0.603)	9	-0.462 (0.249)	8	0.256 (0.678)	5
	Gill Na^+,K^+-ATPase	-0.809 (0.008*)	9	-0.511 (0.195)	8	0.257 (0.677)	5
10 °C treated males	Gross somatic energy	-0.238 (0.412)	14	-0.062 (0.848)	12	0.474 (0.141)	11
	Hematocrit	-0.168 (0.643)	10	-0.229 (0.585)	8	-0.347 (0.446)	7
	Plasma Na^+	-0.098 (0.751)	13	-0.318 (0.341)	11	-0.340 (0.337)	10
	Plasma K^+	-0.050 (0.872)	13	0.139 (0.685)	11	-0.081 (0.825)	10
	Plasma Cl^-	-0.088 (0.775)	13	-0.161 (0.637)	11	0.375 (0.285)	10
	Plasma osmolality	-0.070 (0.821)	13	-0.086 (0.802)	11	-0.298 (0.403)	10
	Plasma glucose	0.118 (0.701)	13	0.302 (0.367)	11	0.372 (0.289)	10
	Plasma lactate	-0.120 (0.696)	13	-0.026 (0.940)	11	-0.173 (0.633)	10
	Plasma cortisol	0.807 (0.005)	10	0.830 (0.011*)	8	0.390 (0.388)	7
	Plasma testosterone	-0.157 (0.686)	9	0.035 (0.942)	7	-0.813 (0.048)*	6
	Plasma 11-keto testosterone	-0.135 (0.730)	9	-0.341 (0.454)	7	-0.791 (0.061)	6
	Gill Na^+,K^+-ATPase	-0.007 (0.982)	14	-0.089 (0.785)	12	0.093 (0.783)	11

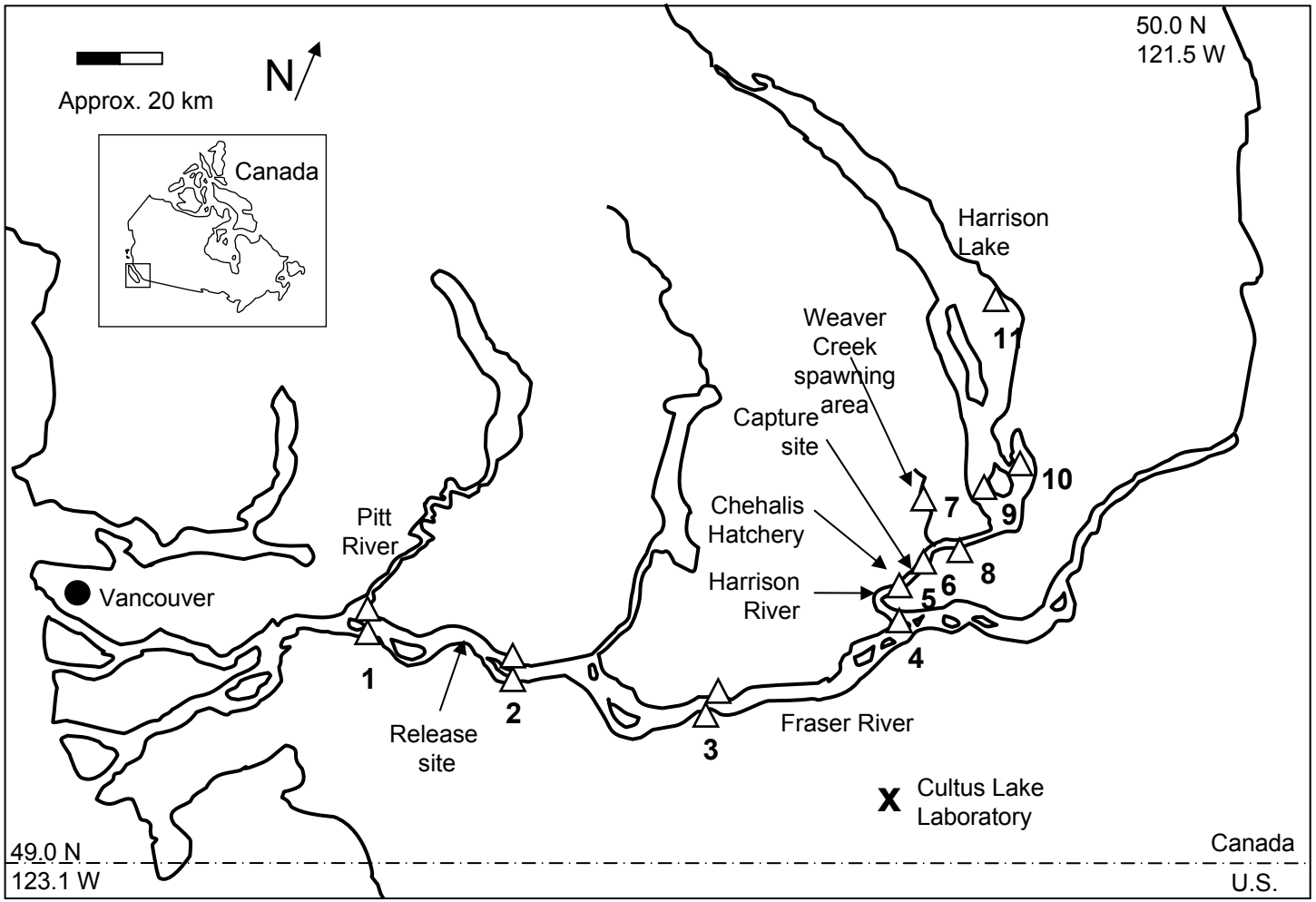

Fig. 6.1: Map of the lower Fraser and Harrison Rivers and the Weaver Creek spawning area in southwestern British Columbia, with an inset map of Canada indicating the location of British Columbia (darkened), and the study site (box). Points of fish capture and release, and sites of laboratory holding facilities are indicated with arrows or symbols. Numbers refer to specific individual and paired VR-2 acoustic receiver sites (triangles) that are listed in the Materials and Methods section. Specific sites of interest are indicated with arrows.

