Factors Affecting Egg Retention and Reproductive Longevity in Spawning Female Sockeye Salmon (*Oncorhynchus nerka*)

by

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Abstract

An individual's physiological and behavioural response to its environment can have fitness implications. To address hypotheses about the roles of physiology and behaviour on spawning success in sockeye salmon (*Oncorhynchus nerka*), I conducted experiments in an artificial spawning channel during three spawning seasons. Experiments involved biopsy sampling and behavioural observations; physiology and behaviour were then related to reproductive longevity and egg retention of spawning females.

Females living longer on the spawning grounds retained a lower proportion of eggs, indicating that females were running out of time to complete spawning. However, several long-lived females (> 7 d) failed to complete spawning before death, indicating that time limitation may not have been a factor for these females.

Physiological changes associated with rapid senescence were characterized for both sexes. Salmon exhibited three major physiological trends during senescence that were independent of sex or reproductive maturity – a large increase in plasma indicators of stress and exercise (i.e., lactate and cortisol), a decrease in major plasma ions (i.e., Cl^- and Na^+) and osmolality, and a decrease in gross somatic energy reserves. Females exhibited a greater magnitude of change than males for gross somatic energy, plasma ions, and reproductive hormones.

Females that arrived at spawning grounds with high plasma lactate and low plasma Cl^- concentrations not only died sooner after arrival, but also retained more eggs at death. Premature mortality was also linked with three other indices of stress and osmoregulatory dysfunction (i.e., elevated plasma glucose concentrations, reduced plasma osmolality, and Na^+ concentrations), suggesting that these fish were stressed and / or senescing prematurely. Levels of reproductive hormones (i.e., testosterone, 17β-estradiol, 17,20β-progesterone) decline as females become reproductively mature and approach senescence. Females lived longer if they arrived with higher reproductive hormone levels, indicating that females that were more reproductively advanced were more likely to die prematurely. My data did not support an energy limitation hypothesis as short-lived females died with greater energy reserves than longer-lived females, indicating something other than energy exhaustion was responsible for mortality.

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List of Abbreviations and Acronyms

11-KT	11-ketotestosterone
17,20β-P	17,20β-progesterone
ADP	adenosine diphosphate
ANOVA	analysis of variance
Cl^-	chloride
d	day
df	degrees of freedom
EMG	electromyogram
FOC	Fisheries and Oceans Canada
GSI_D	gonadosomatic index at death
GSI_E	estimated gonadosomatic index
h	hour
HD	high density
K^+	potassium
lat	latitude
LD	low density
long	longitude
min	minute
MJ	mega joule
Na^+	sodium
nd	no data
NS	not significant
NSERC	Natural Sciences and Engineering Research Council, Canada
POH	post-orbital to hypural length

PSC	Pacific Salmon Commission, Vancouver
SEM	standard error of the mean
SS	sum of squares
UBC	University of British Columbia

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Co-authorship Statement

This dissertation is part of a multi-disciplinary research program investigating the physiological ecology of adult sockeye salmon. The design of experiments, analysis of data, and writing of manuscripts were initiated by me; however, this research was conducted in the framework of a broad research program conducted by Drs. Scott Hinch, Mike Healey, and Tony Farrell, from whom I received guidance and support. Hence, they have been listed as co-authors on the manuscripts that have been developed from Chapters 2-4. Similarly, those who provided additional expertise, methodological support, and field support have also been acknowledged as co-authors. Ultimately, responsibility for the quality of analysis and writing is my responsibility.

Chapter 2: Egg retention in relation to arrival timing and reproductive longevity in female sockeye salmon (*Oncorhynchus nerka*)

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Chapter 3: Influences of sex and activity level on physiological changes in individual adult sockeye salmon during rapid senescence

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Chapter 4: **Blood physiology as a predictor of reproductive longevity and egg retention in female sockeye salmon (*Oncorhynchus nerka*)**

Authors: Kimberly A Hruska, Scott G Hinch, Mike C Healey, David A Patterson, and Anthony P Farrell

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Chapter 1: Introduction

Background

The resources available in an environment impose physiological limitations on animals. For example, energy resources are often limited and must be allocated to metabolism, growth, and reproduction so as to maximize fitness (Calow, 1985). Environmental conditions also influence an animal's behaviour, particularly the timing of behavioural activities and life history transitions (Roff, 2002). Resource limitation, particularly energy limitation, during reproduction can lead to constraints on both animal physiology and behaviour.

Many animals undergo large physiological and morphological changes in preparation for reproduction (Groot and Margolis, 1991; Becker et al., 2002). For example, sexually dimorphic traits like bold patterns and bright colours in some songbirds (Order: Passeriformes) and fish, and other ornaments, such as the antlers of deer (Family: Cervidae) and large kypes of Pacific salmon (*Oncorhynchus* spp.), have been sexually selected through mate choice and intersexual competition for mates (Stearns, 1992). During maturation, endocrine changes act to direct gonadal development and gamete maturation and to prime reproductive behaviour (Becker et al., 2002; Adkins-Regan, 2005; Yaron and Sivan, 2006). Animals that perform long-distance migrations to reach a suitable reproductive environment have additional limitations on their allocation of energy resources (Dingle, 1996). Sockeye salmon (*O. nerka*), which are the subject of this thesis, spawn in freshwater but migrate to the Pacific Ocean to complete their growth and, thus, undertake some of the longest breeding migrations known.

Natural selection can shape reproductive characteristics in unique ways. For example, adult sockeye salmon possess distinct morphological and physiological characteristics that are determined by the distance and elevation of spawning locales (Crossin et al., 2003) and the typical temperature and flow conditions encountered during the river migration (Lee et al., 2003; Farrell et al., 2008). Most animals are also capable of avoiding or mitigating adverse environmental conditions up to a certain point, even during the reproductive period (Wingfield, 2003; Wingfield and Sapolsky, 2003). However, reproduction can be disrupted or irreparably damaged if animals encounter completely unsuitable environmental conditions and are unable to delay or relocate their reproductive activity. A major theme of this thesis is

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how sockeye salmon respond to changing environmental conditions during the reproductive life stage.

The study of reproductive behavioural physiology has helped to elucidate why some individuals are able to succeed in the face of an environmental perturbation where others fail. It has been known for a long time that reproductive hormones can influence animal behaviour (see historic references in Becker et al., 2002). For example, testosterone helps regulate territorial behaviour during reproduction in vertebrates; however, the relationship between testosterone and aggression is not always direct (Adkins-Regan, 2005; Wingfield, 2005). While there is a large amount of literature on the effect of hormones on behaviour, the relationships are not always clear and are often limited to certain life stages and / or environments (Becker et al., 2002; Adkins-Regan, 2005; Soma, 2006).

Behavioural interactions can, in turn, influence an individual's physiology. For example, aggressive behaviour has been shown to affect blood physiology in both dominant and subordinate individuals (Cardwell et al., 1996; Gilmour et al., 2005; Soma, 2006). Several laboratory studies have shown that subordinate individuals exhibit a greater stress response (e.g., higher plasma cortisol concentrations) and tend to take longer to recover after an interaction than dominant individuals (Øverli et al., 1999; Sloman and Armstrong, 2002). The winners of aggressive interactions often exhibit higher testosterone and 11-ketotestosterone concentrations (Cardwell et al., 1996; Elofsson et al., 2000).

Reproductive Behavioural Physiology in Pacific Salmon

Pacific salmon (*Oncorhynchus* spp.) provide an excellent biological model for the study of relationships among reproduction, behaviour and physiology (reviewed in Groot and Margolis, 1991; Groot et al., 1995; Hendry and Stearns, 2004; Hinch et al., 2006). One of their unique features is their high degree of philopatry to spawning grounds (Groot and Margolis, 1991), which has permitted adaptive divergence and genetic drift of populations resulting in population-specific traits such as fish size (Quinn and Foote, 1994; McPhee and Quinn, 1998), size of secondary sex characteristics (Quinn and Foote, 1994; Hendry and Berg, 1999), egg size and number (Kinnison et al., 2001; Crossin et al., 2004b), and the timing of arrival at the spawning grounds (McPhee and Quinn, 1998; Hendry et al., 2004). Recent research has demonstrated that populations also differ in their physiological

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adaptations; adult salmon from populations that make the most arduous upriver migrations have the greatest aerobic and cardiac scope and largest ratio of spongy to compact myocardial muscle (Lee et al., 2003; Farrell et al., 2008; E. Eliason, University of British Columbia, unpublished data)

Another feature of Pacific salmon is their complex mating systems which involve a variety of behavioural classes of spawners. Spawning behaviours have been well-studied. Females compete for access to a suitable site to build a redd (Foote, 1990; Fleming and Gross, 1994; Quinn and Foote, 1994), using a variety of aggressive behaviours including charges, chases and bites (Healey et al., 2003). Larger females tend to be the winners in intra-sexual competition for redds, although prior residence is also an important factor (Foote, 1990; Fleming and Gross, 1994). Males compete with each other for access to spawning females but smaller males adopt a variety of non-aggressive tactics for achieving fertilizations (Schroder, 1982; Foote, 1990; Quinn and Foote, 1994; Healey and Prince, 1998). Male success is typically determined by their dominance in aggressive interactions with other males; the dominant male is often the largest and most aggressive (Quinn and Foote, 1994). Dominant males fertilize a large percentage of eggs (Schroder, 1982; Quinn and Foote, 1994), although in some cases subordinate 'sneaker' males can be as or more successful at fertilizing eggs than dominant males (Schroder, 1982; Foote, 1990; Foote et al., 1997; Mehranvar et al., 2004).

Clearly fish size, aggression and behavioural dominance are important factors involved in spawning success; however, specifically for females, reproductive longevity (i.e., the length of time alive on spawning grounds) also appears to be very important (Morbey and Ydenberg, 2003; Hendry et al., 2004). In essence, a longer time on spawning grounds is an advantage because early arriving females can appropriate the best redd locations and guard her redd from predators and superimposition by later arriving females (Quinn and Foote, 1994; McPhee and Quinn, 1998; Steen and Quinn, 1999).

Reproductive success is a measure of the ability to pass one's genes on to future generations. Fecundity and fertility are key fitness traits of salmon. The fecundity of a female sockeye salmon varies depending on the stock, size, and ocean age of the individual. Average fecundity ranges between 2000 and 5000 eggs per female. All Pacific salmon are semelparous and, thus, a female that does not spawn all her eggs has not maximized her fitness potential. A female that dies without spawning any eggs has a lifetime fitness of zero.

Nevertheless, there is evidence for several salmon species that some females do not spawn all their eggs and, in some instances, up to 20% of individuals fail to spawn any eggs (Gilhousen 1990; Groot and Margolis 1991; Fukushima and Smoker 1997; Reimchen 2000; Quinn et al., 2007). In years of high egg retention, hundreds of thousands of females in some populations may fail to spawn all their eggs, resulting in a potential loss of hundreds of millions of embryos. One of the principle objectives of this thesis is to understand the causes of this incomplete spawning.

Anadromous migrations are physiologically challenging for adults as they involve stressful transitions from salt to freshwater and from cool oceans to warm rivers, exposure to diseases, depletion of energy reserves, and a myriad of physiological changes associated with maturation and senescence (reviewed in Hinch et al., 2006). It is, thus, possible that the physiological state of spawners plays a strong role in reproductive longevity and egg retention. Below I review the concepts of physiological stress, sexual maturation, and energy use in migrating salmonids and describe how these phenomena could affect reproductive longevity and egg retention.

<u>Physiological Stress</u>

The stress response works primarily through two endocrine systems: the sympathetico-chromaffin system, which mediates the release of catecholamines (i.e., epinephrine, norepinephrine, and dopamine) and the hypothalamic-pituitary-interrenal axis, which mediates the release of corticosteroids (i.e., cortisol) (Pickering and Pottinger, 1995; Wendelaar Bonga, 1997). Cortisol is produced in the interrenal tissue in response to most environmental stressors (Pickering and Pottinger, 1995; Mommsen et al., 1999) and its presence affects metabolism (Vijayan et al., 1997), osmoregulation, and immune function (Wendelaar Bonga, 1997). When a fish encounters an acute stressor, plasma cortisol levels dramatically increase over the course of several minutes to an hour, decreasing to basal levels within about a day (Kubokowa et al., 1999). However, when faced with a chronic stressor, fish can acclimate to the stressor such that cortisol levels remain within an 'unstressed' range (Mommsen et al., 1999). In addition, a stressor can stimulate the sympathetico-chromaffin cell axis, leading to an increase in plasma glucose levels (Wendelaar Bonga 1997) via reduced glucose utilization, stimulation of gluconeogenesis, or the stimulation of glycogenolysis (Pickering and Pottinger, 1995). Elevated plasma glucose is characteristic of a variety of long-term stressors, including pollutants (Macfarlane and

Benville, 1986), capture (Laidley and Leatherland, 1988; Rotllant and Tort, 1997), and handling (Pickering et al., 1982). Low environmental oxygen, poor oxygen transport and anaerobiosis during intense exercise can lead to the accumulation of lactate in plasma and muscles (Wood, 1991; McDonald and Milligan, 1992). Thus, high lactate levels are indicative of high levels of activity and hypoxia stress in fish (Pickering et al., 1982; Wood, 1991; Rotllant and Tort, 1997). Stress can also disturb osmoregulation via changes in gill function mediated through increased catecholamine levels (Wood, 1991; Pickering and Pottinger, 1995). When freshwater fish encounter a stressor, plasma osmolality and major ion concentrations (i.e., Cl^-, and Na^+) decline due to a temporary inability to maintain blood ion homeostasis (McDonald and Milligan, 1992; Pickering and Pottinger, 1995; Ackerman et al., 2000). Plasma Cl^- disturbances can be more severe and prolonged with stress (Gingerich et al., 2010).

Adult Pacific salmon migrations are inherently stressful physiologically. The metabolites and compounds associated with stress responses are integral components of migration and maturation in Pacific salmon and these can have both positive and negative effects on survival and spawning. Cortisol is associated with priming the olfactory system and thus assists in homing during the freshwater phase of migration, which is guided by home stream odour (Hasler, 1966; Carruth et al., 2002). Corticosteroids sensitize oocytes to 17,20β-progesterone, so high cortisol concentrations may be critical in assisting oocyte maturation (Jalabert, 1976) and, possibly, in ensuring low levels of egg retention. However, a loss of pituitary control over the interrenal secretion of cortisol leads to its hypersecretion in maturing adults, which may facilitate the process of senescence (Robertson and Wexlar, 1957; Dickhoff, 1989; Schreck et al., 2001) and could lead to shorter reproductive longevity. High cortisol levels have been linked to the degenerative changes in tissues, such as the brain (Maldonado et al., 2000) and muscle tissues (Hendry and Berg, 1999), which occur during upriver migration (Carruth et al., 2002). Moreover, elevated levels of cortisol can suppress immune function (Maule et al., 1996), which may lead to premature mortality (Dickhoff, 1989) and hence decrease reproductive longevity. Elevated cortisol levels can also depress reproductive hormone expression (Schreck et al., 2001; Hinch et al., 2006; Schreck, 2010). Thus, adults that experience continually high levels of migratory stress could have impaired gonad development (Pickering et al., 1987; Tyler and Sumpter 1996; Macdonald, 2000; Schreck et al., 2001), which could lead to high levels of egg retention on spawning grounds. Hyperactive and hypoxic swimming resulting from passage through fast

flows or high temperatures can lead to acidosis arising from an accumulation of plasma lactate (Wood et al., 1983; Tufts et al., 1991) and latent mortality (Hinch and Bratty, 2000; Mathes et al., 2010). Hyperactive swimming on spawning grounds could therefore influence reproductive longevity.

In sum, physiological stress associated with migration prior to reaching the spawning area and on the spawning area itself could influence egg retention and reproductive longevity; although to what extent and whether the influence is positive or negative likely depends on many factors. Because they have only one opportunity to spawn and a limited time for reproduction, Pacific salmon may be adapted to maintain reproduction in the face of significant stressors (Wingfield and Sapolsky, 2003), yet there has been limited field research to assess this.

<u>Sexual Maturation</u>

During migration, Pacific salmon undergo several physiological changes to ensure reproductive maturity upon arrival at the spawning ground, including a general increase in plasma levels of testosterone, 11-ketotestosterone and 17β-estradiol (Truscott et al., 1986; Liley et al., 1993). As females start maturing, steroidogenesis is stimulated in the ovaries through the release of gonadotropins. 17β-estradiol stimulates vitellogenin production from the liver which is released into the plasma and taken up by the oocytes (Tyler et al., 1990). Vitellogenin is the primary means by which energy is transported from energy reserves to the developing oocytes (Tyler et al., 1990). Between freshwater entry and ovulation, ovarian mass increases dramatically. For example, in Weaver Creek sockeye salmon, ovarian mass increased by 26% between freshwater entry and spawning ground arrival (Crossin et al., 2003).

Under normal conditions, adult Pacific salmon reach reproductive maturity near the time they arrive at the spawning grounds and senescence usually occurs long enough after reproductive maturity to allow females time to complete spawning and spend a few additional days guarding the redd (Hendry et al., 2004). However, females that arrive at the spawning grounds in an advanced state of reproductive maturity would be expected to have shorter reproductive longevity. In an experimental study in Atlantic salmon, de Gaudemar and Beall (1998) found that over-ripe females (i.e., beyond reproductive maturity) had lower rates of fertilization success, spawned their eggs more quickly and retained more eggs at

death. Being over-mature on arrival at the spawning grounds could occur if spawning ground access was impeded or if reproductive hormone production occurred uncharacteristically early. Early hormone production has been observed in some Late-run Fraser River sockeye populations (Hinch et al., 2006). On the other hand, females that arrive at spawning grounds not fully mature, may have greater reproductive longevity, but if oocyte growth has been retarded for some reason (e.g., highly stressful migratory conditions; Campbell et al., 1992; Schreck et al., 2001; Patterson et al., 2004), egg retention may also be high. Level of reproductive maturity at spawning ground arrival is variable; some females are mature at spawning ground arrival and others will ovulate within days of arrival (i.e., immature). The proportion of immature females arriving at the spawning grounds is variable among years and through the spawning season, with a lower proportion of immature females arriving near the end of the spawning season (K. Hruska, personal observation).