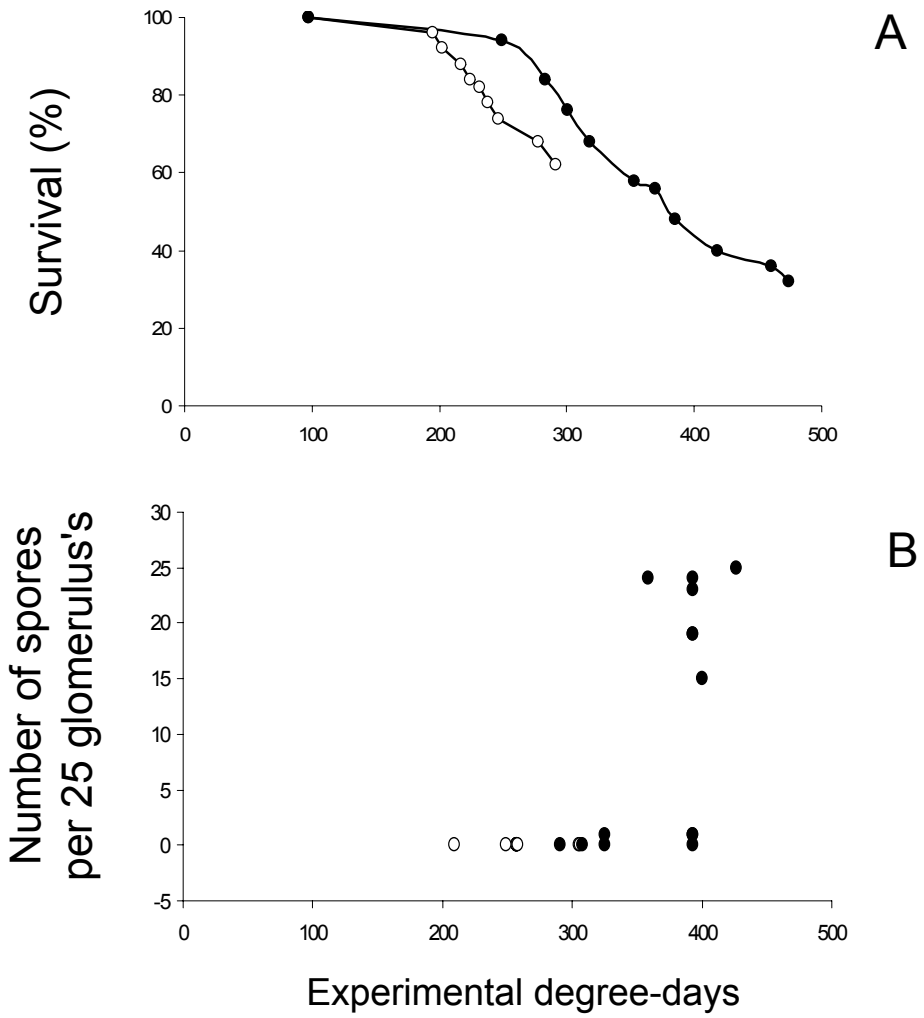

Fig. 6.2: Survival of sockeye salmon (*Oncorhynchus nerka*, Walbaum in Artedi, 1792) during experimental temperature treatments (Panel A) and severity of *Parvicapsula minibicornis* infection in the kidneys of fish that died during temperature treatment (Panel B) relative to the number of degree days accrued over the 24 day holding period at the Cultus Lake Laboratory. The fish had already accrued approximately 97.5 degree days prior to the start of the experiment. Dark circles signify 10 °C-treated sockeye and open circles represent 18 °C-treated sockeye. Severity of infection in Panel B was determined from histological counts of infected glomeruli per 25 glomeruli (ordinate). Many of the dots lie on top of one another: 10 °C-treated sockeye N=13, and 18 °C-treated sockeye N=14.

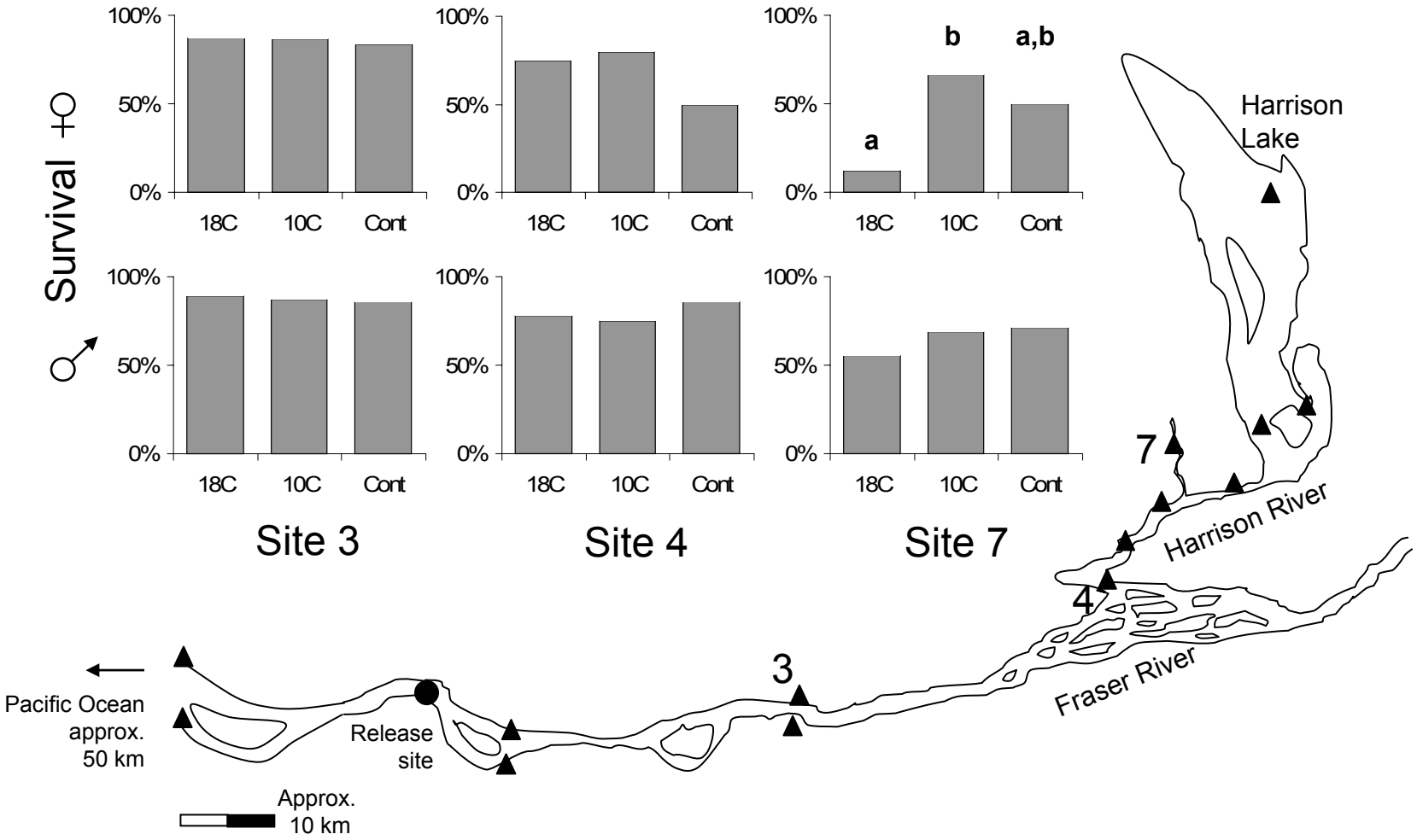

Fig. 6.3: Percent *en route* survival from point of release to various upriver receiver stations for 18 °C-treated, 10 °C-treated, and control sockeye. Site 3 is at Mission, BC, site 4 is at the confluence of the Fraser and Harrison Rivers, and site 7 is at spawning areas at Weaver Creek. Different letters over bars indicates a significant difference in survival between treatments (P=0.032).