Energy Use

Pacific salmon do not eat during up-river migration, and must complete migration and maturation then spawn using energy they had upon freshwater entry. Migration and maturation is energetically expensive, using about 50% of reserves under typical migration conditions prior to arrival at the spawning grounds (Brett, 1995; Hendry and Berg, 1999; Crossin et al., 2004b). The migration is primarily fuelled by lipid catabolism (Idler and Clemens, 1959; Brett and Glass, 1973) with the active metabolic costs of swimming and the allocation of energy to gonad development as the two largest energetic requirements (Rand and Hinch, 1998). In years when migrants encounter high flows or high temperatures, energy is utilized rapidly and individuals can perish due to energy exhaustion prior to reaching spawning grounds (Rand and Hinch, 1998; Rand et al., 2006; Crossin et al., 2008). Thus, it is also possible that fish could arrive on spawning grounds and yet not have sufficient energy reserves to conduct spawning behaviours or complete development of oocytes.

Indeed there are several lines of evidence that suggest that energy limitations can influence spawning success. Mehranvar et al. (2004) showed that pre-spawning energy content was positively correlated with reproductive longevity in both male and female sockeye salmon. Crossin et al. (2003, 2004b) examined body constituents of two populations of pink salmon and five populations of sockeye salmon and consistently found that spawners perished

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when body energy reserves were about 4 MJ•kg^{-1}, suggesting a critical energetic threshold for life. Healey et al. (2003) also provided anecdotal evidence to support the notion that pre-spawning energy levels are important for fuelling reproductive behaviour. Sockeye salmon from the Early Stuart stock returned to the Fraser River at smaller sizes and started using body protein (instead of lipids) for energy at an earlier stage of migration during the years 1997-1999 than they did during the 1950s (Macdonald, 2000; Crossin et al., 2004a). Similarly, the reproductive life spans observed during 1994-1996 (7.6 days for males; 10.6 days for females) were shorter (Healey et al., 2003) than those recorded for fish from the same stream (15-17 days) during the 1950s (Gilhousen, 1980). Body energy concentrations were much greater in sockeye salmon during the 1950s than the 1990s due to changes in oceanic productivity (Crossin et al., 2004a)

Spawners with low energy reserves could thus have reduced reproductive longevity and / or higher egg retention if energy was not available to fully develop and spawn all the eggs. Indeed, spawning activity in Pacific salmon can be energetically expensive (Healey et al., 2003; Crossin et al., 2004b). However, spawners with low energy could adopt energy saving behaviours to increase their reproductive longevity or decrease egg retention. For example, spawners could reduce the frequency of 'high energy' behaviours, such as posture displays (Healey et al., 2003) although such actions might reduce spawning competitiveness. Female sockeye salmon that waited in pools after arriving on the spawning grounds had greater reproductive longevity than those that immediately establish redds, purportedly because they avoided energetically expensive aggressive interactions with other females (Foote, 1990; Morbey and Ydenberg, 2003). Nonetheless, some researchers have found no effects of changes in female aggressiveness on reproductive longevity (e.g., Hendry et al., 2001). Thus, individuals that arrive at spawning grounds with low energy reserves may experience reductions in spawning success due to impacts on reproductive longevity, spawning behaviour, and egg retention.

Thesis Goals and Study System

The primary goal of this thesis was to assess the role of physiological and behavioural factors on female sockeye salmon reproductive longevity and egg deposition success on the spawning grounds. The secondary goal of the thesis was to characterize the physiological

changes that occur in individual salmon during senescence. Clearly physiology, behaviour and environment interact to influence the reproductive success of Pacific salmon, yet there have been no integrative studies to investigate the interrelationships and relative roles of these key factors on reproductive success on the spawning grounds. Figure 1.1 provides a conceptual overview of the factors and their interrelationships that will be assessed in each chapter of this thesis.

The focus of the research is on sockeye salmon because it is one of the best studied species of Pacific salmon in terms of migration physiology (reviewed in Cooke et al., 2004, 2005, 2008; Hinch et al., 2006; Crossin et al., 2008). Moreover, several studies have closely observed the reproductive behaviours of this species in the wild, providing ample baseline information (McPhee and Quinn, 1998; Burgner, 1991; Quinn, 1999; Healey et al., 2003), and have documented the variety of life history strategies and trade-offs that exist among populations (Hendry at al., 2004; Morbey and Abrams, 2004; Crossin et al., 2004b). A further advantage of working on sockeye salmon is that there are well established field protocols for the assessment of physiological condition, thus permitting non-destructive sampling for a variety of stress, metabolic and ionoregulatory indices (Cooke et al., 2008). This biopsy approach, which has been developed and field-validated on sockeye salmon, has been shown to have minimal effects on migration behaviour or survival (Cooke et al., 2005) and has been used extensively to link migratory behaviour, fate and physiological condition (Cooke et al., 2005, 2006a,b, 2008; Young et al., 2006; Crossin et al., 2009; Pon et al., 2009; Mathes et al., 2010). This biopsy technique has also been used successfully on spawning ground fish (Hruska et al., 2007).

All research was conducted on the Weaver Creek sockeye salmon, which is a relatively large coastal population within the Fraser River watershed. Weaver Creek sockeye salmon are part of the late-run stock complex of sockeye salmon in the Fraser River. Late-run sockeye salmon are the last to enter freshwater and, unlike the other run timing groups, they typically hold in the estuary prior to initiating upriver migration (Woodey, 1987). However, over the past decade this group of sockeye salmon stocks has started up-river migration early; this early migration behaviour has been linked with high levels of mortality during migration and on spawning grounds (Cooke et al. 2004).

Weaver Creek is a small tributary of the Harrison River, located about 2 km downstream from Harrison Lake; the Harrison River is a large tributary of the Fraser River, located in

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southwest British Columbia approximately 100 km upstream from the Fraser River mouth (Figure 1.2). The Weaver Creek sockeye salmon population spawns both in Weaver Creek, and in an adjacent artificial spawning channel which affords an ideal location for observing spawning behaviour in free-swimming sockeye salmon. The Weaver Creek Spawning Channel (Figure 1.2) provides optimal spawning habitat for ~20,000 sockeye salmon; smaller numbers of pink (*O. gorbuscha*) and chum (*O. keta*) salmon are also permitted to spawn in the channel (Quinn, 1999). The channel is 2.9 km long (6 m wide) and provides salmon with a layer of gravel substrate of a size suitable for spawning (1.2 - 7.6 cm). The mean water depth is 25-30 cm and the mean current velocity is 0.4 m•s^{-1} (Quinn, 1999). The movement of fish into the channel is controlled by manually operated gates.

Upriver migrations of this population normally start in mid-September when they depart the Pacific Ocean after spending 2, or sometimes 3, years there. Adults travel ~ 125 km up the Fraser and Harrison Rivers to reach the Weaver Creek Spawning Channel (Figure 1.2), a relatively short distance compared to most other populations of Fraser River sockeye (Crossin et al. 2004b). Spawning takes place throughout October, peaking in mid month (Essington et al., 2000). Fish arriving at Weaver Creek must pass through Morris Lake (8 ha; mean depth = 4 m) after moving through Morris Slough which connects to the Harrison River 11 km upriver of the confluence with the Fraser River. Migrants that arrive at Harrison River prior to the opening of the spawning channel in early October will reside in either Harrison River or the nearby Harrison Lake which is large (surface area 220 km^2), deep (mean depth 150 m) and oligotrophic (Mathes et al., 2010). Recent telemetry observations indicate that Weaver adults will mill around in Harrison River or Harrison Lake for several days to weeks prior to entering Weaver Creek (Mathes et al., 2010). Weaver Creek sockeye spawning behaviours has been well characterized by Quinn (1999), Essington et al. (1998), and Mehranvar et al. (2004).

Thesis Chapter Overview and Predictions

In Chapter 2, the relationship between reproductive longevity and egg retention in female sockeye salmon is characterized (Figure 1.1a). I hypothesize that egg retention would be lower among females that live longer after arrival at the spawning grounds and that reproductive longevity would be greater for those females that arrive at the start of the

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spawning season. I also develop a conceptual model to illustrate how timing of events on the spawning grounds could influence the relationship between reproductive longevity and egg retention.

In Chapter 3, I characterize the physiological changes that occur in sockeye salmon during the period between spawning ground arrival and death (Figure 1.1b). Individual changes in blood physiology are measured and correlated with indices of behavioural activity on the spawning grounds. I hypothesize that when they become moribund, sockeye salmon would display elevated levels of stress metabolites (i.e., cortisol, glucose, and lactate) and an imbalance of plasma ions (i.e., osmolality, Na^+, and Cl^-). As individuals undergo senescence they exhibit signs that are consistent with stress and osmoregulatory dysfunction. These physiological patterns are used to set up the predictions for the subsequent chapter.

In Chapter 4, I use blood physiology information from spawning ground females (from Chapter 3) to predict female reproductive longevity and egg retention (Figure 1.1c). I evaluate three hypotheses (i.e., stress / osmoregulation, reproductive maturity, and energy exhaustion) in terms of specific influences of physiology on reproductive longevity and egg retention, and how these processes may be mediated by behaviour. Based on the stress / osmoregulation hypothesis, I predict that females that arrive on the spawning grounds with high levels of physiological stress (e.g., high levels of plasma cortisol, glucose, and lactate) and homeostatic imbalances (e.g., low plasma osmolality, Na^+, and Cl^-) would die shortly after arrival and thus retain more eggs at death. Based on the reproductive maturation hypothesis, I predict that females with lower plasma concentrations of 17β-estradiol, testosterone, and 17,20β-progesterone would start spawning earlier, have shorter reproductive longevity, and have higher levels of egg retention at death. Based on the energy exhaustion hypothesis, I predict that all females would die with similarly low energy levels regardless of reproductive longevity or levels of egg retention. The findings show no evidence to support the energy exhaustion hypothesis. The stress / osmoregulation hypothesis and reproductive maturation are both supported by the data.

In Chapter 5, I summarize the key results of the thesis and discuss the relative importance of the factors affecting reproductive longevity and egg retention in female sockeye salmon.
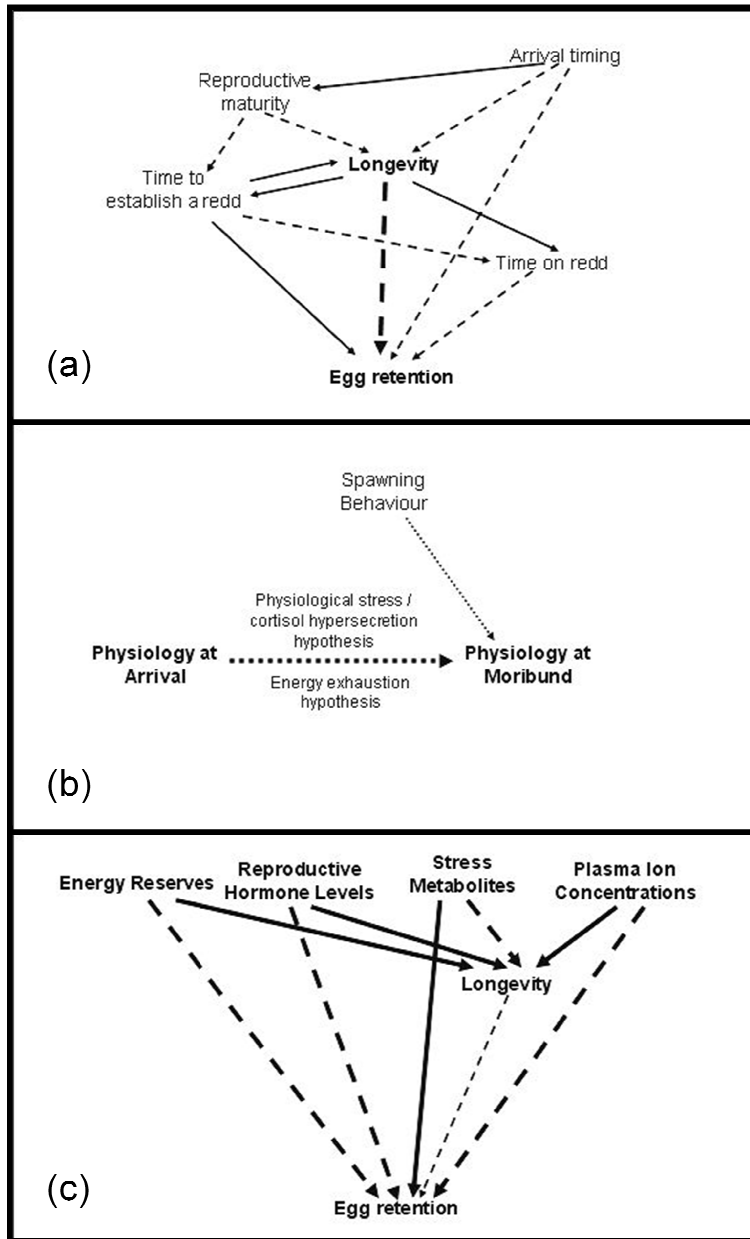
Figure 1.1: Conceptual models of the interrelationships between the factors investigated in Chapter 2 (a), Chapter 3 (b), and Chapter 4 (c). The primary relationships of interest in each chapter are indicated by bold and larger arrow(s) in each panel. Dashed arrows indicate negative relationships; solid arrows indicate positive relationships.
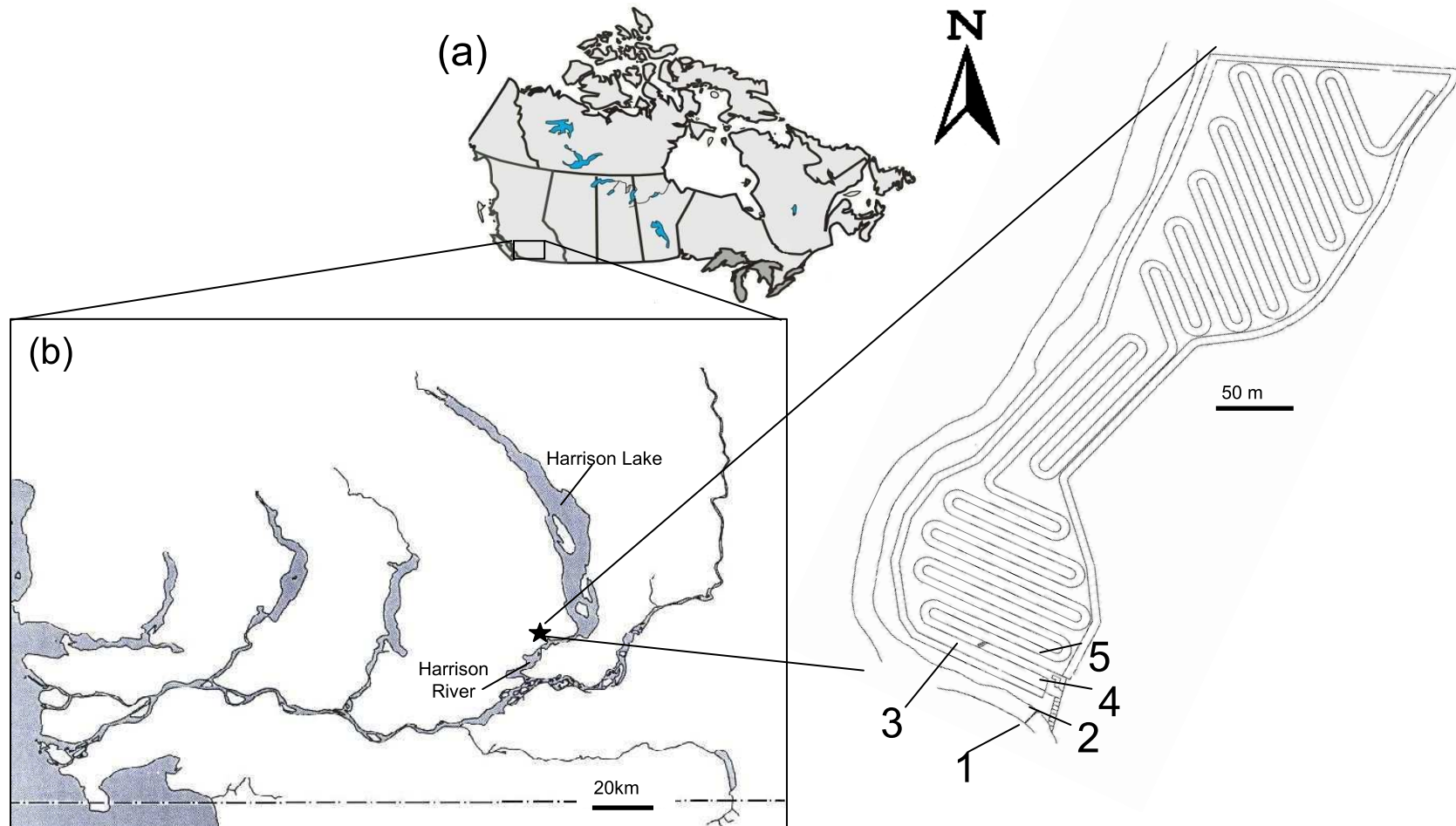
Figure 1.2: Map of British Columbia, Canada (a) showing the lower Fraser River (b). The star indicates the location of the Weaver Creek Spawning Channel. The Spawning Channel (lat: 49º32'N, long: 121º88'W) was built next to Weaver Creek (1). Salmon move from the creek up a fish ladder (2) into the entrance of the channel and hold below the splitter shed (3) before being allowed into the spawning channel. Fish were captured from the holding pool (4), tagged and released into the spawning channel (5).

Literature Cited

Ackerman PA, Forsyth RB, Mazur CF, and Iwama GK. 2000. Stress hormones and the cellular stress response in salmonids. Fish Physiology and Biochemistry 23:327-336.

Adkins-Regan E. 2005. Hormones and social behavior. Princeton University Press, Princeton.

Becker JB, Breedlove SM, Crews D, McCarthy MM. 2002. Behavioural Endocrinology. MIT Press, Cambridge, MA.