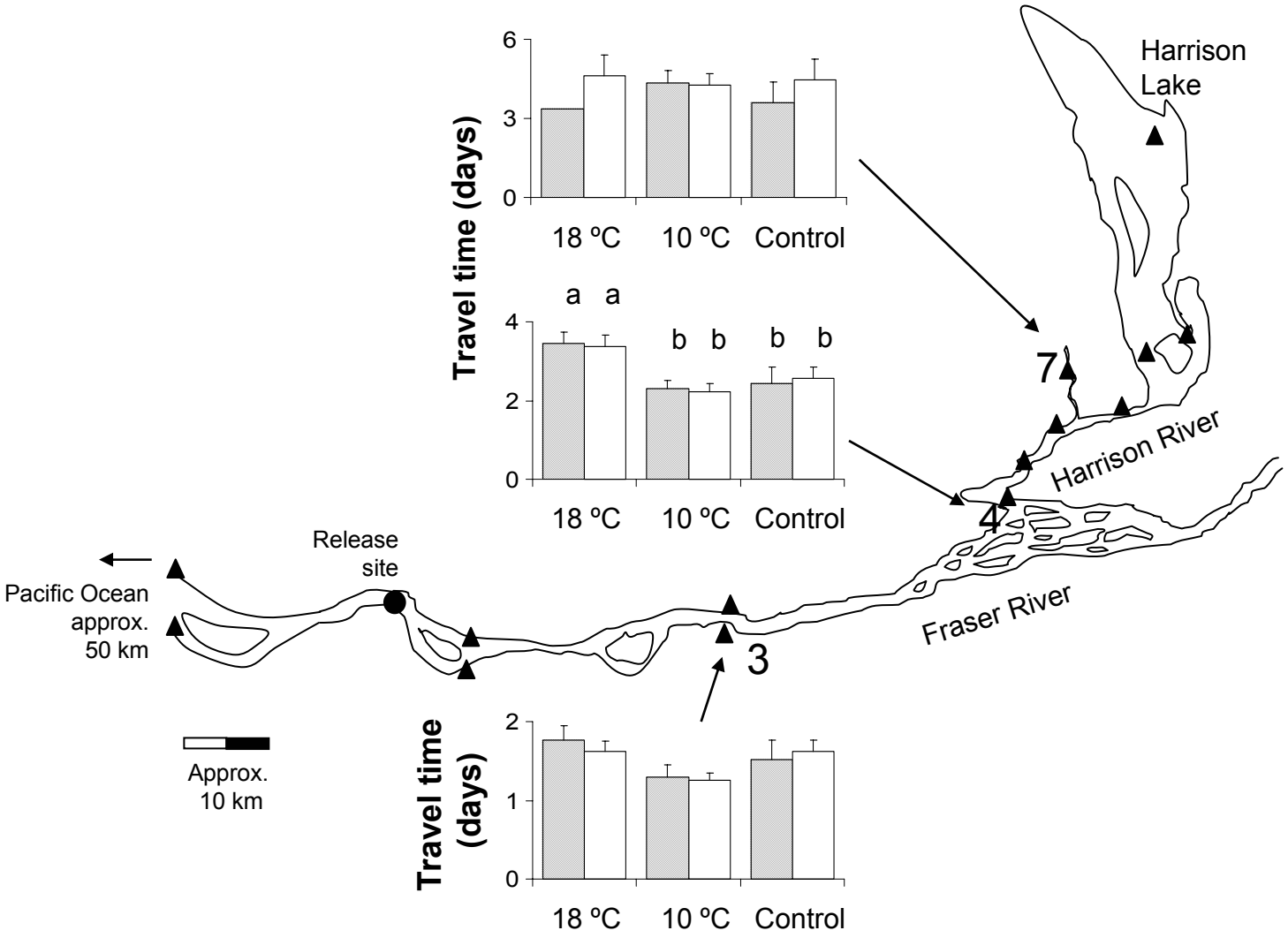

Fig. 6.4: Travel times from point of release to various upriver locales for 18 °C-treated, 10 °C treated, and control sockeye. Site 3 is at Mission, BC, site 4 is at the confluence of the Fraser and Harrison Rivers, and site 7 is at spawning areas at Weaver Creek. Distance from the Fraser River release site to spawning grounds is approximately 90 km. Striped bars are females and white bars are males. Differing letters represent significant difference at $P<0.05$. Error bars are +1 SEM.

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CHAPTER 7
CONCLUSION

In the early 1990s, the reknown salmon biologist William Pearcy noted that the oceanic life-history of Pacific salmon was a virtual 'black-box' into which our understanding had been limited by the enormous difficulty of studying salmon on the high seas (Pearcy 1992). Since then, many studies have sought to open that box (Welch et al. 1998; Tanaka et al. 2000; Hinch et al. 2006), and advances in telemetry are making possible the study of salmon at broad spatio-temporal scales (Tanaka et al. 2000; Cooke et al. 2006a,b; Cooke et al. 2008; Chapter 5- Crossin et al. in review). This dissertation has provided some new insights to the baseline physiology of ocean salmon, and the physiological bases of their behaviour whilst homing along coastal margins to natal rivers.

Throughout my studies, I have consistently observed a modulating effect of testosterone on migration timing. Endogenous, circannual rhythms are known to underlie important life-history events like reproductive migrations (Dingle 1996), and though many factors indeed work synergistically to control the timing of migrations toward and into freshwater, testosterone consistently showed the strongest associations (Chapters 2, 3 and 5). The high levels of testosterone seen far at sea is indicative of a seasonal, photoperiodic activation of hypothalamic GnRH (Table 7.1). Indeed, testosterone at all stages of migration was strongly correlated with Julian day of sampling. Though I did not detect population differences in female testosterone levels, nor a difference in egg production at the early stage of migration at sea, population differences in male testosterone and in testes mass varied in a way that was in direct proportional to population specific dates of river entry (Early Summer-run males, which enter the river earliest each summer, had the highest [T] and the heaviest testes. In contrast, Late-run males had the lowest [T] and the smallest testes). These patterns of testosterone secretion also seemed to exert a pleitrophic effect on travel times over a 490 km stretch of the Pacific coast. Males with the highest [T] were covered this distance in half the time (~8 days) relative to males with the lowest [T]. This pattern is consistent with other studies showing that males are faster migrants both in marine (Chapter 2- Crossin et al. 2007) and freshwater locales (Hanson et al. 2008).

In Chapter 2, I speculated that the faster swim speeds of males might enable them to arrive at spawning areas in advance of females (i.e. protandrous migration), for increased time on the spawning grounds theoretically increases a male's opportunity for reproduction. Male fitness is constrained in part by the number of females that an individual spawns with, so faster

migrations may be a tactic to maximize fitness (Trivers 1972; Gross 1984). My results suggest that testosterone secretion is a key mechanism supporting this tactic. What is fascinating is that despite the positive correlations between testosterone levels and travel speeds in males salmon homing through the ocean and upriver, it is the females that appear to dictate the key decision of when to enter the river, a decision that is also positively correlated with testosterone (Chapters 2 and 5). Whilst homing from Johnstone Strait, males from Late-run populations swam faster than co-migrating females to the Fraser River mouth. But once at the mouth, both males and females appeared to slow their migration and began milling about the estuary in the characteristic way that Late-run sockeye do, for as little as 4 days to as many as 3 weeks before entering the river. If the holding pattern that Late-run sockeye exhibit prior to river entry has indeed evolved to minimize exposure to sub-optimal river temperatures, as has been speculated (Hodgson and Quinn 2002), then perhaps Late-run females, who invest comparatively more energy to gamete production than males (Crossin et al. 2004), have evolved timing behaviours to minimize the energetic risk associated with migration into warm, mid-summer water. Because egg production is much more metabolically demanding than sperm production (Gilhousen 1980; Jonsson et al. 1997), females could be at greater risk when temperatures are high. When I manipulated the thermal experience of sockeye migrating upriver during the normal migration window (when the Fraser was cool; Chapter 6- Crossin et al. 2008), females exposed to a temperature that is by no means extreme- 18 ºC- showed the greatest susceptibility to thermal stress and only 1 of 9 (11%) survived to reach spawning areas. In contrast, 9 of 14 or 64% of females exposed to 10 ºC reached spawning grounds.

I sought to directly test the effects of GnRH and testosterone on rates of marine migration, but unfortunately, due to the reasons outlined in Chapter 3 (i.e. fisheries! though initial physiological state and environmental stressors cannot be discounted), I cannot make a full assessment of this. Of the 12 fish for which I had travel time information, exogenous GnRH and testosterone appeared to have little effect, though small samples sizes raise the possibility of a Type II error. Nevertheless, as stated previously, initial (i.e. untreated) testosterone levels were strongly correlated with travel times among those 12 fish. In the laboratory, I documented a profound positive effect of GnRH and T co-administration on circulating [T] in males and females (Chapter 4), so it seems reasonable to predict that I may have seen an effect on migration rates if the Chapter 3 study had not been complicated by various factors. This remains an interesting subject to explore experimentally.

These studies are interesting from a life-history and evolutionary point of view, but they do have broader significance. As mentioned above, river entry timing may have evolved, in part, to minimize thermal stress during migration. Knowing that high temperature can deleteriously affect river migration survival, and knowing too that testosterone is a key driver of migration timing, support emerges for the hypothesis that advanced reproductive cycles are a proximate cause of the early migration and high mortality phenomenon that has been afflicting Late-run Fraser sockeye for the past 13 years (i.e. the Maturation Hypothesis; Cooke et al. 2004). If this is the case, the ultimate question is what is responsible for this change?

The distribution of sockeye on the high seas is constrained by thermal boundaries within which energy intake exceeds standard and active metabolism, thus supporting growth (Welch et al. 1997). Sea-surface temperatures in the north Pacific Ocean have been steadily rising over the past 50 years (Cox and Hinch 1997) due to increasing green-house gas emission, and scores of climatological and oceanographic models predict that SSTs will continue to increase in the coming decades. Atmospheric CO_2 concentrations are expected to continue increasing too (Körner 2000). Welch et al. (1997) forecasted the seasonal distribution of sockeye under a doubled CO_2 scenario and found that the area of habitat suitable for sockeye growth was profoundly shifted northward to the Bering Sea, and the magnitude of that shift varied seasonally. If such a prediction were to hold true, and if sockeye had no other means of supporting growth (e.g. vertical shifts in ocean residency and foraging), then the distribution of sockeye would gradually be pushed to higher latitudes. A consequence of this would be exposure to earlier and longer photoperiods in spring and summer which would presumably phase the activation of the HPG axis to earlier than normal times and possibly lead to earlier migrations. Certainly, HPG functionality can be readily advanced with photo-manipulation, a practice that is commonly used in salmon aquaculture (Norberg et al. 2004). Whether earlier activation translates to earlier migration, an hypothesis I tried to test in Chapter 3, is as yet unknown in wild ocean salmon.

Reciprocal transplant experiments with birds lend some support to this idea. African stonechats (*Saxicola torquata axillaris*) experience a near constant annual photoperiod in the equatorial regions they inhabit. When experimentally transplanted to temperate latitudes in northern Europe, changes in gonadal cycles and moult were in phase with those of their European conspecifics (*S. t. rubecula*) (reviewed by Coppack 2007). They also exhibited patterns of nocturnal locomotory activity and *zugenrhue* in tandem with the migratory forms of European stonechats. This suggests that the latitudinal variation in breeding and migration

schedules may well be due to differing photoperiods. Though birds were not physiologically sampled, I would predict a pattern of hypothalamic GnRH secretion and testosterone circulation indicative of HPG activation.

Counter examples can be found however. No effect of photoperiod on gonadal cycles and pre-migratory locomotor activities were observed in reciprocally transplanted populations of garden warblers (*Sylvia borin*, from Finland and Germany) when experimentally maintained under photoperiods common to the other population (see Coppack 2007). Thus, the timing of life reproductive events and migratory restlessness in this study was attributed to genetic rather than photoperiodic control.

Gwinner and Scheuerlein (1999) have suggested that the response of the transplanted African stonechats to photoperiod was due to their high degree of phenotypic plasticity. When plasticity is retained within a lineage, it can allow animals to deal with environmental perturbations. Salmon and their relatives exhibit an exceptional degree of plasticity, and the average life-history response to environmental change can vary over comparatively short time scales (one generation to another) (reviewed by Hutchings 2004; see also Kinnison et al. 2001). If Late-run salmon are experiencing altered photoperiods in northward contracting ocean distribution, then perhaps the early migration "problem" is an expected outcome. Whatever the course of future research is this area, it is likely that reciprocal transplant experiments, telemetry (especially at sea), and physiological sampling will be important tools.