Brett JR. 1995. Energetics. Pages 1-68 in Groot C, Margolis L, and Clarke C (eds). Physiological ecology of Pacific salmon. UBC Press, Vancouver, BC.

Brett JR and Glass NR. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. Journal of the Fisheries Research Board of Canada 30:379-387.

Burgner RL. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). Pages 1-117 in Groot C and Margolis L (eds). Pacific salmon life histories. UBC Press, Vancouver, BC.

Calow P. 1985. Adaptive aspects of energy allocation. Pages 13-31 in Tytler P and Calow P (eds). Fish Energetics, New Perspectives. Croom Helm, London.

Campbell PM, Pottinger TG, and Sumpter JP. 1992. Stress reduces the quality of gametes produced by rainbow trout. Biology of Reproduction 47:1140-1150.

Cardwell JR, Sorensen PW, van der Kraak GJ, and Liley NR. 1996. Effect of dominance status on sex hormone levels in laboratory and wild-spawning male trout. General and Comparative Endocrinology 101:333-341.

Carruth LL, Jones RE, and Norris DO. 2002. Stress and Pacific salmon: a new look at the role of cortisol in olfaction and home-stream migration. Integrative and Comparative Biology 42:574-581.

Cooke SJ, Hinch SG, Farrell AP, Lapointe MF, Jones SRM, MacDonald JS, Patterson DA, Healey MC, and van der Kraak G. 2004. Abnormal migration timing and high en route mortality of sockeye salmon in the Fraser River, British Columbia. Fisheries 29:22-33.

Cooke SJ, Crossin GT, Patterson DA, English KK, Hinch SG, Young JL, Alexander RF, Healey MC, van der Kraak G, and Farrell AP. 2005. Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behaviour and survivorship of sockeye salmon: development and validation of a technique. Journal of Fish Biology 67:1342-1358.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Healey MC, Macdonald JS, Shrimpton JM, van Der Kraak G, and Farrell AP. 2006a. Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. Ecology 87:1575-1586.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Shrimpton JM, van Der Kraak G, and Farrell AP. 2006b. Physiology of individual Late-run Fraser River sockeye salmon (*Oncorhynchus nerka*) sampled in the ocean correlates with fate during spawning migration. Canadian Journal of Fisheries Aquatic Sciences 63:1469-1480.

Cooke SJ, Hinch SG, Farrell AP, Patterson DA, Miller-Saunders K, Welch DW, Donaldson MR, Hanson KC, Crossin GT, Mathes MT, Lotto AG, Cooperman MS, Hruska KA, Olsson IC, Wagner GN, Thomson R, Hourston R, English KK, Larsson S, Shrimpton JM, and van der Kraak G. 2008. Developing a mechanistic understanding of fish migrations by linking telemetry with physiology, behavior, genomics and experimental biology: an interdisciplinary case study on adult Fraser River sockeye salmon. Fisheries 33:321-338.

Crossin GT, Hinch SG, Farrell AP, Whelley MP, and Healey MC. 2003. Pink salmon (*Oncorhynchus gorbuscha*) migratory energetics: response to migratory difficulty and comparisons with sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Zoology 81:1986-1995.

Crossin GT, Hinch SG, Farrell AP, Higgs DA, and Healey MC. 2004a. Somatic energy of sockeye salmon *Oncorhynchus nerka* at the onset of upriver migration: a comparison among ocean climate regimes. Fisheries Oceanography 13:345-349.

Crossin GT, Hinch SG, Farrell AP, Higgs DA, Lotto AG, Oakes JD, and Healey MC. 2004b. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. Journal of Fish Biology 65:788-810.

Crossin GT, Hinch SG, Cooke SJ, Welch DW, Lotto AG, Patterson DA, Jones SRM, Leggatt RA, Mathes MT, Shrimpton JM, van Der Kraak G, and Farrell AP. 2008. Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migrations. Canadian Journal of Zoology 86:127-140.

Crossin GT, Hinch SG, Cooke SJ, Cooperman MS, Patterson DA, Welch DW, Hanson KC, Olsson I, English KK, and Farrell AP. 2009. Mechanisms influencing the timing and success of reproductive migration in a capital breeding semelparous fish species, the sockeye salmon. Physiological and Biochemical Zoology 82:635-652.

de Gaudemar B and Beall E. 1998. Effects of overripening on spawning behaviour and reproductive success of Atlantic salmon females spawning in a controlled flow channel. Journal of Fish Biology 53:434-446.

Dickhoff WW. 1989. Salmonids and annual fishes: death after sex. Pages 253-266 in Schreibman MP and Scanes CG (eds). Development, Maturation, and Senescence of Neuroendocrine Systems: A Comparative Approach. Academic Press, New York, NY.

Dingle H. 1996. Migration: The Biology of Life on the Move. Oxford University Press, Cambridge, MA.

Elofsson UOE, Mayer I, Damsgård B, and Winberg S. 2000. Intermale competition in sexually mature Arctic charr: effects on brain monoamines, endocrine stress responses, sex hormone levels, and behavior. General and Comparative Endocrinology 118:450-460.

Essington TE, Quinn TP, and Ewert VE. 2000. Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences 57:205-213.

Farrell AP, Hinch SG, Cooke SJ, Patterson DA, Crossin GT, Lapointe M, Mathes MT. 2008. Pacific salmon in hot water: applying metabolic scope models and biotemetry to predict the success of spawning migrations. Physiological and Biochemical Zoology 81:697-708.

Fleming IA and Gross MR. 1994. Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. Evolution 48:637-657.

Foote CJ. 1990. An experimental comparison of males and female spawning territoriality in a Pacific salmon. Behaviour 115:283-314.

Foote CJ, Brown GS, and Wood CC. 1997. Spawning success of males using alternative tactics in sockeye salmon, *Oncorhynchus nerka*. Canadian Journal of Fisheries and Aquatic Sciences 54:1785-1795.

Fukushima M and Smoker WW. 1997. Determinants of stream life, spawning efficiency, and spawning habitat in pink salmon in the Auke Lake system, Alaska. Canadian Journal of Fisheries and Aquatic Sciences 54:96-104.

Gilhousen P. 1980. Energy sources and energy expenditures in Fraser River sockeye salmon during their spawning migration. International Pacific Salmon Fisheries Commission Bulletin 22.

Gilhousen P. 1990. Prespawning mortalities of sockeye salmon in the Fraser River system and possible causal factors. International Pacific Salmon Fisheries Commission Bulletin 26.

Gilmour KM, Wilson RW, and Sloman KA. 2005. The integration of behaviour into comparative physiology. Physiological and Biochemical Zoology 78:669-678.

Gingerich AJ, Philipp DP, and Suski CD. 2010. The effects of nutritional status on metabolic rate, exercise and recovery in a freshwater fish. Journal of Comparative Physiology B 180:371-377.

Groot C and Margolis L. 1991. Pacific salmon life histories. UBC Press, Vancouver, BC.

Groot C, Margolis L, and Clarke C. 1995. Physiological ecology of Pacific salmon. UBC Press, Vancouver, BC.

Hasler AD. 1966. Underwater guideposts: homing of salmon. The University of Wisconsin Press, Milwaukee, WI.

Healey MC and Prince A. 1998. Alternative tactics in the breeding behaviour of male coho salmon. Behaviour 135:1099-1024.

Healey MC, Lake R, and Hinch SG. 2003. Energy expenditures during reproduction by sockeye salmon (*Oncorhynchus nerka*). Behaviour 140:161-182.

Hendry AP and Berg OK. 1999. Secondary sexual characteristics, energy use, senescence, and the cost of reproduction in sockeye salmon. Canadian Journal of Zoology 77:1663-1675.

Hendry AP, Berg OK, and Quinn TP. 2001. Breeding location choice in salmon: causes (habitat, competition, body size, energy stores) and consequences (life span, energy stores). Oikos 93:407-418.

Hendry AP, Morbey YE, Berg OK, and Wenburg JK. 2004. Adaptive variation in senescence: reproductive lifespan in a wild salmon population. Proceedings of the Royal of Society of London Series B 271:259-266.

Hendry AP and Stearns SC. 2004. Evolution Illuminated: Salmon and Their Relatives. Oxford University Press, Oxford.

Hinch SG and Bratty JM. 2000. Effects of swim speed and activity pattern on success of adult sockeye salmon migration through an area of difficult passage. Transactions of the American Fisheries Society 129:604-612.

Hinch SG, Cooke SJ, Healey MC, and Farrell AP. 2006. Behavioural physiology of fish migrations: salmon as a model approach. Pages 240-296 in Sloman KA, Wilson RW, and Balshine S (eds). Behaviour and Physiology of Fish. Elsevier, Amsterdam.

Hruska KA, Hinch SG, Healey MC, and Farrell AP. 2007. Electromyogram telemetry, non-destructive physiological biopsy, and genetic markers: linking recent technologies with behavioural observations for the study of reproductive success in sockeye salmon mating systems. Pages 17-29 in Woody CA (ed). Sockeye Salmon Ecology, Evolution, and Management. American Fisheries Society, Symposium 54. Bethesda, MD.

Idler DR and Clemens WA. 1959. The energy expenditures of Fraser River sockeye salmon during the spawning migration to Chilko and Stuart Lakes. International Pacific Salmon Fisheries Commission Progress Report 6.

Jalabert B. 1976. In vitro oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*), and goldfish (*Carassius auratus*). Journal of the Fisheries Research Board of Canada. 33:974-988.

Kinnison MT, Unwin MJ, Hendry AP, and Quinn TP. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution 55:1656-1667.

Kubokawa K, Watanabe T, Yoshioka M, and Iwata M. 1999. Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breeding season. Aquaculture 172:335-349.

Laidley CW and Leatherland JF. 1988. Cohort sampling, anaesthesia and stocking-density effects on plasma cortisol, thyroid hormone, metabolite and ion levels in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 33:73-88.

Lee CG, Farrell AP, Lotto A, MacNutt MJ, Hinch SG, and Healey MC. 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) coho (*O. kisutch*) salmon stocks. Journal of Experimental Biology 206:3239-3251.

Liley NR, Olsén KH, Foote CJ, and van der Kraak GJ. 1993. Endocrine changes associated with spawning behaviour in male kokanee salmon (*Oncorhynchus nerka*) and the effects of anosmia. Hormones and Behavior 27:470-487.

Macdonald JS. 2000. Mortality during migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): a study of the effect of ocean and river environmental conditions in 1997. Canadian Technical Report of Fisheries and Aquatic Sciences 2315.

Macfarlane RB and Benville PE. 1986. Primary and secondary stress responses of striped bass (*Morone saxitilis*) exposed to benzene. Marine Biology 92:245-254.

Maldonado TA, Jones, RE, and Norris DO. 2000. Distribution of β-amyloid and amyloid precursor protein in the brain of spawning (senescent) salmon: a natural, brain-aging model. Brain Research 858:237-251.

Mathes MT, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Lotto AG, and Farrell AP. 2010. Effect of water temperature, timing, physiological condition and lake thermal refugia on migrating adult Weaver Creek sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 67:70-84.

Maule AG, Schrock R, Slater C, Fitzpatrick MS, and Schreck CB. 1996. Immune and endocrine responses of adult chinook salmon during freshwater immigration and sexual maturation. Fish and Shellfish Immunology 6:221-233.

McDonald DG and Milligan CL. 1992. Chemical properties of blood. Pages 55-133 in Hoar WS, Randall DJ, and Farrell AP (eds). The Cardiovascular System. Academic Press, San Diego, CA.

McPhee MV and Quinn TP. 1998. Factors affecting the duration of nest defense and reproductive lifespan of female sockeye salmon, *Oncorhynchus nerka*. Environmental Biology of Fishes 51:369-375.

Mehranvar L, Healey M, Farrell A, and Hinch S. 2004. Social versus genetic measures of reproductive success in sockeye salmon, *Oncorhynchus nerka*. Evolutionary Ecology Research 6:1167-1181.

Mommsen TP, Vijayan MM, and Moon TW. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9:211-268.

Morbey YE and Ydenberg RC. 2003. Timing games in the reproductive phenology of female Pacific salmon (*Oncorhynchus* spp.). The American Naturalist 161:284-298.

Morbey YE and Abrams PA. 2004. The interaction between reproductive lifespan and protandry in seasonal breeders. Journal of Evolutionary Biology 17:768-778.

Øverli Ø, Harris CA, and Winberg S. 1999. Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. Brain Behavior and Evolution 54:263–275.

Patterson DA, Macdonald JS, Hinch SG, Healey MC, and Farrell AP. 2004. The effect of exercise and captivity on energy partitioning, reproductive maturation, and fertilization success in adult sockeye salmon. Journal of Fish Biology 64:1039-1059.

Pickering AD, Pottinger TG, and Christie P.1982. Recovery of the brown trout, Salmo trutta L., from acute handling stress: a time-course study. Journal of Fish Biology 20:229-244.

Pickering AD, Pottinger TG, Carragher J, and Sumpter JP. 1987. The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. General and Comparative Endocrinology 68:249-259.

Pickering AD and Pottinger TG. 1995. Biochemical effects of stress. Pages 349-379 in Hochachka PW and Mommsen TP (eds). Biochemistry and Molecular Biology of Fishes. Vol. 5. Environmental and Ecological Biochemistry. Elsevier, Amsterdam.

Pon LB, Hinch SG, Cooke SJ, Patterson DA, and Farrell AP. 2009. Physiological, energetic and behavioural correlates of successful fishway passage of adult sockeye salmon *Oncorhynchus nerka* in the Seton River, British Columbia. Journal of Fish Biology 74:1323-1336.

Quinn TP and Foote CJ. 1994. The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon, *Oncorhynchus nerka*. Animal Behaviour 48:751-761.

Quinn TP. 1999. Variation in Pacific salmon reproductive behaviour associated with species, sex, and levels of competition. Behaviour 136:179-204.

Quinn TP, Eggers DM, Clark JH, and Rich HB Jr. 2007. Density, climate, and the processes of prespawning mortality and egg retention in Pacific salmon (*Oncorhynchus* spp.). Canadian Journal of Fisheries and Aquatic Sciences 64:574-582.

Rand PS and Hinch SG. 1998. Swim speeds and energy use of upriver-migrating sockeye salmon (*Oncorhynchus nerka*): simulating metabolic power and assessing risk of energy depletion. Canadian Journal of Fisheries and Aquatic Sciences 55:1832-1841.

Rand PS, Hinch SG, Morrison J, Foreman MGG, MacNutt MJ, Macdonald JS, Healey MC, Farrell AP and Higgs DA. 2006. Effects of river discharge, temperature, and future climates on energetics and mortality of adult migrating Fraser River sockeye salmon. Transactions of the American Fisheries Society 135:655-667.

Reimchen TE. 2000. Some ecological and evolutionary aspects of bear-salmon interactions in coastal British Columbia. Canadian Journal of Zoology 78:448-457.

Robertson OH and Wexler BC. 1957. Pituitary degeneration and adrenal tissue hyperplasia in spawning Pacific salmon. Science 125:1295-1296.

Roff DA. 2002. The Evolution of Life Histories: Theory and Analysis. Chapman and Hall, New York, NY.

Rotllant J and Tort L. 1997. Cortisol and glucose responses after acute stress by net handling in the sparid red porgy previously subjected to crowding stress. Journal of Fish Biology 51:21-28.

Schreck CB, Contreras-Sanchez W, and Fitzpatrick MS. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197:3-24.

Schreck CB. 2010. Stress and fish reproduction: the roles of allostasis and hormesis. General and Comparative Endocrinology 165:549-556.

Schroder S. 1982. The influence of intrasexual competition on the distribution of chum salmon in an experimental stream. Pages 275-285 in Brannon EL and Salo EO (eds). Proceedings of the salmon and trout migratory symposium. University of Washington Press, Seattle, WA.

Sloman KA and Armstrong JD. 2002. Physiological effects of dominance hierarchies: laboratory artefacts or natural phenomena? Journal of Fish Biology 61:1-23.

Soma KK. 2006. Testosterone and aggression: Berthold, birds and beyond. Journal of Neuroendocrinology 18:543-551.

Stearns SC. 1992. The Evolution of Life Histories. Oxford University Press, Oxford.

Steen RP and Quinn TP. 1999. Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. Canadian Journal of Zoology 77:836-841.

Truscott B, Idler DR, So YP, and Walsh JM. 1986. Maturational steroids and gonadotropin in upstream migratory sockeye salmon. General and Comparative Endocrinology 62:99-110.

Tufts BL, Tang Y, Tufts K, and Boutilier RG. 1991. Exhaustive exercise in wild Atlantic salmon: acid-base regulation and blood gas transport. Canadian Journal of Fisheries and Aquatic Sciences 48:868-874.

Tyler CR, Sumpter JP, and Bromage NR. 1990. An *in-vitro* culture system to study vitellogenic growth of ovarian follicles of the rainbow trout, *Salmo gairdneri*. Journal of Experimental Biology 248:199-206.

Tyler CR and Sumpter JP. 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6:287-318.

Vijayan MM, Pereira C, Grau EG, and Iwama GK. 1997. Metabolic responses associated with confinement stress in tilapia: the role of cortisol. Comparative Biochemistry and Physiology 116C:89-95.

Wendelaar Bonga SE. 1997. The stress response in fish. Physiological Reviews 77:591-625.

Wingfield JC. 2003. Control of behavioural strategies for capricious environments. Animal Behaviour 66:807-816.

Wingfield JC and Sapolsky RM. 2003. Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology 15:711-724.

Wingfield JC. 2005. A continuing saga: the role of testosterone in aggression. Hormones and Behavior 48:253-255.

Wood CM, Turner JD, and Graham MS. 1983. Why do fish die after severe exercise? Journal of Fish Biology 22:189–201.

Wood CM. 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. Journal of Experimental Biology 160:285-308.

Woodey JC. 1987. In-season management of Fraser River sockeye salmon (*Oncorhynchus nerka*): meeting multiple objectives. Pages 367-374 in Smith HD, Margolis L, and Wood CC (eds). Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Canadian Special Publications of Fisheries and Aquatic Sciences 96.

Yaron Z and Sivan B. 2006. Reproduction. Pages 343-386 in Evans DH and Claiborne JB (eds). The Physiology of Fishes, 3rd ed. CRC Press, Boca Raton, FL.