One issue that is unavoidable in studies of migratory salmon is that of handling stress. Capturing salmon in nets, drawing blood and other physiological biopsies from them, and then applying telemetry devices are cumulative stressors. The question begs- at what point does this cumulative stress exert a deleterious effect on survival? Handling procedures can push some individuals to the point of lactic acidosis or other related physiological conditions that lead inevitably to post-release mortality (e.g. Wood 1991). Salmon that die after being released generally have higher (though not always statistically significant) levels of plasma lactate than survivors. In laboratory settings, a threshold of approximately 12 $mmol \cdot L^{-1}$ of lactate has been identified as the point beyond which salmonids have difficulty recovering from anaerobic activity (Jain and Farrell 2003), and our methodologies in field settings have sought to minimize the likelihood of anaerobic stress. However, this is not usually possible due to the severity of purse- and beach-seine capture, and it is not uncommon to observe lactate concentrations as high as 20 $mmol \cdot L^{-1}$ (see Fig. 5.2). Despite this, I have found that sockeye with lactate as high as 18 $mmol \cdot L^{-1}$ and released in marine areas are capable of reaching distant freshwater spawning

grounds. What is emerging is the idea that salmon are well equipped to deal with acute stress during migration, and that the thresholds previously identified in the lab may be very conservative in field settings.

Despite a technique validation study conducted by colleagues and me that found no differences between biopsied and un-biopsied sockeye in rates of travel or in survivorship to in-river telemetry checkpoints over 200 kms away (Cooke et al. 2004), some sockeye nevertheless die after being released. The Chapter 2, in which sockeye were released in marine waters approximately 215 km from the Fraser River mouth when no commercial harvest was occurring, yielded a survivorship was 85%. Thus, 15 % mortality occurred before entering the Fraser River. In the Chapter 5 study (Crossin et al. in review), we had acoustic receiver lines placed within a day's swim from the points of marine release. Assuming that handling related mortality would occur within a day post-handling (as per English et al. 2005), mortality to this acoustic line was ~17%. It is not possible to determine the degree that predation and other natural forms of mortality lends to this 15-17% mortality. However, a controlled laboratory study of marine captured sockeye held in iso-osmotic marine water yielded mortality of only 10% in large, predator free tanks (M.S. Cooperman, University of British Columbia, Vancouver, BC, pers. comm.) Thus, a conservative estimate of handling mortality in marine waters may be as little as 10%. In contrast, handling-related mortality can be as low as 0% in freshwater captured fish (no fish died in a 2-day period after capture and sampling in the Chapter 6 study [Crossin et al 2008], and no fish captured at a local some 200km upriver from the ocean died in the 2-day period after biopsy and the more invasive EMG telemetry tag implantation, which requires surgery (L.B. Pon, University of Britihs Columbia, Vancouver, BC, pers. comm.). This suggests that there is something fundamentally stressful about the transition from salt-to-freshwater. Indeed, sockeye work harder in saltwater as standard metabolism is higher than in freshwater (Wagner et al. 2006), and major physiological changes are occurring as fish home into their natal river. In Table 7.1, a peaking of plasma cortisol titres can be seen as fish more toward and into the Fraser River, and a concomitant dip in plasma sex steroids (e.g. testosterone) occurs which is a typical stress response. Using best handling practices, it is perhaps reasonable to anticipate a baseline handling-related mortality of 10% in marine environments, but in freshwater as little as 0% mortality may occur.

Table 7.1: Studies reporting the physiological profiles of Fraser River sockeye salmon sampled at various locales. For ease of presentation, all data were rounded, and in some cases SEMs were not presented. See source documents below for precise values and variances. Studies marked with an asterisk examine Summer- and Late-run sockeye, and the study marked with a dagger examines Summer-run only. All others examine Late-run sockeye. Empty cells are variables that were not measured in the specific study.