Young JL, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Farrell AP, van der Kraak G, Lotto AG, Lister A, Healey MC, and English KK. 2006. Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 63:1067-1077.

Chapter 2: Egg retention in relation to arrival timing and reproductive longevity in female sockeye salmon (*Oncorhynchus nerka*)[1]

Introduction

All adult Pacific salmon (*Oncorhynchus* spp.) die after spawning, yet some females fail to completely deposit eggs prior to death (Gilhousen, 1990; Quinn et al., 2007). In healthy sockeye salmon (*O. nerka*), egg retention is typically quite low (i.e., less than 5%; Burgner, 1991), yet extreme spawning failure, in which many females die without depositing any eggs (pre-spawning mortality), can reach 90% within some populations in the Fraser River watershed in some years (Gilhousen, 1990), resulting in a potential loss of hundreds of millions of unspawned eggs. High levels of egg retention (i.e., >20%) have also been observed in sockeye salmon populations from other watersheds (Quinn et al., 2007) and in the four other species of North American Pacific salmon (Groot and Margolis, 1991; Fukushima and Smoker, 1997; Reimchen, 2000). Yet despite the tremendous conservation and economic consequences associated with this phenomenon, its causes are still largely unknown.

Extreme environmental conditions can affect travel rates and migratory success in Pacific salmon (Lee et al., 2003; Crossin et al., 2008; Farrell et al., 2008). High water temperatures or high water discharge can increase physiological stress and incidence of disease, accelerate energy depletion and senescence, and slow or stop migrations (Rand and Hinch, 1998; Hinch et al., 2006; Farrell et al., 2008; Keefer et al., 2008). Given that sockeye salmon must complete migration and spawning using stored energy reserves, delays in migration may exhaust energy reserves prior to spawning completion (Keefer et al., 2004). Thus, sockeye salmon that experience extreme environmental conditions during migration may have reduced length of life on the spawning grounds (i.e., reproductive longevity).

Few studies have examined the links between reproductive longevity and spawning success or egg retention in salmonids; however, several studies have shown a seasonal decline in reproductive longevity (van den Berghe and Gross, 1989; Morbey and Ydenberg, 2003; Hendry et al., 2004). Based on observations on non-anadromous kokanee (*O. nerka*),

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Morbey and Ydenberg (2003) conjectured that early arriving females live longer to protect their redds from superimposition by later arrivals. Some authors also report that egg retention was highest near the start of the spawning season and declined quickly thereafter (Gilhousen, 1990; Fukushima and Smoker, 1997). But, as far as I know, none of the above relationships have been subject to experimentation; it is all observation.

Other behavioural and environmental factors may also influence reproductive longevity and egg retention in female Pacific salmon (Figure 1.1a). Females that are not fully mature at spawning ground arrival will complete the process of sexual maturation prior to redd establishment and, hence, live relatively longer on the spawning grounds (Morbey and Ydenberg, 2003; Morbey and Guglielmo, 2006). Fish size can also affect reproductive success in salmonids (van den Berghe and Gross, 1986; Foote, 1990; Steen and Quinn, 1999) and, thus, may also influence egg retention and reproductive longevity. Specifically, larger females have been observed to arrive and establish redds earlier (Blanchfield and Ridgway, 1997; Rich et al., 2006), obtain better redd locations (van den Berghe and Gross, 1989; Foote, 1990), and guard redds longer (van den Berghe and Gross, 1986) than smaller females. However, other studies have shown that the largest females have reduced fecundity (Healey and Heard, 1984; Healey, 1987). Thus, experiments to examine egg retention and reproductive longevity must consider female size and reproductive maturity.

The time it takes to establish a redd may influence not only the length of time spent on the redd, but also reproductive longevity and egg retention. By waiting to establish a redd and, thus, conserving energy and avoiding aggressive interactions, a female salmonid may increase her reproductive longevity (Blanchfield and Ridgway, 1997; Morbey and Ydenberg, 2003). Increased time on the redd reduces the likelihood of redd superimposition, improving reproductive success (Fukushima et al., 1998). Conversely, establishing a redd and spawning take time; a female will be unable to complete spawning if she dies or is displaced prior to the completion of these activities.

The first objective of this study was to characterize the relationship between reproductive longevity and egg retention in female sockeye salmon. I hypothesized that the potential causes of egg retention outlined above would be mediated through effects on reproductive longevity. Thus, I predicted that egg retention would be lower for females that lived longer after arrival at the spawning grounds. My second objective was to quantify how reproductive longevity and egg retention, as well as the relationship between these two variables,

changed over the spawning season. I hypothesized that females that arrived earlier in the spawning season would live longer. My third objective was to quantify how other factors, such as reproductive maturity of females, behaviour, and fish size, affect egg retention and reproductive longevity. I hypothesized that: 1) immature females would establish redds later and live longer than mature females; 2) females that establish their redds shortly after arrival would live longer on the spawning grounds and be more likely to complete spawning; and 3) females that spend more time on their redds would be more likely to complete spawning.

Methods

The study took place at the Weaver Creek Spawning Channel (lat: 49°32'N, long: 121°88'W), which is approximately 125 km east of Vancouver, British Columbia, Canada (Figure 1.2) and focused on sockeye salmon. The spawning channel was constructed in 1965 to provide spawning habitat for approximately 20,000 sockeye salmon; smaller numbers of pink (*O. gorbuscha*) and chum (*O. keta*) salmon are also permitted to spawn in the channel. Weaver Creek sockeye are a well-studied population; see Quinn (1999), Essington et al. (2000), Mehranvar et al. (2004), and Mathes et al. (2010) for more details. The entrance to the channel is manually operated and fish are counted into the channel daily throughout the spawning season.

In 2006 the spawning channel was opened on October 5 and spawning took place through the month of October. Peak female abundance in the spawning channel occurred on October 14. Total female abundance in the channel was 18,837 (R Stitt, Weaver Creek Spawning Channel, FOC, personal communication). To assess temporal changes in reproductive longevity and egg retention I tagged (details below) and released two hundred and fifty female sockeye salmon during three sampling periods (Oct. 5-6, Oct. 13, and Oct. 19), which spanned the main period of salmon entry into the channel. Median annual pre-spawning mortality for sockeye salmon in the Weaver Creek Spawning Channel (1996-2005) was 13.3% (range: 3.75 to 21.05%); median abundance during this time period was 15,739 (range: 2,854 to 20,789; R Stitt, Weaver Creek Spawning Channel, FOC, personal communication). My objective was to obtain data on at least 20 females that retained > 75% of their eggs at death, thus I anticipated having to tag up to 500 females; however after the first week of observations it was evident that egg retention was higher than expected so I

scaled back my planned tagging. In total, I tagged 139, 71, and 40 females in the first, second and third sampling periods, respectively.

Water temperature in the spawning channel declined from 15.0°C to 7.4°C at about 1 to 1.5 °C per week through October. Fish from the first sampling period experienced mean spawning ground temperatures of 14.1 ± 0.1°C; the second group of fish experienced mean temperatures of 13.3 ± 0.2°C; the third arrival group experienced mean temperatures of 12.1 ± 0.2°C (R Stitt, Weaver Creek Spawning Channel, FOC, personal communication).

Salmon were captured from the holding pool at the top of the fish ladder below the entrance to the spawning channel (Figure 1.2). I assumed that all fish in the holding pool had an equal likelihood of entering the spawning channel when the gates were open. All captured fish were used in the study, regardless of condition, so that data were representative of fish condition at arrival. Fish were captured by dip net and immediately placed, ventral side up, in a V-shaped trough with continuous flow-through water from the spawning channel for processing. Fish were biopsy-sampled (i.e., 3 ml blood sample and 0.03 g gill biopsy; 30-90 s duration) for another study (Chapter 4), measured to fork length for body size, and tagged with individually marked Peterson discs. This bio-sampling procedure has been validated and used in several recent adult sockeye salmon studies and has minimal impacts on the migration speed or survival of the fish during upstream migration (Cooke et al., 2005; Crossin et al., 2007). The abdomen of each female was palpated to determine maturity: a female was considered mature if the eggs felt loose within the abdomen and / or eggs were extruded from the vent with the application of gentle pressure and immature if the eggs felt tight within the abdomen and eggs were not extruded with the application of gentle pressure (Craik and Harvey, 1984; de Gaudemar and Beall, 1998). Each fish was released into the spawning channel (Figure 1.2) immediately after processing. I recorded total handling time from capture to release in seconds. Handling procedures were in accordance with animal care protocols (protocol number A05-0424; Appendix 2).

The spawning channel was surveyed daily to map the locations of tagged individuals. Each fish was observed for approximately 20-30 s to assess the status of redd establishment and spawning activity. For each female I calculated two behavioural indices, time to establish redd and time on redd, based on: the last day observed before redd establishment, first day observed on redd, last day observed on redd, and first day observed off redd. The day of redd establishment was calculated as the midpoint between the last day before redd

establishment and the first day on redd. The day off redd was calculated as the midpoint between the last day on redd and the first day off redd. If more than two days of observations were missed between either of these check points, the female was excluded from the analysis. Time to establish redd was calculated as the number of days between the first day in the channel and the day of redd establishment. Time on redd was calculated as the number of days between the day of redd establishment and the day off redd.

Dead fish were collected daily. Reproductive longevity was calculated as the number of days between arrival and death. Eleven of the 250 tagged females were not recovered from the channel and were excluded from reproductive longevity calculations. There was evidence of scavenging by gulls (*Larus* spp.) on eight additional females so these fish were excluded from egg retention analyses due to the potential bias in final egg retention values. Total and gonad mass were measured for each dead fish. Gonadosomatic index at death (GSI_D) was calculated by dividing the remaining gonad mass by the somatic mass at death (i.e., total body mass minus gonad mass). Expected gonadosomatic index (GSI_E) was calculated based on the relationship between gonadosomatic index and somatic mass for unspawned females in this population. For unspawned females in this population, gonad mass typically ranges from about 350 to 500 g and is positively related to fish size. Egg retention was then calculated as GSI_D / GSI_E * 100%.

All statistics were calculated using either SAS 9.1 or JMPIN 4.0.4 (SAS Institute, USA). Effects were considered significant at $\alpha = 0.05$. Values are given as mean ± SEM unless otherwise indicated. Due to multiple comparisons, I applied Bonferroni corrections to minimize the chance of a Type II error (Rice, 1989). However, Bonferroni corrections are highly conservative, so I also indicated effects at $\alpha = 0.05$ to allow the reader to define for themselves effects that are biologically meaningful (Cabin and Mitchell, 2000).

There were only two females that arrived immature in the final sampling period, thus I was unable to analyse the data using a two-way ANOVA (i.e., maturity × sample period). Instead, one-way ANOVAs were used to test for differences in fork length and reproductive longevity between maturity classes and then between sampling periods for mature and immature females separately. Due to reproductive longevity differences between maturity and sampling groups, I chose not to pool data across these groups for the remaining analyses (see below).

A preliminary exploration of the data was done to assess whether data fit the assumptions of normality and homogeneity of variances. Time on redd data were transformed (square root) to normalize the residuals. Egg retention could not be normalized due to the large number of zero values in the dataset; similarly, time to redd establishment data could not be normalized due to the large number of unity values. Thus, Kruskal-Wallis tests were used to test for differences between maturity and arrival timing groups for these two parameters. All egg retention and time to redd values are reported as medians.

Due to the large number of zero values in the dataset, egg retention and time to establish redd data were categorized to facilitate further analysis. For egg retention, females were categorized as either completely spawned (≤0.5% egg retention at death) or not completely spawned (>0.5% egg retention at death). A small number of eggs may occasionally become trapped behind an organ and thus be retained in the body cavity. During preliminary data analysis I explored the use of a range of thresholds for categorizing these data (i.e., 0 eggs, 0.5% egg retention, 1% egg retention, 5% egg retention, 25% egg retention, 50% egg retention, and 75% egg retention). Each of these thresholds resulted in similar negative relationships between reproductive longevity and egg retention, although the relationships were not as tight when higher proportions of egg retention were used. For time to establish redd data, females were categorized as: established a redd within ≤ 2 d, established a redd in > 2 d (range: 3 to 8 d), or never observed establishing a redd. Two days was chosen in order to increase the probability of selecting all females that established a redd shortly after arrival. Chi-square analysis was used to determine whether egg retention differed by either maturity class, sampling period, or redd establishment category, whether redd establishment category differed by either maturity class or sampling period, and whether maturity class differed by sampling period. Logistic analysis was used to determine whether spawning success differed by fork length.

Females were grouped by their maturity and arrival group for analysis of relationships between reproductive longevity and egg retention. Females were similarly grouped for logistic regression analysis of the probability of complete spawning as a function of reproductive longevity. Similar analyses were done to analyze for correlations between time on redd and egg retention.

To determine whether handling impacted spawning success in the tagged fish, pre-spawning mortality was calculated for the tagged fish following the methods in Gilhousen

(1990). The values for tagged fish in the study were compared to values for all sockeye salmon females in the spawning channel.

Results

Handling time ranged from 2 to 5 min and was not correlated with either reproductive longevity (F = 0.16; p = 0.690) or egg retention (F = 0.23; p = 0.630). In this study year, pre-spawning mortality in the Weaver Creek population was 28.4% (R Stitt, Weaver Creek Spawning Channel, FOC, personal communication). The pre-spawning mortality for the tagged fish in this study was 24%, which was lower than in the spawning channel at large indicating that the handling had minimal impacts on the behaviour and spawning success of the females in this study. Median egg retention for tagged fish was 9.2%.

There was no difference in fork length between mature and immature females (F = 2.06; p = 0.153; mature = 58.9 ± 0.3 cm; immature = 59.6 ± 0.4 cm). Females that arrived in the first sampling period were significantly smaller than females that arrived in the last sampling period (F = 5.92; p = 0.003; fork length: Oct. 5-6 = 58.5 ± 0.3 cm; Oct. 13 = 59.7 ± 0.4 cm; Oct. 19 = 60.6 ± 0.7 cm).

The females that were immature at arrival lived approximately 1 d longer than mature females (F = 9.13; p = 0.003; mature = 6.3 ± 0.2 d; n = 172; immature = 7.4 ± 0.3 d; n = 70). Among mature females, those that arrived in the first sampling session lived longer on the spawning grounds than those that arrived in the latter two sampling periods (F = 8.35; p < 0.001; Oct. 5-6 = 7.0 ± 0.2 d; n = 93; Oct. 13 = 5.9 ± 0.4 d; n = 42; Oct. 19 = 5.3 ± 0.4 d; n = 37). This result was similar for immature females: those that arrived in the first sampling session lived significantly longer than those that arrived in the second sampling session (F = 10.67; p = 0.002; Oct. 5-6 = 8.1 ± 0.3 d; n = 44; Oct. 13 = 6.1 ± 0.5 d; n = 24). Fork length was related to reproductive longevity (p = 0.043; r = -0.137); however, this relationship was not significant when we considered the three sampling periods separately (Oct. 5-6: p = 0.438; Oct. 13: p = 0.804; Oct. 19: p = 0.185), indicating that the relationship was likely driven by differences in fish size across arrival periods.

Percent egg retention did not differ across sampling periods (χ^2 = 1.26; p = 0.534; median: Oct. 5-6 = 8.2%; Oct. 13 = 14.6%; Oct. 19 = 10.8%) or maturity categories (χ^2 = 0.27; p =

0.604; median: mature = 11.0%; immature = 7.6%). There was one female that was completely unspawned (i.e., ~ 100% egg retention) and several others that were completely spawned (i.e., <0.5% egg retention at death) in each of the sampling periods and maturity groups. There was no difference in the proportions of mature and immature females that were categorized as completely spawned vs. not completely spawned (χ^2 = 0.86; p = 0.353; mature = 22% were completely spawned; immature = 28% were completely spawned). Similarly, there was no difference in the proportions of females captured in each of the three sampling sessions that were categorized as completely spawned or not completely spawned (χ^2 = 2.80; p = 0.246; Oct. 5-6 = 29% were completely spawned; Oct. 13 = 20% were completely spawned; Oct. 19 = 22% were completely spawned). Fork length was unrelated to egg retention (χ^2 = 0.96; p = 0.326; completely spawned = 58.75 ± 0.39 cm; not completely spawned = 59.27 ± 0.27 cm).

For the mature females in the first sampling period, there was a significant negative correlation between percent egg retention and reproductive longevity (Figure 2.1; p < 0.001; r = -0.442). This relationship was also significant in all other sampling periods / maturity groups, except among immature females from the first sampling period (Table 2.1). There was considerable variability in these relationships, largely resulting from females that lived for more than 7 d in the spawning channel yet died with high egg retention (Figure 2.2). Some individuals had greater egg retention than would be expected for the length of time they spent in the spawning channel.

Among mature females in the first sampling period, individuals had a higher probability of being completely spawned the longer they lived in the spawning channel (Figure 2.1; χ^2 = 14.70; p < 0.001). Similarly, females that arrived in the last sampling session more frequently spawned completely if they lived longer (Table 2.1), although not so for the immature females in the first sampling group and for both maturity groups in the second sampling session (p > 0.10). There were no completely spawned fish that died before the fourth day in the spawning channel.

On average, > 70% of the females were observed in the channel each day, which provided data to calculate the time to establish redds for 145 females and the time on redds for 143 females. In addition, 14 females were classified as never forming redds. There was no difference between mature and immature fish in the time to establish a redd (χ^2 = 0.06; p = 0.808; median; mature: 1.5 d; immature: 1.5 d) or time on redd (F = 0.70; p = 0.404: mean ±

30

SEM; mature: 4.2 ± 0.2 d; immature: 4.6 ± 0.3 d). There was also no difference among the three sampling periods of the time to establish a redd (χ^2 = 4.38; p = 0.112; median; Oct. 5-6: 1.5 d; Oct. 13: 1.3 d; Oct. 19: 2.0 d) or the time on redd (F = 2.73; p = 0.069; mean ± SEM; Oct. 5-6: 4.7 ± 0.3 d; Oct. 13: 4.0 ± 0.3 d; Oct. 19: 3.9 ± 0.4 d).