	Salt-water						Salt- & Fresh-water	Fresh-water				
Distance (km) from Fraser River estuary	-850	-215	-215	-215	-215	-215	0	+140	+150	+150	+200	+250
Variable	Study 1 (**Chapter 3**)	Study 2 *	Study 3	Study 4 (**Chapter 2**)	Study 5 (**Chapter 5**)	Study 6	Study 7	Study 8 (**Chapter 6**)	Study 9	Study 10	Study 11	Study 12 †
Somatic Energy ($MJ \cdot kg^{-1}$)	♂ 10.6 – 11.2 ♀ 10.3 – 11.4	9.1 – 9.5	7.9 – 7.9 H 8.9 – 9.2 A	7.8 – 9.4	♂ 8.6 A ♂ 9.1 C ♀ 8.8 – 8.7 A ♀ 8.7 – 8.8 C	8.1 – 8.6 H 9.1 – 9.3 A	6.8 ± 0.5 F	5.42 – 6.12		♂ 3.66 ± 0.05 ♀ 3.48 ± 0.12	6.6 ± 0.4	6.08 ± 0.16
Na^+ ($mmol \cdot L^{-1}$)	179.3 – 182.4	183	186 ± 1.0	178 –184	173 – 180 A 176 – 178 C	186.3 – 188.0	153.7 ± 1.6 F 204.4 ± 9.7 S	150 – 160	♂ 152.7 ± 0.8 ♀ 153.1 ± 0.8	♂ 125.8 ± 4.4 ♀ 106.4 ± 2.6	166 ± 3.0	146 ± 1.0
K^+ ($mmol \cdot L^{-1}$)		1.3 – 1.8	1.5 ± 0.1	0.7 – 1.3		1.51 – 1.82	2.6 ± 0.5 F 4.1 ± 1.2 S	1.4 – 2.0	♂ 2.51 ± 0.13 ♀ 2.02 ± 0.13	♂ 4.49 ± 0.56 ♀ 2.62 ± 0.27	1.1 ± 0.6	2.9 ± 0.2
Cl^- ($mmol \cdot L^{-1}$)	151.8 – 155.6	146 – 147	148 ± 0.5	145 – 149	151 – 153 A 150 – 151 C	147.3 – 148.9	124.2 ± 3.6 F 168.6 ± 8.3 S	130 – 141	♂ 124.6 ± 0.5 ♀ 130.5 ± 0.5	♂ 93.2 ± 3.3 ♀ 79.0 ± 2.3	132 ± 2.0	136 ± 1.3
Osmolality ($mOsm \cdot kg^{-1}$)	381.0 – 386.0	340 – 344	345 ± 2	350 – 359	371 – 373 A 371 C	344.2 – 348.2	288.8 ± 4.8 F 419.9 ± 21.7 S	300 – 320	♂ 283.7 ± 1.2 ♀ 288.4 ± 1.2	♂ 264.6 ± 5.9 ♀ 253.3 ± 7.0	321 ± 10	304 ± 2
Cortisol ($ng \cdot ml^{-1}$)	369.7 – 373.9	450 – 515	458 ± 33		401 – 430 A 415 – 433 C	415.4 – 553.1	125.4 ± 17.9 F 354.2 ± 95.1 S	♂ 108.6 – 119.8 ♀ 325.4 – 362.4	♂ 90.8 ± 16.3 ♀ 350 ± 60	♂ 740 ± 140 ♀ 1 287 ± 84	56 ± 101	238 ± 27
Lactate ($mmol \cdot L^{-1}$)	11.0 – 11.2	6.8 – 8.9	9 ± 0.4	8.2 – 10.6	7.1 – 10.4 A 8.2 – 9.4 C	8.75 – 9.39	2.0 ± 0.9 F 9.6 ± 3.6 S	5.6 – 7.4	♂ 1.23 ± 0.08 ♀ 1.73 ± 0.23	♂ 13.5 ± 1.2 ♀ 12.7 ± 1.2	4.5 ± 0.1	2.4 ± 0.2
Glucose ($mmol \cdot L^{-1}$)	7.2 – 7.8	6.5 – 7.2	7 ± 0.2	6.7 – 7.4	♂ 5.6 – 7.2 A ♂ 5.8 – 7.6 C ♀ 4.9 – 6.5 A ♀ 5.2 – 7.1 C	7.07 – 7.44	6.4 ± 0.4 F 6.4 ± 0.1 S	5.3 – 6.5	♂ 4.76 ± 0.09 ♀ 5.50 ± 0.12	♂ 4.9 ± 0.7 ♀ 5.1 ± 1.4	4.7 ± 0.3	5.2 ± 0.5
Gill Na^+,K^+-ATPase (μmol ADP·mg protein^{-1}·h^{-1})	3.19 – 3.79	2.5 – 2.8	2.6 ± 0.1	1.6 – 2.1	3.4 – 3.7 A 4.1 C	2.74 – 2.86	1.8 ± 0.1 F	1.8 ± 0.1	♂ 2.46 ± 0.08 ♀ 2.42 ± 0.11	♂ 2.40 ± 0.2 ♀ 2.23 ± 0.1		

	Salt-water						Salt- & Fresh-water	Fresh-water				
Distance (km) from Fraser River estuary	-850	-215	-215	-215	-215	-215	0	+140	+150	+150	+200	+250
Variable	Study 1 (**Chapter 3**)	Study 2 *	Study 3	Study 4 (**Chapter 2**)	Study 5 (**Chapter 5**)	Study 6	Study 7	Study 8 (**Chapter 6**)	Study 9	Study 10	Study 11	Study 12 †
Testosterone ($ng \cdot ml^{-1}$)	♂ 89.0 ES ♂ 46.3 S ♂ 38.7 L ♀ 121.0 ES ♀ 86.8 S ♀ 78.5 L	♂ 12.1 ± 0.39 ♀ 13.6 ± 0.61	♂ 10.6 ± 0.5 ♀ 11.9 ± 0.5	♂ 4.3 – 8.5 ♀ 4.4 – 8.0	♂ 25.8 – 26.4 A ♂ 17.3 – 25.3 C ♀ 38.5 – 40.8 A ♀ 31.3 – 41.2 C	♂ 10.4 – 10.4 ♀ 11.7 – 12.2		♂ 11.1 – 12.6 ♀ 17.5 – 19.0	♂ 23.8 ± 1.0 ♀ 40.2 ± 1.8	♂ 9.6 ± 1.0 ♀ 5.9 ± 0.9	♂ 17.5 ± 6.1 ♀ 34.9 ± 7.7	♂ 84 ± 0.02 ♀ 319 ± 0.1
11-ketotestosterone ($ng \cdot ml^{-1}$)		♂ 5.7 ± 0.2 ♀ 1.0 ± 0.04	♂ 5.4 ± 0.3 ♀ 0.9 ± 0.04			♂ 5.0 ♀ 0.8 – 1.0			♂ 14.9 ± 0.7 ♀ 1.9 ± 0.1	♂ 6.7 ± 0.9 ♀ 0.4 ± 0.04	♂ 7.9 ± 4.4 ♀ 1.8 ± 0.2	pending
17β estradiol ($ng \cdot ml^{-1}$)	♀ 5.0 ES ♀ 4.5 S ♀ 3.6 L	♂ 0.4 ± 0.03 ♀ 4.9 ± 0.28	♂ 0.4 ± 0.03 ♀ 4.3 ± 0.26	♀ 2.5 – 5.5	♀ 7.9 – 13.6 A ♀ 4.5 – 11.6 C	♂ 0.3 – 0.4 ♀ 4.3 – 4.5		♀ 5.6 – 13.0	♂ 0.8 ± 0.13 ♀ 2.4 ± 0.14	♀ 0.7 ± 0.19	♀ 6.0 ± 2.5	♀ 3.9
17α,20β dihydroxy-4-pregen-3-one ($ng \cdot ml^{-1}$)								Pending	♂ 0.1 ± 0.004 ♀ 0.8 ± 0.1	♂ 0.2 ± 0.04 ♀ 0/2 ± 0.03		