Fork length was negatively correlated with time spent on redd among females that arrived in the first sampling session (p = 0.008; r = - 0.306) but not for the other two sampling periods (Oct. 13: p = 0.946; r = 0.011; Oct. 19: p = 0.288; r = 0.-250). Fork length was not related to whether a female established a redd within 2 d, or in more than 2 d for any of the sample periods (all p > 0.45).

Seventy-five percent (109 of 145) of the females that were observed to establish redds did so within 2 d of arrival. Median egg retention by these females was 3%. Of the 109 females that established a redd within 2 d, 38 (35%) were considered completely spawned. In contrast, only 22% of the females that took longer than 2 d to establish redds were completely spawned. Median egg retention among the females that took longer than 2 d to establish a redd was 30%. In addition, the females that established redds within 2 d were on their redds longer than the females that took more than 2 d to establish a redd (F = 22.98; p < 0.001; mean ± SEM; redd in ≤ 2 d: 4.8 ± 0.2 d on redd; redd in > 2 d: 3.0 ± 0.3 d on redd). However, the females that took more than 2 d to establish redds lived significantly longer in the spawning channel than did females that established redds within 2 d of arrival (F = 8.58; p = 0.004; mean ± SEM; redd in ≤ 2 d: 6.6 ± 0.2 d; redd in > 2 d: 7.6 ± 0.3 d). Females that were not observed on a redd lived a similar length of time (6.6 ± 0.8 d; range 1-13 d) as the females that established a redd.

Among mature females in the first sampling period, there was a negative correlation between egg retention and time spent on redd (Figure 2.3; Table 2.2; p < 0.001; r = -0.691), and the probability of being completely spawned increased with increasing time spent on redd (Figure 2.3; Table 2.2; χ^2 = 19.40; p < 0.001). These relationships were also significant during all the other sampling period / maturity groups (Table 2.2) except for the females from the last sampling period, for which the probability of being completely spawned did not increase with increasing time on redd. None of the females that were observed on redds for 2 d or less were completely spawned at death.

Discussion

While several studies have looked at redd guarding benefits of greater reproductive longevity in Pacific salmon (Quinn and Foote, 1994; Morbey and Ydenberg, 2003; Hendry et al., 2004), to my knowledge no study has examined the relationship between reproductive longevity and the ability of females to complete spawning. In general I found, as I had predicted, that egg retention was lower among females that lived longer after arrival at the spawning grounds and reproductive longevity was higher among early arriving individuals.

I observed a significant decline in egg retention with an increasing number of days on the spawning grounds for most maturity / arrival timing groups. Completely spawned females lived approximately 2 d longer on the spawning grounds than females that retained at least some eggs at death. This result supports my hypothesis that some females were running out of time to complete spawning; females that lived longer after arrival had a greater probability of being completely spawned. This benefit to reproductive success was in addition to any benefit derived from guarding the redd from superimposition by later arriving females (McPhee and Quinn, 1998; Morbey and Ydenberg, 2003).

The 4 d minimum reproductive longevity for complete spawning in this population is consistent with observations of spawning behaviour in Weaver Creek, as well as other populations of sockeye salmon. After arrival, a female must spend time searching for a suitable spawning location, fighting for that location and then digging her redd. In addition, female sockeye salmon do not spawn all of their eggs in a single spawning episode; rather, they deposit a portion of their eggs during each of several spawning events and then pause to cover the eggs and dig a new nest within the redd (reviewed by Burgner, 1991). My data indicate that a female spent more than 2 d on her redd to complete spawning. Thus, 2 d may be the minimum amount of time a female requires to perform the three to seven spawning episodes necessary to deposit all of her eggs. The mean time females spent on their redds (4.4 d) is similar to results from other Fraser River sockeye salmon (e.g. 3.5 d; Early Stuart sockeye, Gluskie Creek, BC; Healey et al., 2003). In contrast, Clark (1959) found that most sockeye salmon females in a population on Kodiak Island were completely spawned within 2 d of establishing a redd. The Kodiak Island population experiences intense predation pressure from bears (*Ursus arctos*), suggesting that sockeye salmon can complete spawning quicker than I observed if risk of reduced fitness due to predation is extremely high. Predation of sockeye salmon within the Weaver Creek Spawning Channel is generally

quite low; very few bear sightings were observed in the area in 2006 (K Hruska, personal observation).

My results were consistent with the idea that females can increase time on spawning grounds by waiting to establish a redd (Blanchfield and Ridgway, 1997; Morbey and Ydenberg, 2003). Females that started a redd more than 2 d after arrival were alive in the spawning channel longer (~1 d) than females that established a redd within 2 d. Similarly, Morbey and Ydenberg (2003) observed an increase in spawning ground lifespan of 0.65 d for every day a female kokanee salmon delayed in establishing a redd. Increased reproductive longevity can be important for preventing redd reuse by later-arriving females, as redd superimposition can be an important source of egg mortality (Fukushima et al., 1998; Steen and Quinn, 1999). While a longer period of redd guarding would be more advantageous for females that arrive early in the spawning season, I did not observe any difference in the time to establish a redd among the three sampling periods. Similarly, while I predicted that immature females would delay establishing a redd longer than would mature females, there was no difference in the length of time to establish a redd between the two maturity classes.

Females that established redds within 2 d of arrival spent longer (> 1.5 d) on redds than females that took more than 2 d to establish redds, suggesting a trade-off between reproductive longevity and time on redd. A small number of females were observed off their redds for at least 1 d before death, indicating that redd departure may have been due to displacement rather than death for some females. Egg retention was strongly correlated with time spent on the redd; the females that established a redd sooner after arrival generally had lower levels of egg retention, possibly due to the greater time spent on the redd. Waiting to establish a redd may be a successful strategy if the additional day spent in the channel provides a large benefit in terms of redd guarding, an effect that was not measured in my study. However, as my data show, waiting is not always the most effective strategy for maximizing reproductive success (Morbey and Ydenberg, 2003). For example, predation on the spawning grounds may increase the risks of waiting (Clark, 1959; Quinn and Kinnison, 1999). In populations like Weaver Creek, sockeye salmon can encounter high temperatures during upriver migration and arrive at spawning grounds with high levels of physiological stress and advanced stages of naturally occurring diseases (Wagner et al., 2005; Farrell et al., 2008; Crossin et al., 2008); these environmental conditions may also increase the risks

associated with waiting. However, this population can also wait in the cooler Harrison Lake prior to arrival at the spawning grounds (Mathes et al., 2010).

I found no difference in egg retention across the spawning season. This finding contrasts with the conclusions of Gilhousen (1990) who stated that pre-spawning mortality was highest in the early part of the season for all Fraser River sockeye salmon stocks. One reason for this apparent contradiction could be methodological. Gilhousen (1990) related egg retention with date of death without knowing the date of arrival for individual fish. In contrast, I tagged females at three distinct times during the spawning season in order to directly measure the effects of arrival timing on egg retention and reproductive longevity. Williams (1973) tagged females at the start, middle, and end of the spawning season and, like me, found no differences in egg retention throughout the spawning season. Gilhousen (1990) likely observed a decline in egg retention throughout the spawning season due to the lower levels of egg retention in longer-lived females, which created the impression that earlier arriving females had higher levels of egg retention.

The relationship between reproductive longevity and egg retention was negative across all maturity / sampling groups, except among immature females that arrived in the first sampling period, and it appears to be largely driven by females that died shortly after arrival at the spawning channel. There were no immature females that arrived in the first sampling period and died earlier than 5 d after arrival at the spawning channel, which may explain the lack of relationship between reproductive longevity and egg retention in this group. There was also a negative relationship between time on redd and egg retention in all maturity / sampling groups, indicating that, regardless of arrival timing or maturity status, the length of time a female spends on her redd is an important factor in spawning success.

The hypothesis that larger females have reduced egg retention was unsupported here. Instead, larger females from the first sampling group spent less time on their redds than smaller females. This finding is contrary to findings by van den Berghe and Gross (1986) who found that larger females guard their redds longer than smaller females. However, the effects of fish size are not always clear, as other studies have also failed to find links between female size and reproductive success parameters (Holtby and Healey, 1986; Quinn and McPhee, 1998; Morbey and Ydenberg, 2003). The range in female size in this study was small relative to other studies (Holtby and Healey, 1986; van den Berghe and Gross, 1989), which may have contributed to the limited number of size effects observed.

Egg retention observations in my study were consistent with those in other studies of spawning sockeye salmon (Gilhousen, 1990; Burgner, 1991; Hendry et al., 2004). Several authors have observed high levels of egg retention in association with high spawner densities (van den Berghe and Gross, 1989; Quinn et al., 2007). However, spawner density was not likely a major factor contributing to the high levels of egg retention observed in this study. First, the stocking density in the spawning channel in 2006 was within the normal historical range and similar to years with much lower levels of pre-spawning mortality (R Stitt, Weaver Creek Spawning Channel, FOC, personal communication, 2006). Second, Essington et al., (2000) did not observe density-dependent effects on egg retention in the Weaver Creek Spawning Channel. Third, experimental manipulation of Weaver Creek sockeye salmon density during spawning did not result in effects on egg retention (Appendix 1).

It is beneficial for females to live as long as possible, not only to complete spawning, but also to protect their redds from later-arriving females. Consistent with several other studies of salmonids (van den Berghe and Gross, 1989; Morbey and Ydenberg, 2003; Hendry et al., 2004), reproductive longevity was highest in females that arrived at the start of the spawning season. The maximum reproductive longevity observed in this study was 14 d, which is low but not outside the normal range of reproductive longevities observed in sockeye salmon populations. Reproductive longevity of sockeye salmon on the spawning grounds is generally variable among populations within a given year, ranging from about 5 to 26 d (Healey et al., 2003; Hendry et al., 2004; Mehranvar et al., 2004). The discrepancy between this and other studies may be an artefact of the conditions in the spawning channel or may be unique to coastal, late-run stocks of sockeye salmon.

Why some females died soon after arrival at the spawning grounds and why some failed to spawn even after spending up to two weeks in the spawning channel remain unresolved questions. It is possible that females possess physiological states related to the environmental conditions encountered during migration or on the spawning grounds that predispose them to specific behaviour strategies on the spawning grounds. High water temperatures on spawning grounds may have contributed to the observed high levels of egg retention; however environmental conditions on the spawning grounds were quite uniform so it is unlikely that these factors explain the in-season patterns of reproductive longevity. Recent physiological telemetry studies of adult Fraser River sockeye have found that osmoregulatory, stress, and energetic states of individuals predict migratory success (Cooke

et al., 2006 Young et al., 2006). Incorporating physiological assessments into spawning ground behavioural studies (e.g., Chapters 3 and 4; as suggested in Hruska et al., 2007) may help to elucidate the relative contribution of behaviour and physiology to egg retention, as well as other aspects of spawning success, in sockeye salmon.

Table 2.1: Correlations between reproductive longevity and egg retention at death by sample period / maturity groups in female sockeye salmon (*Oncorhynchus nerka*) at the Weaver Creek Spawning Channel in 2006. The probability of completely spawning as a function of reproductive longevity was determined by logistic regression for each of the sample period / maturity groups. Bold p-values indicate significant effects with Bonferroni correction (α = 0.008). * indicate significant effects at α = 0.05.

Group	Correlation between egg retention and reproductive longevity		n	Probability of Complete Spawning	
	r	p- value		χ^2	p-value
Oct. 5-6 Mature	-0.442	**<0.001**	85	14.70	**<0.001**
Oct. 5-6 Immature	-0.028	0.861	42	2.75	0.097
Oct. 13 Mature	-0.543	**<0.001**	40	3.66	0.056
Oct. 13 Immature	-0.690	**<0.001**	21	3.26	0.071
Oct. 19 Mature	-0.837	**<0.001**	30	4.39	0.036*
All Females Pooled	-0.440	**<0.001**	219	29.08	**<0.001**

Table 2.2: Correlations between time spent on redd and egg retention at death by sample period / maturity groups in female sockeye salmon (*Oncorhynchus nerka*) at the Weaver Creek Spawning Channel in 2006. The probability of completely spawning as a function of time on redd was determined by logistic regression for each of the sample period / maturity groups. Bold p-values indicate significant effects with Bonferroni correction ($\alpha = 0.008$). * indicate significant effects at $\alpha = 0.05$.

Group	Correlation			Logistic Regression	
	r	p- value	n	χ^2	p-value
Oct. 5-6 Mature	-0.691	**<0.001**	50	19.40	**<0.001**
Oct. 5-6 Immature	-0.590	**0.002**	25	10.22	**0.001**
Oct. 13 Mature	-0.759	**<0.001**	24	12.33	**<0.001**
Oct. 13 Immature	-0.648	**0.012**	14	6.13	0.016*
Oct. 19 Mature	-0.839	**<0.001**	21	0.18	0.673
All Females Pooled	-0.669	**<0.001**	135	42.24	**<0.001**
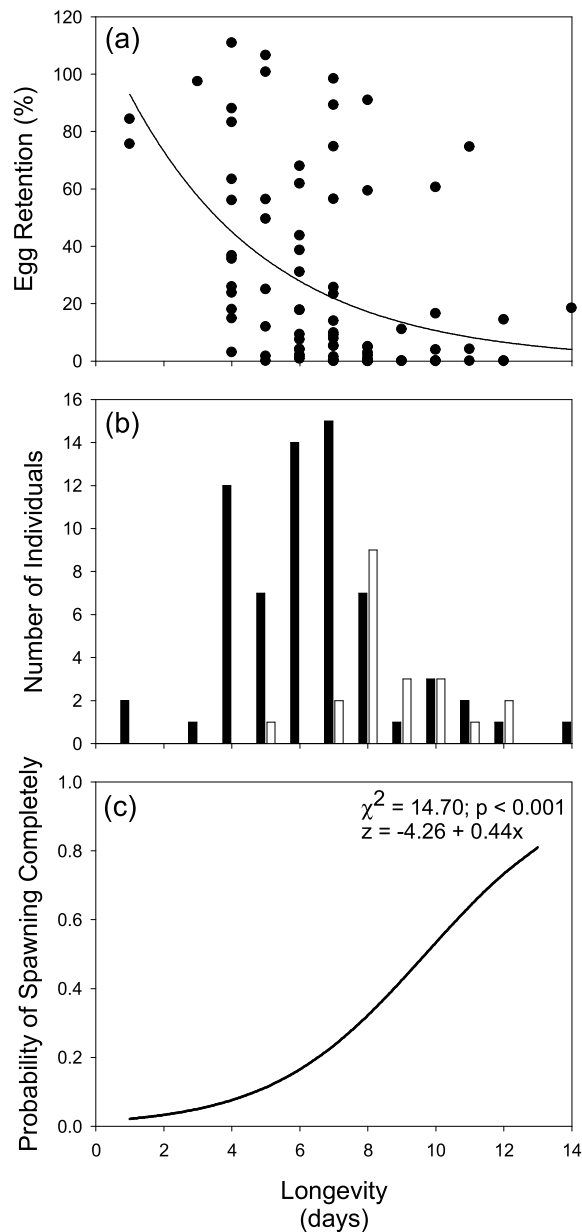
Figure 2.1: (a) Scatterplot of egg retention vs. reproductive longevity for mature female sockeye salmon (*Oncorhynchus nerka*) that were tagged at the Weaver Creek Spawning Channel on October 5-6, 2006. An exponential decay trend line is indicated on the graph. (b) Bar graph of the number of females that were classified as completely spawned (white bars) or not completely spawned (black bars) by reproductive longevity. (c) The logistic probability of females spawning completely as a function of reproductive longevity. χ^2 and p values and equation for the logit are indicated in the upper right corner of the graph.
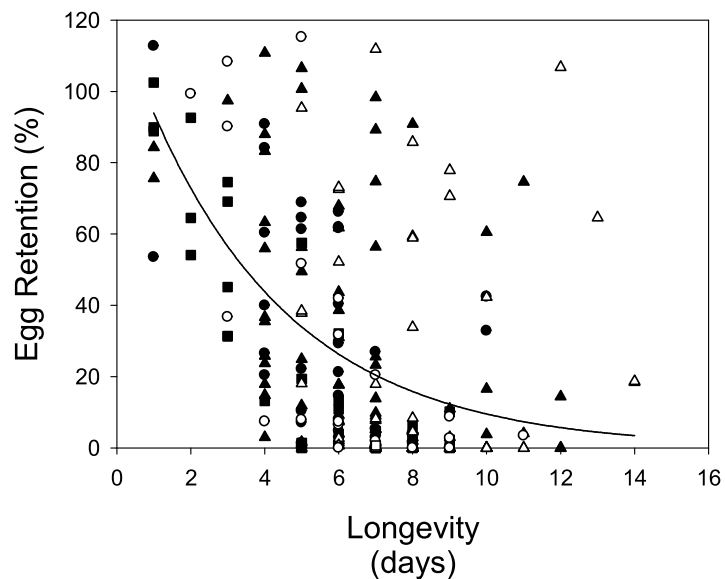
Figure 2.2: Scatterplot of egg retention vs. reproductive longevity for female sockeye salmon (*Oncorhynchus nerka*) captured at the start (Oct. 5-6; triangles), middle (Oct. 13; circles), and end (Oct. 19; squares) of the spawning season at Weaver Creek Spawning Channel in October, 2006. Females which were mature at arrival are indicated by black symbols; immature females are indicated by open symbols. An exponential decay trend line is indicated on the graph.
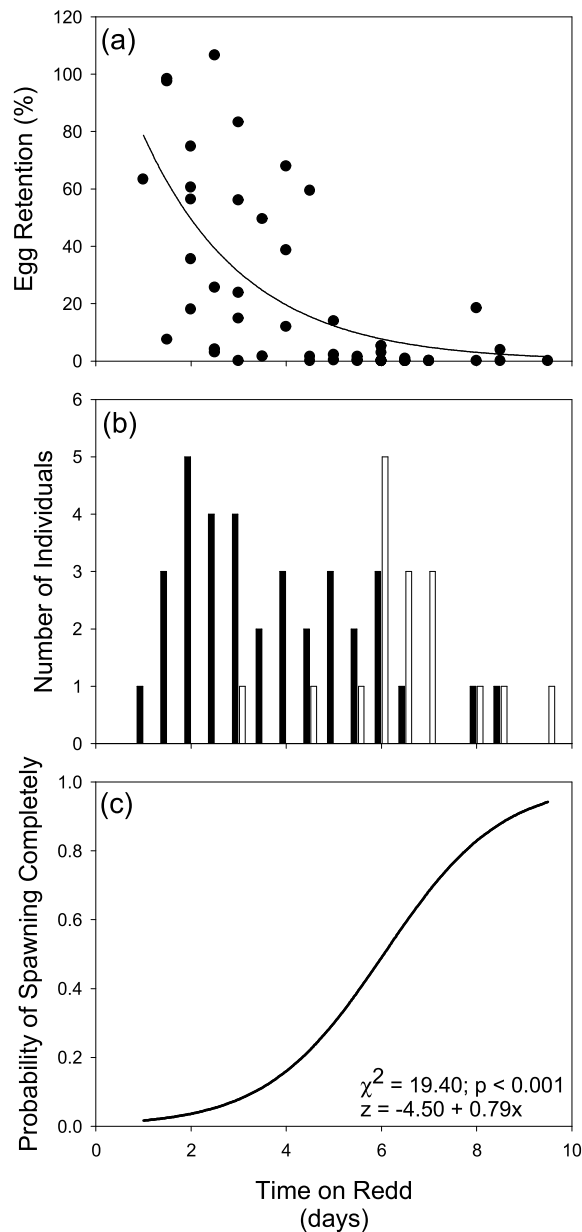
Figure 2.3: (a) Scatterplot of egg retention vs. time on redd (n = 50) for mature female sockeye salmon (*Oncorhynchus nerka*) tagged at the Weaver Creek Spawning Channel on October 5-6, 2006. An exponential decay trend line is indicated on the graph. (b) Bar graph of the number of females that were classified as completely spawned (white bars) or not completely spawned (black bars) by reproductive longevity. (c) Logistic probability of a female sockeye salmon spawning completely as a function of the time spent on the redd. χ^2 and p values and equation for the logit are indicated in the lower right corner of the graph.

Literature Cited

Blanchfield PJ and Ridgway MS. 1997. Reproductive timing and use of redd sites by lake-spawning brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 54:747-756.

Burgner RL. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). Pages 1-117 in Groot C and Margolis L (eds). Pacific salmon life histories. UBC Press, Vancouver, BC.

Cabin RJ and Mitchell RJ. 2000. To Bonferroni or not to Bonferroni: when and how are the questions. Bulletin of the Ecological Society of America 81:246-248.

Clark WK. 1959. Kodiak bear-red salmon relationships at Karluk Lake, Alaska. Transactions of the North American Wildlife Conference 24:337-345.

Cooke SJ, Crossin GT, Patterson DA, English KK, Hinch SG, Young JL, Alexander RF, Healey MC, van der Kraak G, and Farrell AP. 2005. Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behaviour and survivorship of sockeye salmon: development and validation of a technique. Journal of Fish Biology 67:1342-1358.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Healey MC, Macdonald JS, Shrimpton JM, van der Kraak G, and Farrell AP. 2006. Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. Ecology, 87:1575-1586.

Craik JCA and Harvey SM. 1984. Egg quality in rainbow trout: the relation between egg viability, selected aspects of egg composition, and time of stripping. Aquaculture 40:115-134.

Crossin GT, Hinch SG, Cooke SJ, Welch DW, Batten SD, Patterson DA, van der Kraak G, Shrimpton JM, and Farrell AP. 2007. Behaviour and physiology of sockeye salmon homing through coastal waters to a natal river. Marine Biology 152:905-918.

Crossin GT, Hinch SG, Cooke SJ, Welch DW, Lotto AG, Patterson DA, Jones SRM, Leggatt RA, Mathes MT, Shrimpton JM, van der Kraak G, and Farrell AP. 2008. Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migrations. Canadian Journal of Zoology 86:127-140.

de Gaudemar B and Beall E. 1998. Effects of overripening on spawning behaviour and reproductive success of Atlantic salmon females spawning in a controlled flow channel. Journal of Fish Biology 53:434-446.

Essington TE, Quinn TP and Ewert VE. 2000. Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences 57:205-213.

Farrell AP, Hinch SG, Cooke SJ, Patterson DA, Crossin GT, Lapointe M, and Mathes MT. 2008. Pacific salmon in hot water: applying metabolic scope models and biotelemetry to predict the success of spawning migrations. Physiological and Biochemical Zoology 81:697-708.

Foote CJ. 1990. An experimental comparison of male and female spawning territoriality in a Pacific salmon. Behaviour 115:283-314.

Fukushima M and Smoker WW. 1997. Determinants of stream life, spawning efficiency, and spawning habitat in pink salmon in the Auke Lake system, Alaska. Canadian Journal of Fisheries and Aquatic Sciences 54:96-104.

Fukushima M, Quinn TP, and Smoker WW. 1998. Estimation of eggs lost from superimposed pink salmon (*Oncorhynchus gorbuscha*) redds. Canadian Journal of Fisheries and Aquatic Sciences 55:618-625.

Gilhousen P. 1990. Prespawning mortalities of sockeye salmon in the Fraser River system and possible causal factors. International Pacific Salmon Fisheries Commission Bulletin 26.

Groot C and Margolis L. 1991. Pacific salmon life histories. UBC Press, Vancouver, BC.

Healey MC and Heard WR. 1984. Inter- and intra-population variation in the fecundity of chinook salmon (*Oncorhynchus tshawytscha*) and its relevance to life history theory. Canadian Journal of Fisheries and Aquatic Science 41:476-483.

Healey MC. 1987. The adaptive significance of age and size at maturity in female sockeye salmon (*Oncorhynchus nerka*). Pages 110-117 in Smith HD, Margolis L, and Wood CC (eds). The Sockeye Salmon. Canadian Special Publication of Fisheries and Aquatic Science 96.

Healey MC, Lake R, and Hinch SG. 2003. Energy expenditures during reproduction by sockeye salmon (*Oncorhynchus nerka*). Behaviour 140:161-182.

Hendry AP, Morbey YE, Berg OK, and Wenburg JK. 2004. Adaptive variation in senescence: reproductive lifespan in a wild salmon population. Proceedings of the Royal Society of London Series B. 271:259-266.

Hinch SG, Cooke SJ, Healey MC, and Farrell AP. 2006. Behavioural physiology of fish migrations: salmon as a model approach. Pages 240-296 in Sloman KA, Wilson RW, and Balshine S (eds). Behaviour and physiology of fish. Elsevier, Amsterdam.

Holtby LB and Healey MC. 1986. Selection for adult size in female coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 43:1946-1959.

Hruska KA, Hinch SG, Healey MC, and Farrell AP. 2007. Electromyogram telemetry, nondestructive physiological biopsy, and genetic markers: linking recent techniques with behavioral observations for the study of reproductive success in sockeye salmon mating systems. Pages 17-29 in Woody CA (ed). Sockeye salmon ecology, evolution, and management. American Fisheries Society, Bethesda, MD.

Keefer ML, Peery CA, Jepson MA, and Stuehrenberg LC. 2004. Upstream migration rates of radio-tagged adult Chinook salmon in riverine habitats of the Columbia River basin. Journal of Fish Biology 65:1126–1141.

Keefer ML, Peery CA, and Heinrich MJ. 2008. Temperature-mediated en route migration mortality and travel rates of endangered Snake River sockeye salmon. Ecology of Freshwater Fish 17:136–145.

Lee CG, Farrell AP, Lotto A, MacNutt MJ, Hinch SG, and Healey MC. 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. Journal of Experimental Biology 206:3239-3251.

Mathes MT, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Lotto AG, and Farrell AP. 2010. Effect of water temperature, timing, physiological condition, and lake thermal refugia on migrating adult Weaver Creek sockeye salmon (*Oncohrynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 67:70-84.

McPhee MV and Quinn TP. 1998. Factors affecting the duration of nest defense and reproductive lifespan of female sockeye salmon, *Oncorhynchus nerka*. Environmental Biology of Fishes 51:369-375.

Mehranvar L, Healey M, Farrell A, and Hinch S. 2004. Social versus genetic measures of reproductive success in sockeye salmon, *Oncorhynchus nerka*. Evolutionary Ecology Research 6:1167-1181.

Morbey YE and Ydenberg RC. 2003. Timing games in the reproductive phenology of female Pacific salmon (*Oncorhynchus* spp.). The American Naturalist 161:284-298.

Morbey YE and Guglielmo CG. 2006. Evidence of a novel reproductive tactic in female kokanee *Oncorhynchus nerka*. Journal of Fish Biology 69:1731-1743.

Quinn TP and Foote CJ. 1994. The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon, *Oncorhynchus nerka*. Animal Behaviour 48:751-761.

Quinn TP and McPhee MV. 1998. Effects of senescence and density on the aggression of adult female sockeye salmon. Journal of Fish Biology 52:1295-1300.

Quinn TP. 1999. Variation in Pacific salmon reproductive behaviour associated with species, sex, and levels of competition. Behaviour 136:179-204.

Quinn TP and Kinnison MT. 1999. Size-selective and sex-selective predation by brown bears on sockeye salmon. Oecologia 121:273-282.

Quinn TP Eggers DM, Clark JH, and Rich HB Jr. 2007. Density, climate, and the processes of prespawning mortality and egg retention in Pacific salmon (*Oncorhynchus* spp.). Canadian Journal of Fisheries and Aquatic Sciences 64:574-582.

Rand PS and Hinch SG. 1998. Swim speeds and energy use of upriver-migrating sockeye salmon (*Oncorhynchus nerka*): simulating metabolic power and assessing risk of energy depletion. Canadian Journal of Fisheries and Aquatic Sciences 55:1832-1841.

Reimchen TE. 2000. Some ecological and evolutionary aspects of bear-salmon interactions in coastal British Columbia. Canadian Journal of Zoology 78:448-457.

Rice WR. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rich HB Jr, Carlson SM, Chasco BE, Briggs KC, and Quinn TP. 2006. Movements of male sockeye salmon, *Oncorhynchus nerka*, on spawning grounds: effects of in-stream residency, density and body size. Animal Behaviour. 71:971-981.

Steen RP and Quinn TP. 1999. Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and selection on female body size. Canadian Journal of Zoology 77:836-841.

van den Berghe EP and Gross MR. 1986. Length of breeding life of coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Zoology 64:1482-1486.

van den Berghe EP and Gross MR 1989. Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution 43:125-140.

Wagner GN, Lotto AG, Kuchel L, Jones SRM, Patterson DA, Cooke SJ, Macdonald JS, van der Kraak G, Healey MC, Shrimpton M, English K, Hinch SG, and Farrell AP. 2005. Metabolic rates and swimming performance of adult Fraser River sockeye salmon (*Oncorhynchus nerka*) after a controlled infection with *Parvicapsula minibicornis*. Canadian Journal of Fisheries and Aquatic Sciences 62:2124–2133.

Williams IV. 1973. Investigation of the prespawning mortality of sockeye in Horsefly River and McKinley Creek in 1969. International Pacific Salmon Commission, Progress Report 27. Part II: 42 p.

Young JL, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Farrell AP, van der Kraak G, Lotto AG, Lister A, Healey MC, and English KK. 2006. Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 63:1067-1077.

Chapter 3: Influences of sex and activity level on physiological changes in individual adult sockeye salmon during rapid senescence[2]

Introduction

Semelparity, wherein an organism dies shortly after breeding once, is a life history characteristic found in taxonomic groups as divergent as insects, fishes and dasyurid marsupials (Dickhoff, 1989; Finch, 1990). Shortly after mating, semelparous animals undergo a period of rapid deteriorative change involving a loss of homeostasis, decreased ability to respond to stressors, and increased risk of disease. These physiological changes, a process often referred to as senescence, are believed to be responsible for organism mortality (Finch, 1990). While all semelparous individuals die quickly after spawning, the length of the period of senescence can have important life history consequences. For example, female Pacific salmon (*Oncorhynchus* spp.) that live longer on the spawning grounds, not only have lower levels of egg retention at death (Chapter 2), but are also able to guard their redds longer against superimposition by later arriving females (Morbey and Ydenberg, 2003; Hendry et al., 2004). Therefore, there is considerable interest in the mechanisms that govern the senescence process. This study examines individual sockeye salmon (*O. nerka*) during residence on the spawning grounds to characterize the physiological changes associated with senescence.

The behavioural physiology of migration is well studied in adult Fraser River sockeye salmon (Hinch et al., 2006; Cooke et al., 2008). Reproductive hormone and osmoregulatory indices suggest fish are preparing for entry into freshwater and spawning > 700 km from the Fraser River mouth (Crossin et al., 2009). Symptoms of immuno-suppression and disease are also becoming evident at this phase of their migration (Miller et al., 2009). As sockeye salmon get closer to the Fraser River, increases in plasma sex steroid levels advance reproductive maturity (Crossin et al., 2009) and gill function changes in preparation for freshwater entry (Shrimpton et al., 2005; Hinch et al., 2006). High plasma lactate and cortisol concentrations during the transition from saltwater to freshwater (Cooke et al., 2006a,b; Crossin et al., 2009) indicate this is a particularly stressful and active phase of their migration. Extreme freshwater conditions (e.g., high temperatures or flows) cause additional physiological stress

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and even metabolic collapse (Farrell et al., 2008; Mathes et al., 2010). During freshwater migration, plasma sex steroid levels continue to rise, further advancing sexual maturation and leading to development of secondary sexual characteristics (Truscott et al., 1986; Young et al., 2006). Fish become immuno-compromised and disease states emerge as fish approach and enter spawning areas (Wagner et al., 2005; Crossin et al., 2008; Miller et al., 2009). In all cases, sockeye were more likely to perish before reaching spawning grounds if they were more stressed, diseased, or ill-prepared for freshwater osmoregulation.

While attention has been focused on understanding the physiological basis of mortality during coastal and riverine migrations of Pacific salmon (e.g. Cooke et al., 2006a,b; Young et al., 2006; Crossin et al., 2009), the physiological changes that occur during rapid senescence on spawning grounds have received little attention. There are prominent hypotheses that deserve scrutiny as causes of rapid senescence and death: the physiological stress / cortisol hypersecretion model and the energy exhaustion model of Pacific salmon senescence. These models may not be mutually exclusive, however.

It has been proposed that senescence in Pacific salmon results from elevated cortisol levels as a result of hypersecretion (Robertson and Wexler, 1957; McBride et al., 1965; Dickhoff, 1989; Stein-Behrens and Sapolsky, 1992). Cortisol, the major glucocorticoid in fish, is released from the hypothalamus-pituitary-interrenal cascade and represents a primary stress response (Barton, 2002). Hyperadrenocorticism in maturing Pacific salmon can result from hyperplasia of the interrenal cells (Idler et al., 1959; Robertson et al., 1961), a state similar to Cushing's disease in humans, which results in hypersecretion of corticosteroids into the circulatory system (Schreck et al., 2001). High plasma cortisol levels, which have been measured in adult Pacific salmon during migration and spawning (Carruth et al., 2000) have also been linked with high levels of circulating reproductive steroids during maturation (van Overbeeke and McBride, 1971), decreased responsiveness of the negative feedback system for cortisol and reduced ability to clear cortisol from the circulation (Schreck et al., 2001). Long term elevation of plasma cortisol can lead to tissue degeneration, suppression of the immune system, and loss of homeostasis, which will eventually lead to death. Cortisol levels in semelparous salmon are typically higher than in iteroparous salmon (Barry et al., 2001).

Elevated cortisol levels have also been observed in adult Pacific salmon experiencing difficult conditions during upriver migration. Environmental stressors, such as high water

velocities, can lead to not only high plasma cortisol concentrations, but also to a loss of homeostatic balance during migration (Hinch et al., 2006; Nadeau et al, 2010). Marine and river biopsy telemetry research has found that migrating adult Pacific salmon were less likely to reach spawning grounds if fish displayed indices of ionoregulatory or metabolic stress (e.g., high plasma concentrations of Na^+, osmolality, and lactate; Cooke et al., 2006a,b; Young et al., 2006; Crossin et al., 2008). In addition, salmon that arrived at spawning grounds with relatively low levels of major plasma ions and relatively high levels of plasma lactate were more likely to die shortly after arrival (Chapter 4). However, the role of environmental stressors in the process of senescence in Pacific salmon has not been fully explored.

The energy exhaustion hypothesis, which suggests that rapid senescence and death on spawning grounds is due to dwindling energy reserves (Dickhoff, 1989), is supported in part by life history observations and measurements of gross somatic energy in migrating salmon. Pacific salmon stop eating before entering freshwater so migration, spawning, and the physiological and morphological changes associated with maturity and the transition to freshwater are all fuelled by endogenous energy reserves (Hinch et al., 2006). Measurements of whole body energy reserves in adult sockeye and pink (*O. gorbuscha*) salmon have found, across several different populations, that moribund adults on the spawning grounds all have similar gross somatic energy levels (~ 4 MJ•kg^{-1}), suggesting a common energetic threshold to support life (Crossin et al., 2003, 2004). Similarly, energy reserves at the start of spawning in sockeye salmon were positively correlated with reproductive longevity on the spawning grounds (Mehranvar, 2002). McBride et al. (1965) extended the lifespan of maturing or spawned sockeye salmon by up to 10 weeks (but not indefinitely) by force feeding the fish. In addition, large-scale marine and river biopsy telemetry programs with Pacific salmon have uncovered links between an individual's physiological state and their migration fate – females with relatively low levels of gross somatic energy tended to perish before reaching spawning areas (Cooke et al., 2006a,b; Young et al., 2006; Crossin et al., 2008).

Overlying these physiological mechanisms are individual animal behaviours, which can induce stress and increase energy exhaustion as a result of increased locomotory activities. The activity patterns of individual spawning salmon may affect the rate and degree of physiological changes during rapid senescence. For example, aggressive behaviour has been shown to affect blood physiology, such as plasma hormone (e.g., testosterone, 11-

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ketotestosterone, cortisol) concentrations, in both dominant and subordinate individuals (Cardwell et al., 1996; Gilmour et al., 2005); the winners of aggressive interactions often exhibit higher testosterone and 11-ketotestosterone concentrations (Cardwell et al., 1996; Elofsson et al., 2000). In Pacific salmon, activity levels may affect energy use and reproductive longevity of an individual (van den Berghe and Gross, 1986), however, other studies have not found support for this relationship (Foote, 1990; Hendry et al., 2001; Healey et al., 2003; Morbey and Ydenberg, 2003).