1. Crossin et al. – **Chapter 3, this dissertation**. Data ranges are for Early Summer (ES), Summer (S) and Late-summer (L) Fraser River sockeye sampled in Rennell Sound, Queen Charlotte Island in 2006, near where they make continental landfall during homeward migration from high seas regions. Means have been adjusted for Julian date.
2. Cooke et al. (2006a). Data are the range reported for Chilcotin, Quesnel, Nechako stock (Summer-run sockeye) individuals sampled in the ocean and which survived to river entry in 2003. Values for ocean and in-river mortalities are in source document.
3. Cooke et al. (2006b). Data are ± 1 SE for Late-run sockeye sampled in Johnstone Strait and which survived to river entry in 2003. Hormone levels are from sockeye which survived to natal watershed. Data for in-river failures are in source document.
4. Crossin et al. (2007) - **Chapter 2, this dissertation**. Data are the range of means for five Late-run sockeye stocks sampled in Browns Bay, Johnstone Strait in 2003.
5. Crossin et al. (2008a) - **Chapter 5, this dissertation**. Adams (A) and Chilko (C) sockeye were sampled in both Juan de Fuca Strait (early) and Johnstone Strait (later) in 2006. Range means have been adjusted for Julian date. See Table 5.2.
6. Cooke et al. (2008). Weaver-Harrison and Adams populations captured in Johnstone Strait in 2003. See Table 6 in manuscript.
7. Wagner et al. (2006). Cannulated sockeye were swum in a respirometer and performance was measured in both salt- (S) and fresh-water (F). Sockeye were Late-run Fraser River populations.
8. Crossin et al. (2008b) - **Chapter 6, this dissertation**. Data are the range observed in approximately 48 Weaver (Late-run) sockeye caught in freshwater near Weaver Creek in 2004.
9. K. Hruska, University of British Columbia, unpublished data. Data are mean ± 1 SE for Weaver (Late-run) sockeye collected at entrance to Weaver Creek spawning channel Oct 12-13 and Oct 25, 2004.

10. K. Hruska, University of British Columbia, unpublished data. Data are mean ± 1 SE for Weaver (Late-run) sockeye collected while moribund in Weaver Creek spawning channel enclosures Oct 16-30, 2004.
11. Young et al. (2006). Data are mean ± 1 SE for "normal river entry timed" Late-run sockeye sampled in Thompson River and were successful at reaching spawning grounds in 2003. Data on "early entry," and normal-timed Late-runs which failed to reach spawning grounds are in source document.
12. L.B. Pon, University of British Columbia, unpublished data. Data are means ± 1 SE for Gates Creek sockeye (Summer-run) captured at the top of the Seton Dam Fishway August 9-29, 2005.

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APPENDIX

A University of British Columbia Research Ethics Board certificate of approval was issued to Professor Scott Hinch (my research supervisor) which permitted me to conduct research on wild salmon. This document is attached below.

THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A05-0424

Investigator or Course Director: Scott G. Hinch

Department: Forest Sciences

Animals:

Salmon Sockeye salmon (O. nerka), Gates Creek stock 140
Trout wild Rainbow trout (Oncorhynchus mykiss) 1000
Salmon Sockeye salmon (O. nerka), Late Shuswap stock 320
Salmon Sockeye salmon (O. nerka), Early Stuart or Early Shuswap stock 70
Salmon Sockeye salmon (O. nerka) (Late Shuswap stock, wild fertilized eggs; juveniles) 10000

Start Date: December 4, 2001 **Approval Date:** June 8, 2007

Funding Sources:

Funding Agency:	Natural Sciences and Engineering Research Council of Canada (NSERC)
Funding Title:	Abnormal migration and premature mortality in Pacific salmon
Funding Agency:	Natural Sciences and Engineering Research Council of Canada (NSERC)
Funding Title:	Energetics, behaviour and fitness of anadromous migrating fish
Funding Agency:	Forestry Innovation Investment Ltd.
Funding Title:	Long-term stream habitat and rainbow trout responses to alternative riparian management in north-central British Columbia.
Funding Agency:	British Columbia Hydro and Power Authority

Funding Title:	The Seton Dam fishway and power house water diversion: factors limiting production of sockeye salmon
Funding Agency:	Pacific Salmon Commission
Funding Title:	Investigations to determine the cause of early migration behaviour and magnitude of in-river survival and losses above Mission for adult Late-run Fraser River sockeye.
Funding Agency:	British Columbia Pacific Salmon Forum
Funding Title:	Migrations, spawning behaviours, and physiology of wild adult sockeye salmon in the Fraser River: impacts of global warming scenario
Unfunded title:	N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

Office of Research Services and Administration
102, 6190 Agronomy Road, Vancouver, BC V6T 1Z3
Phone: 604-827-5111 Fax: 604-822-5093