My main objective was to examine the rapid senescence phenomenon in terms of physiological stress / cortisol hypersecretion and energy exhaustion hypotheses. I also wanted to determine whether activity patterns of individual fish affect the physiological changes observed while on the spawning grounds. I biopsied sockeye salmon on arrival at the spawning grounds and when they became moribund. I observed their activity patterns and reproductive longevity during residence on the spawning grounds and extent of egg retention at death. The biopsy procedure assessed key ionoregulatory / stress indicators (e.g., plasma ions, cortisol, lactate, glucose, osmolality, gill Na^+/K^+ ATPase), energy indicators (e.g., plasma glucose, gross somatic energy), and reproductive indicators (e.g., plasma reproductive hormones).

The physiological stress / cortisol hypersecretion hypothesis predicts that fish will display elevated levels of stress metabolites (i.e., cortisol, glucose, lactate) and an imbalance of plasma ions (i.e., osmolality, Na^+, and Cl^-) when they become moribund. Furthermore, individuals with a higher level of physiological stress should die earlier after arrival at the spawning grounds. In particular, those individuals that arrive with high levels of plasma cortisol would be expected to die sooner after arrival and exhibit more pronounced changes in other physiological parameters. Individuals which are less dominant (i.e., those giving fewer attacks and receiving more attacks) should be more stressed and have a shorter period of senescence.

The energy exhaustion hypothesis predicts that spawners will die with energy reserves of less than 4 $MJ \cdot kg^{-1}$. I predict that fish that die due to energy exhaustion would exhibit hypoglycaemia (i.e., plasma glucose concentrations <4 $mmol \cdot L^{-1}$) due to an inability to mobilize energy reserves. I further predict that heightened individual activity (i.e., high frequency of aggressive encounters) would deplete energy reserves more quickly and shorten the period of senescence.

Materials and Methods

The study was carried out at the Weaver Creek Spawning Channel, which is located about 125 km east of Vancouver, British Columbia, Canada (Figure 1.2). The channel is 2.9 km long and 6 m wide, with a layer of gravel substrate of a size suitable for spawning (1.2 - 7.6 cm). The mean water depth is 25-30 cm and the mean current velocity is 0.4 m•s^{-1} (see Quinn, 1999 for a complete description of the channel). The movement of fish into the channel is controlled by manually operated gates. Fish arrival at the spawning channel occurs throughout October, peaking in the middle of the month (Essington et al., 2000).

Four enclosures (3.0 m wide by 7.5 m long) were constructed in the channel. The walls of the enclosures were constructed of wooden frames covered with Vexar$^©$ (Masternet Ltd., Mississauga, ON). Each wall was buried 35 cm into the gravel so that walls extended about 65 cm out of the gravel and approximately 35 cm above the water surface. The ends of the enclosures (perpendicular to the flow) were covered with 50 mm x 50 mm Vexar$^©$ which maximized through flow of water relative to strength, whereas, the sides were covered with 20 mm x 20 mm Vexar$^©$ to prevent fish from snagging their tags on the side. Vexar$^©$ (20 mm x 20 mm) was laid across the tops of the enclosures, overhanging the edges of the frames by 15-20 cm to prevent fish from jumping out.

On October 12 and 13, 2004, 56 adult sockeye salmon (28 females, 28 males) were individually captured by dip net from the entrance to the spawning channel and immediately placed, ventral side up in a padded V-shaped trough with a continuous supply of flow-through water from the spawning channel. A 3 ml blood sample was collected from the caudal vein (Houston, 1990) using a heparanized vacutainer (1.5 inch, 21 gauge). The blood sample was placed in an ice-water slurry for a maximum of 20 minutes, pending further processing. Pressure was applied to the puncture site to facilitate blood clotting. A small gill biopsy (tips of 5-8 gill filaments, approximately 0.03 g) was taken from the first gill arch using sharpened end-cutter pliers. The gill sample was immediately transferred to an Eppendorf tube and placed on dry ice until the samples could be transferred to a -80ºC freezer for long-term storage. This biopsy procedure has been used on adult sockeye salmon at various stages of the upriver migration (Cooke et al., 2006a,b); in these studies the salmon are not anaesthetized prior to the biopsy procedure due to the human health risks (e.g., some of these fish may be captured by First Nations fishers after we are done observing them for sale of roe or rendering) and post-release survivorship issues of releasing anaesthetized fish

back into the river. This biopsy procedure has been shown to have minimal impacts on the behaviour and success of adult sockeye salmon (Cooke et al., 2006a,b) and is in accordance with animal care protocols (protocol number A05-0424; Appendix 2). Also in this study, I chose to biopsy individuals without anaestheia in order to maintain consistency between my results and those in other studies on migrating adult sockeye salmon (e.g., Cooke et al., 2006a; Crossin et al., 2008; Mathes et al., 2010). Finally, tricaine methanesulfanate (the only anaesthetic approved in Canada for use on fish) can have significant effects on plasma cortisol, glucose, and lactate concentrations (Molinero and Gonzalez, 1995).

After tissue samples were collected, fish were anaesthetized in 60 mg•L^{-1} tricaine methanesulfanate for 120-150 s to obtain an anaesthesia level of 5 (i.e., loss of gross body movements and cessation of opercular movements) for the remaining measurements and / or for the electromyogram (EMG) surgery. Each fish was weighed to the nearest gram. Fork length was measured to the nearest 0.5 cm. Body energy reserves were assessed by a Distell model 692 Fish Fatmeter (Distell Inc., West Lothian, Scotland) following the procedures in Crossin and Hinch (2005).

Electromyogram radio transmitters (cylindrically shaped, 53 mm length, 16 mm diameter, 18.5 g mass Lotek Wireless Inc., Newmarket, ON) were randomly allocated to half the males and half the females destined for each enclosure for another study. Electromyogram transmitters were implanted in males following the procedures in Hinch et al. (1996). For females, there was concern that water would get into the body cavity during surgery, resulting in a hardening of eggs and abnormal spawning activity. Thus, in females I implanted the transmitters between the skin and musculature on the left side of the body, midway between the lateral line and the ventral midline of the body and anterior to the pelvic fins (Healey et al., 2003). The electrode positioning was the same as in the males. The surgeries to implant the transmitters took approximately 5 min. Electromyogram transmitters were used to explore individual patterns of energy use for another study (K Hruska, unpublished data).

Each fish was tagged with an individually marked Peterson disc through the dorsal musculature anterior to the dorsal fin and revived for at least 5 min in coolers (57 L) full of aerated, clean, ambient water while transported to the enclosures (<500 m). All fish restored their righting reflex before release into the enclosures. After recovery, fish were allocated to

one of two treatments in the enclosures, a high density (9 males and 9 females) and a low density treatment (5 males and 5 females); fish were allocated to the two densities for another study (Appendix 1). All fish swam vigorously on release into the enclosures.

Activity level was assessed for each fish by daily 5 min observations. The length of the observations was determined based on the work of Mehranvar et al. (2004) wherein similar observation periods were sufficient to successfully identify indices of social reproductive success and link pre-spawning energy levels with reproductive behaviour. I used similar procedures, study site, and enclosure design to study the same stock of sockeye salmon as Mehranvar et al. (2004). During each observation period, the type of interaction (see Healey et al., 2003), duration, interacting fish, and status as attacker or recipient were recorded for each behavioural interaction observed. Number of attacks given were summed for each fish and divided by the total minutes of observation for that fish to calculate the frequency of attacks given. Within each enclosure, the males and females were ranked in ascending order according to their frequencies of attacks given (i.e., 0 to 4 in the low density enclosures and 0 to 8 in the high density enclosures). To standardize the two density treatments along the same scale, the rankings of the fish from low density enclosures were multiplied by 2. The same procedure was used to rank the fish according to the number of attacks received.

As fish became moribund (defined as still ventilating but unable to hold position or remain upright), they were captured and re-sampled for blood and gill tissue. Any fish pinned against the rear wall of the enclosure and still ventilating was righted and turned into the water flow. A fish was considered moribund if it could not maintain its position in the channel or equilibrium. The biopsy procedure was repeated on all moribund fish. When fish were found showing no signs of life they were considered dead and removed from the enclosures.

Fork length, total mass and gonad mass were measured for all fish after they had died. A piece (~200 g) of dorsal musculature extending from the operculum to the dorsal insertion and down from the dorsal midline was removed from the left side of the fish for estimation of gross somatic energy reserves at death. Tissues were stored in air-tight plastic bags at -20ºC until further processing.

Reproductive longevity was calculated as the number of days between initial sample and death. Gonadosomatic index at death (GSI_D) was calculated by dividing the remaining

gonad mass by the somatic mass at death (i.e., total body mass minus gonad mass). Expected gonadosomatic index (GSI_E) was calculated based on the relationship between gonadosomatic index and somatic mass for unspawned females in this population (K Hruska, unpublished data). Egg retention was then calculated as GSI_D / GSI_E * 100%.

<u>Tissue and data analysis</u>

Dorsal muscle samples were homogenized and proximate constituent analysis performed on a sub-sample of the homogenate following procedures of Crossin et al. (2004). Blood samples were centrifuged for 5 min to separate plasma from cellular components. Three 0.5 ml samples of plasma were collected and immediately stored on dry ice until the samples could be transported to and stored in a -80°C freezer pending further processing. Plasma ion, cortisol, and osmolality were measured following the procedures described by Farrell et al. (2000). Plasma testosterone, 17β-estradiol, 17,20β-progesterone, and 11-ketotestosterone were measured by radioimmunoassay (van der Kraak and Chang, 1990; McMaster et al., 1992).

All data were reported as mean ± SEM unless otherwise indicated. All statistics were calculated using either SAS 9.1 or JMPIN 4.0.4 (SAS Institute, USA). I used α = 0.05 for all tests. Due to multiple comparisons, I applied Bonferroni corrections to minimize the chance of a Type II error (Rice, 1989). However, Bonferroni corrections are highly conservative, so I indicated effects at α = 0.05 to allow the reader to define for themselves effects that are biologically meaningful (Cabin and Mitchell, 2000).

Paired t-tests were performed for each physiological parameter to determine whether physiological status differed between arrival and the moribund state. F-tests were performed to determine whether the mean values of parameter changes differed between the sexes. Due to differences in the blood physiology between the sexes, males and females were treated separately for the remaining analyses. T-tests were used to test for effects of EMG transmitter implantation and enclosure on reproductive longevity, egg retention, and the net changes in physiology.

Plasma testosterone, chloride, cortisol, and glucose were selected to illustrate individual patterns of physiological change during senescence. I chose chloride because it is one of the major ions found in the plasma and is expected to change in response to stressors. I selected testosterone because it is a major reproductive hormone and is detected at high

concentrations in both male and female fish (McDonald and Milligan, 1992). Glucose was selected because it is an indicator of physiological stress in fish and tends to increase in response to stimulation of the sympathetico-chromaffin cell axis (Wendelaar Bonga, 1997). Cortisol was selected because it is the major stress hormone in fish which has been linked to the senescent changes in Pacific salmon and is released in response to activation of the hypothalamus-pituitary-interrenal axis (McDonald and Milligan, 1992; Wendelaar Bonga, 1997).

Pearson correlation analyses were used to explore the individual-level patterns in senescence. Specifically, all parameter values at arrival were compared with values at moribund, gross somatic energy reserves were compared with plasma ion concentrations in moribund fish, plasma cortisol concentrations at spawning ground arrival were compared with net changes in all other parameters, and activity level rankings were compared with net changes in all physiological parameters.

Results

All 56 of the study fish died while in the enclosures; 23 of these individuals (i.e., 13 females and 10 males) were observed in the moribund state and re-biopsied. All physiological parameters, with the exceptions of plasma glucose and gill Na^+/K^+-ATPase activity, exhibited a net change over the spawning life of the fish (Table 3.1). Plasma concentrations of K^+, and especially lactate and cortisol, increased in both sexes, and 17,20β-progesterone increased in males (Table 3.1). In contrast, plasma osmolality and plasma concentrations of Cl^-, Na^+, and especially testosterone, 11-ketotestosterone, and 17β-estradiol decreased significantly in both sexes; 17,20β-progesterone decreased significantly in females (Table 3.1).

Females exhibited larger decreases in ions and energy during senescence than males. For example, at arrival mean values for females were either significantly higher than (i.e., Cl^-) or not significantly different from (i.e., osmolality, Na^+, energy) male values, but moribund females had significantly lower values. Similarly, females exhibited larger decreases for all four reproductive hormones (Table 3.1), irrespective of whether they arrived with higher (i.e., testosterone, 17,20β-progesterone, 17β-estradiol) or lower (i.e., 11-ketotestosterone)

plasma concentrations than males. In fact, 17,20β-progesterone increased in males during senescence.

Reproductive longevity was similar for females and males, 5.3 ± 0.5 d and 5.2 ± 0.4 d, respectively. For all 56 salmon, reproductive longevity was 5.9 ± 0.4 d and 6.3 ± 0.3 d for females and males, respectively. Median egg retention of females was 1.8% (mean = 10.2 ± 5.7%; range = 0 to 75%); only one female retained more than 25% of eggs at death. Median GSI_D for males was 1.4% (range = 0.6 - 2.6%). The males that underwent the EMG surgery had shorter reproductive longevity (F = 20.8; p = 0.002; EMG = 4.33 ± 0.30 d; no EMG = 6.50 ± 0.37 d) and had lower plasma osmolality (F = 8.70; p = 0.019; EMG = - 24.83 ± 4.66 mmol•L^{-1}; no EMG = -3.12 ± 5.70 mmol•L^{-1}). There were no significant differences in any endpoint in females between individuals with EMG transmitters and those without at α = 0.05. There were no significant differences in reproductive longevity, egg retention, or net change in plasma physiology between high and low density enclosures for either males or females at α = 0.05. Therefore, I was able to pool results across density and transmitter groups for the remaining analysis.

Individual-level Patterns

When I compared the value of a physiological parameter in an individual fish at spawning ground arrival with the value of the same parameter when the fish was moribund I found four significant correlations for females (i.e., glucose, 17β-estradiol, 17,20β-progesterone, and gill Na$^+$/K$^+$ ATPase activity; Table 3.2). However, with the application of Bonferroni corrections only one of these correlations was still significant (females: 17β-estradiol p = 0.005). Glucose was the only parameter for which a significant negative correlation between arrival and moribund values was detected (Table 3.2; p = 0.026; r = -0.612); females that arrived at the spawning channel with relatively high plasma glucose (> ~5.5 mmol•L^{-1}) concentrations tended to exhibit a decrease in plasma glucose concentrations when they were moribund (Table 3.2).

In females, there was a positive correlation between gross somatic energy reserves and plasma glucose concentrations in the moribund state (Figure 3.1; p = 0.028; r = 0.607). Females with final energy reserves less than ~3.5 MJ•kg^{-1} had final plasma glucose concentrations less than 4 mmol•L^{-1}. All of the females with plasma glucose concentrations less than 4 mmol•L^{-1} at moribund exhibited a net decrease in plasma glucose concentration

between arrival and the moribund state (Figure 3.2). These data provide evidence for a link between low energy reserves (i.e., < 3.5 MJ•kg^{-1}) and an inability to mobilize glucose as some fish became moribund, suggesting a potential mechanism for mortality in these individuals. However, there was one female that did not conform to this pattern: the female with the highest gross somatic energy (4.05 MJ•kg^{-1}) had a low plasma glucose concentration (1.94 mmol•L^{-1}). This female was also anomalous due to her high level of egg retention at death (egg retention = 75%; range of all other females = 0 to 21%). The females that died with low gross somatic energy levels (< 3.5 MJ•kg^{-1}) also had significantly lower plasma concentrations of Cl$^-$ and Na$^+$ and osmolality in the moribund state (Figure 3.1). In moribund males, there were no significant relationships between energy reserves and plasma glucose, Cl$^-$, or Na$^+$ concentrations or plasma osmolality. Males died with significantly higher energy reserves than females.

In males, there were significant correlations between plasma cortisol concentrations at arrival and the net change in plasma lactate (r = 0.820; p = 0.004), K$^+$ (r = -0.785; p = 0.007), and 11-ketotestosterone (r = -0.762; p = 0.010) concentrations based on Bonferroni corrections (Figure 3.3). There was also a positive correlation between cortisol concentrations at arrival and the standardized ranking of the frequency of attacks received while on the spawning grounds in males (r = 0.709; p = 0.022); males with high cortisol levels at the start of spawning were the recipients of a greater frequency of attacks during their residence on the spawning grounds. There were no significant correlations between plasma cortisol concentrations at arrival and any physiological or activity measure in females.

After Bonferroni corrections a significant negative correlation existed between the frequency of attacks received by a male and the net change in 11-ketotestosterone during senescence (r = -0.785; p = 0.007). Females that participated in a greater frequency of aggressive interactions had a greater increase in plasma cortisol concentrations (r = 0.598; p = 0.040) as well as greater decreases in plasma testosterone (r = 0.630; p = 0.028) and 11-ketotestosterone (r = 0.621; p = 0.031) during senescence, but these relationships did not reach significance after Bonferroni correction.

Discussion

I observed major changes in the blood physiology of individual sockeye salmon during the senescent period between spawning ground arrival and death. In general, there were large increases in plasma indicators of stress and activity (i.e., lactate and cortisol), decreases in the major plasma ions (i.e., Cl^- and Na^+) and osmolality, and decreases in gross somatic energy reserves. Many of the physiological changes that I observed followed my predictions based on previous studies on senescence in Pacific salmon (Roberston and Wexler, 1957; Dickhoff, 1989; Finch, 1990; Stein-Behrens and Sapolsky, 1992). In addition to the expected sex differences in reproductive hormones and cortisol, I also observed differences between males and females in major plasma ions changes. The repeated sampling of blood physiology in individual fish allowed me to characterize the interplay between physiological changes and activity levels and reproductive longevity.

<u>Physiological Stress / Cortisol Hypersecretion Hypothesis</u>

The physiological stress / cortisol hypersecretion model predicts that fish will show signs of physiological stress as they senesce. This prediction is supported by my data. The sockeye salmon in my study exhibited large decreases in plasma osmolality, Na^+, and Cl^- during residence on the spawning grounds. When freshwater fish encounter a chronic stressor, plasma osmolality and major ion concentrations (i.e., Cl^-, and Na^+) typically decrease (McDonald and Milligan, 1992; Pickering and Pottinger, 1995; Ackerman et al., 2000). Substantial decreases in plasma Cl^- and Na^+ can exceed 15 mmol•L^{-1} when fish face a severe stressor in freshwater (McDonald and Robinson, 1993), although the degree of osmotic disturbance is dependent on the severity, duration, and type of stressor (McDonald and Robinson, 1993; McDonald and Milligan, 1997). In this study, the mean net change in plasma electrolyte concentrations in females was substantial (e.g., mean ΔCl^- ~ 50 mmol•L^{-1} for females); ion loss was less in males and similar to values reported for salmonids following a confinement stress (McDonald and Robinson, 1993). In moribund fish, the concentrations of the major plasma ions were well below the normal range of values typically observed in freshwater salmonids and, in some individuals, were at or below levels that are considered life-threatening. For example, plasma Cl^- concentrations lower than 90 mmol•L^{-1} can be lethal for salmonids (Wedemeyer et al., 1990); 85% of females and 10% of males in our study had plasma Cl^- concentrations <90 mmol•L^{-1} in the moribund state.

Values recorded in the moribund fish for other physiological parameters, such as lactate, cortisol, and hematocrit, were also supportive of the physiological stress hypothesis.

At spawning ground arrival, cortisol levels were elevated in both sexes, a trend which was more pronounced in females (350 ng•ml^{-1}) than in males (91 ng•ml^{-1}). Plasma cortisol concentrations in adult Pacific salmon were well above basal plasma cortisol levels in other salmonids (e.g., 5-10 ng•ml^{-1}; Pickering and Pottinger, 1989), but were similar to concentrations recorded following an acute stressor (e.g., 30-300 ng•ml^{-1}; Barton, 2002). However, my results were consistent with values reported in the literature for migrating and sexually maturing Pacific salmon both in terms of concentrations and sex differences (Fagerlund et al., 1995; Pottinger et al., 1995; Carruth et al., 2000; Patterson et al., 2004). For example, plasma concentrations of up to 639 ± 56 ng•ml^{-1} were measured in a landlocked population of kokanee salmon (*O. nerka*) during their migration to spawning grounds (Carruth et al., 2000). Cortisol levels in adult sockeye salmon have also been shown to be elevated during the transition from saltwater to freshwater (Crossin, 2008) and during difficult portions of the upriver migration (Hinch et al., 2006). The elevated cortisol concentrations at arrival were not likely due to handling stress as plasma cortisol levels would not be expected to increase until 5-10 minutes after the blood samples were taken. Kubokawa et al. (1999) showed significant effects of handling stress on cortisol at 15 minutes after the stressor; the processing of my fish was completed well before these effects might be observed. Despite the elevated levels at spawning ground arrival, plasma cortisol concentrations still exhibited large-scale increases (~300% in females; ~800% in males) in the moribund fish. The increase in plasma cortisol concentrations in moribund fish is consistent with previous studies which have shown a second peak in plasma cortisol concentrations in post-spawning individuals (Carruth et al., 2000).

I predicted that salmon with higher plasma cortisol concentrations at arrival would exhibit greater magnitudes of change in other plasma variables during residence on the spawning grounds than the salmon with relatively low plasma cortisol concentrations. This prediction was supported for three physiological variables in males; I observed correlations between initial cortisol and net changes in plasma lactate (+), K$^+$ (-), and 11-ketotestosterone (-). However, I found no correlation between plasma cortisol at arrival and the net change in plasma concentrations of any of the major ions, which was contrary to my predictions. I also found no significant correlations between cortisol and any physiological or behavioural measures in females, which was contrary to my expectations considering the very high

plasma cortisol concentrations in females and the larger degree of variability in plasma cortisol concentrations in females at arrival. These results indicate that the physiological changes associated with senescence may not be cortisol-dependent.

In summary, the physiological changes that were observed in the sockeye salmon as they underwent senescence were consistent with a physiological stress response, indicating that the fish were stressed during this period of their lives. However, I did not find evidence to support the predictions that these physiological changes were cortisol-dependent, indicating that the cortisol hypersecretion may be associated with senescence, but may not drive the physiological changes associated with this phenomenon.

Energy Exhaustion Hypothesis

Exhaustion of energy reserves has also been suggested as a factor leading to the death of Pacific salmon after spawning (Dickhoff, 1989), as these fish stop eating prior to initiating their freshwater migration (reviewed by Burgner, 1991). I expected that males would use more of their energy reserves on the spawning grounds than females because males tend to be more active (Healey et al., 2003; K Hruska, unpublished data). However, my data did not conform to these expectations. Females exhibited a greater decrease in energy reserves than males, even though males and females lived for a similar amount of time on the spawning grounds. Energy partitioned in the gonads was not measured in either of my methods for estimating energy reserves as many of the females had already ovulated or were near ovulation at spawning ground arrival, thus the energy-rich ovary tissue would not be responsible for the greater change in somatic energy reserves in females. The decrease in energy reserves in both males and females during senescence is consistent with my expectations and previous studies (Crossin et al., 2003, 2004; Hendry and Beall, 2004). However, the energy reserves remaining in moribund fish were somewhat lower, although within the range of, the approximately 4 MJ•kg^{-1} that I expected based on the results in Crossin et al. (2003, 2004).

Many of the fish in my study experienced hypoglycaemia during senescence, which would be expected if these fish had exhausted energy stores. Thus, I predicted a positive correlation between plasma glucose concentrations and gross somatic energy reserves in moribund fish. This relationship was found in females but not in males. The absence of a correlation in males may be due to the higher gross somatic energy reserves remaining in

males in the moribund state, indicating that the males in this study may not have reached their lower energetic threshold. In general, the degree of glucose mobilization in response to a stressor is linked to hepatic glycogen reserves (McDonald and Milligan, 1992). In sockeye salmon, the glycolytic pathway tends to be down-regulated during migration but is up-regulated again during spawning, indicating that glycolysis is important for fuelling activity on the spawning grounds (French et al., 1983; Miller et al., 2009). The females that had the lowest energy reserves at death also had significantly lower levels of osmolality, Cl^-, and Na^+ when they were moribund. Thus, while my results provide evidence of energy exhaustion in these individuals, the results also suggest that mortality in these females may have resulted from extremely low plasma ion concentrations. This pattern of low energy, low plasma ions, and low plasma glucose concentrations in some females suggests differing mechanisms of mortality for some of the fish.

Other Factors

My results indicated that there were multiple mechanisms of mortality in semelparous salmon. Indeed, both energy exhaustion and ionoregulatory dysfunction may be factors in the mortality of some of my sockeye salmon. There are other factors that I did not specifically examine, such as disease, parasite loads, or injury, which may be additional mechanisms of mortality in some individuals on the spawning grounds. For instance, *Saprolegnia* lesions were observed on most of the dead fish. High cortisol and testosterone levels can compromise the immune system of salmonids (Slater and Schreck, 1993; Maule et al., 1996); an immuno-compromised fish is more susceptible to diseases and parasites. Several parasites, such as the myxosporean parasite *Parvicapsula minibicornis*, have been detected in spawning and moribund salmon. *Parvicapsula minibicornis*, which is contracted when sockeye salmon enter the Fraser River, tends to develop more quickly at high temperatures (Wagner et al., 2005) and was detected at high rates in most of the moribund sockeye salmon at Weaver Creek in 2004 (K Hruska, unpublished data).

The EMG transmitter implantation had effects on the males but not on the females. The greater net decrease in plasma osmolality in males with transmitters supports my observations of differences between the sexes in physiological changes during senescence. Transmitter-implanted males had plasma osmolality values at moribund that were more similar to females than the non-transmitter males. Thus, if transmitter-implanted males were

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excluded from the analysis, greater differences between the sexes in net change in plasma osmolality would be evident.

I found evidence of a link between activity levels and physiology in sockeye salmon on the spawning grounds. In particular, males that received a lower frequency of attacks while alive on the spawning grounds had a smaller net decrease in 11-ketotestosterone than males that received more attacks. 11-ketotestosterone has been strongly associated with aggressive behaviour in males and tends to increase in the winners of aggressive interactions, potentially as a mechanism to prime the winner for the next interaction (Elofsson et al., 2000). Physiological stress can also down-regulate the plasma concentrations of reproductive hormones (Schreck et al., 2001); greater stress in the losers of aggressive interactions may also be a factor contributing to these results. It is interesting that I was able to observe a significant correlation between frequency of attacks received and 11-ketotestosterone but no correlation between frequency of attacks given and either androgen, indicating that the role of recipient in aggressive interactions had a greater effect on androgen levels than the role of attacker. In females there were significant positive correlations between both number of interactions and number of attacks given and androgen levels.

In some years and for some populations, sockeye salmon experience high levels of mortality during migration and spawning (Cooke et al., 2004). Despite the use of physiological biopsy telemetry in several studies on migrating adults (e.g., Cooke et al., 2006 a,b; Crossin et al., 2009), the physiological causes of such mortality have been difficult to ascertain because individuals are only biopsied at the start of the observation period. This is the first field study to follow the fate of individual sockeye salmon and relate mortality to changes in physiological condition and activity levels. The results of my study should provide a baseline model of the blood physiology changes that sockeye salmon undergo as they senesce and die. Pacific salmon are experiencing increasingly stressful conditions during their freshwater migration due to anthropogenic factors such as climate change, angling, and loss of thermal refugia (Farrell et al., 2008; Mathes et al., 2010). Further research is needed to determine whether migratory experience can have resulting reproductive consequences on Pacific salmon after spawning ground arrival.

Table 3.1: Mean (SEM and n) of all physiological measures from male and female sockeye salmon (*Oncorhynchus nerka*) at spawning ground arrival and in moribund state in the Weaver Creek Spawning Channel in October, 2004. F, t, and p values for paired samples t-tests, grouped by sex, are indicated. Bold p-values indicates significant effects with Bonferroni correction[1]. * indicate significant effects at α = 0.05.

Category	Parameter	Sex	Sample Period	n	Mean ± SEM	Comparison	p-value
Ions	**Osmolality** $(mmol \cdot L^{-1})$	Female	Arrival	13	291.8 ± 3.0	Sample Period: t = -6.77	**<0.001**
			Moribund	13	242.0 ± 6.1	Sex	
		Male	Arrival	10	284.7 ± 3.1	Change: F = 18.56	**<0.001**
			Moribund	10	268.5 ± 4.9	Mean: F = 3.37	0.080
	Cl⁻ $(mmol \cdot L^{-1})$	Female	Arrival	13	129.8 ± 1.1	Sample Period: t = -14.71	**<0.001**
			Moribund	13	80.7 ± 2.3	Sex	
		Male	Arrival	10	124.1 ± 0.6	Change: F = 39.56	**<0.001**
			Moribund	10	95.9 ± 2.2	Mean: F = 6.42	0.019*
	Na⁺ $(mmol \cdot L^{-1})$	Female	Arrival	13	151.7 ± 1.9	Sample Period: t = -10.22	**<0.001**
			Moribund	13	108.6 ± 2.5	Sex	
		Male	Arrival	10	151.4 ± 1.8	Change: F = 16.03	**<0.001**
			Moribund	10	129.1 ± 3.3	Mean: F = 20.57	**<0.001**
	K⁺ $(mmol \cdot L^{-1})$	Female	Arrival	13	2.01 ± 0.19	Sample Period: t = 3.75	**0.001**
			Moribund	13	2.78 ± 0.28	Sex	
		Male	Arrival	10	2.59 ± 0.20	Change: F = 2.47	0.131
			Moribund	10	4.34 ± 0.60	Mean: F = 9.01	**0.007**
	Gill Na⁺/K⁺ ATPase Activity[2]	Female	Arrival	11	2.28 ± 0.19	Sample Period: t = -1.11	0.284
			Moribund	13	2.16 ± 0.15	Sex	
		Male	Arrival	7	2.44 ± 0.22	Change: F = 0.03	0.868
			Moribund	10	2.37 ± 0.26	Mean: F = 0.56	0.467
Stress Metabolites	**Lactate** $(mmol \cdot L^{-1})$	Female	Arrival	12	1.53 ± 0.13	Sample Period: t = 14.05	**<0.001**
			Moribund	13	12.66 ± 1.16	Sex	
		Male	Arrival	10	1.26 ± 0.17	Change: F = 0.32	0.577
			Moribund	10	13.55 ± 1.21	Mean: F = 0.05	0.820
	Glucose $(mmol \cdot L^{-1})$	Female	Arrival	13	5.58 ± 0.21	Sample Period: t = -0.16	0.876
			Moribund	13	5.08 ± 1.39	Sex	
		Male	Arrival	10	4.59 ± 0.13	Change: F = 0.19	0.666
			Moribund	10	4.91 ± 0.74	Mean: F = 0.55	0.468
	Cortisol $(ng \cdot ml^{-1})$	Female	Arrival	12	350 ± 60	Sample Period: t = 9.18	**<0.001**
			Moribund	10	1287 ± 84	Sex	
		Male	Arrival	10	91 ± 16	Change: F = 4.54	0.049*
			Moribund	9	737 ± 136	Mean: F = 20.12	**<0.001**

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Category	Parameter	Sex	Sample Period	n	Mean ± SEM	Comparison	p-value
Reproductive Hormones	**Testosterone** (ng•ml[-1])	Female	Arrival	13	39.3 ± 2.7	Sample Period: t = -8.22	**<0.001**
			Moribund	13	5.6 ± 0.9	Sex	
		Male	Arrival	10	21.3 ± 1.6	Change: F = 31.92	**<0.001**
			Moribund	10	9.3 ± 1.1	Mean: F = 17.04	**<0.001**
	11-KT[3] (ng•ml[-1])	Female	Arrival	12	1.922 ± 0.092	Sample Period: t = -5.29	**<0.001**
			Moribund	13	0.424 ± 0.039	Sex	
		Male	Arrival	10	11.494 ± 0.665	Change: F = 17.28	**<0.001**
			Moribund	10	6.180 ± 0.755	Mean: F = 276.99	**<0.001**
	17,20β-P[4] (ng•ml[-1])	Female	Arrival	13	832 ± 177	Sample Period: t = 2.80	**0.010**
			Moribund	13	174 ± 34	Sex	
		Male	Arrival	10	63 ± 5	Change: F = 16.18	**<0.001**
			Moribund	10	149 ± 40	Mean: F = 11.80	**0.003**
	17β-Estradiol (ng•ml[-1])	Female	Arrival	13	2.61 ± 0.15	Sample Period: t = -6.35	**<0.001**
			Moribund	13	0.73 ± 0.17	Sex	
		Male	Arrival	9	0.61 ± 0.18	Change: F = 54.71	**<0.001**
			Moribund	10	0.34 ± 0.13	Mean: F = 32.56	**<0.001**
Other	**Energy** (MJ•kg[-1])	Female	Arrival	13	4.74 ± 0.05	Sample Period: t = 12.17	**<0.001**
			Moribund	13	3.45 ± 0.11	Sex	
		Male	Arrival	10	4.63 ± 0.09	Change: F = 7.59	**0.012**
			Moribund	10	3.78 ± 0.10	Mean: F = 1.25	0.275

[1] Bonferroni corrections: Ions α = 0.010, Stress metabolites α = 0.017, Reproductive hormones α = 0.013, Other α = 0.050
[2] Units for Gill Na^+/K^+ ATPase Activity are μmol ADP mg^{-1} protein h^{-1}
[3] 11-KT refers to 11-ketotestosterone
[4] 17,20β-P refers to 17,20β-progesterone

Table 3.2: Correlations between values at spawning ground arrival and in moribund state for physiological parameters measured in wild male and female sockeye salmon (*Oncorhynchus nerka*) at the Weaver Creek Spawning Channel in October, 2004. Sample sizes, p-values, and correlation coefficients are indicated. Bold p-values indicate significant effects with Bonferroni correction[1]. * indicate significant effects at $\alpha = 0.05$.

Category	Parameter	Females			Males		
		n	r	p-value	n	r	p-value
Ions	Osmolality (mmol•L^{-1})	13	0.372	0.211	10	0.296	0.406
	Cl$^-$ (mmol•L^{-1})	13	0.188	0.538	10	0.108	0.767
	Na$^+$ (mmol•L^{-1})	13	-0.136	0.657	10	-0.214	0.553
	K$^+$ (mmol•L^{-1})	13	0.054	0.861	10	0.284	0.426
	Gill Na$^+$/K$^+$ ATPase Activity[2]	11	0.729	0.011*	7	-0.177	0.704
Stress Metabolites	Lactate (mmol•L^{-1})	12	0.194	0.546	10	0.430	0.215
	Glucose (mmol•L^{-1})	13	-0.612	0.026*	10	-0.172	0.635
	Cortisol (ng•ml^{-1})	9	0.219	0.572	9	0.391	0.298
Reproductive Hormones	Testosterone (ng•ml^{-1})	13	0.315	0.294	10	-0.261	0.467
	11-ketotestosterone (ng•ml^{-1})	12	-0.120	0.710	10	0.001	0.997
	17β-estradiol (ng•ml^{-1})	13	0.728	**0.005**	9	0.360	0.341
	17,20β-progesterone (ng•ml^{-1})	13	0.602	0.030*	10	0.011	0.975
Other	Energy (MJ•kg^{-1})	13	0.076	0.806	10	0.428	0.217

[1] Bonferroni corrections: Ions $\alpha = 0.010$, Stress metabolites $\alpha = 0.017$, Reproductive hormones $\alpha = 0.013$, Other $\alpha = 0.050$
[2] Units for Gill Na$^+$/K$^+$ ATPase Activity are μmol ADP mg^{-1} protein h^{-1}
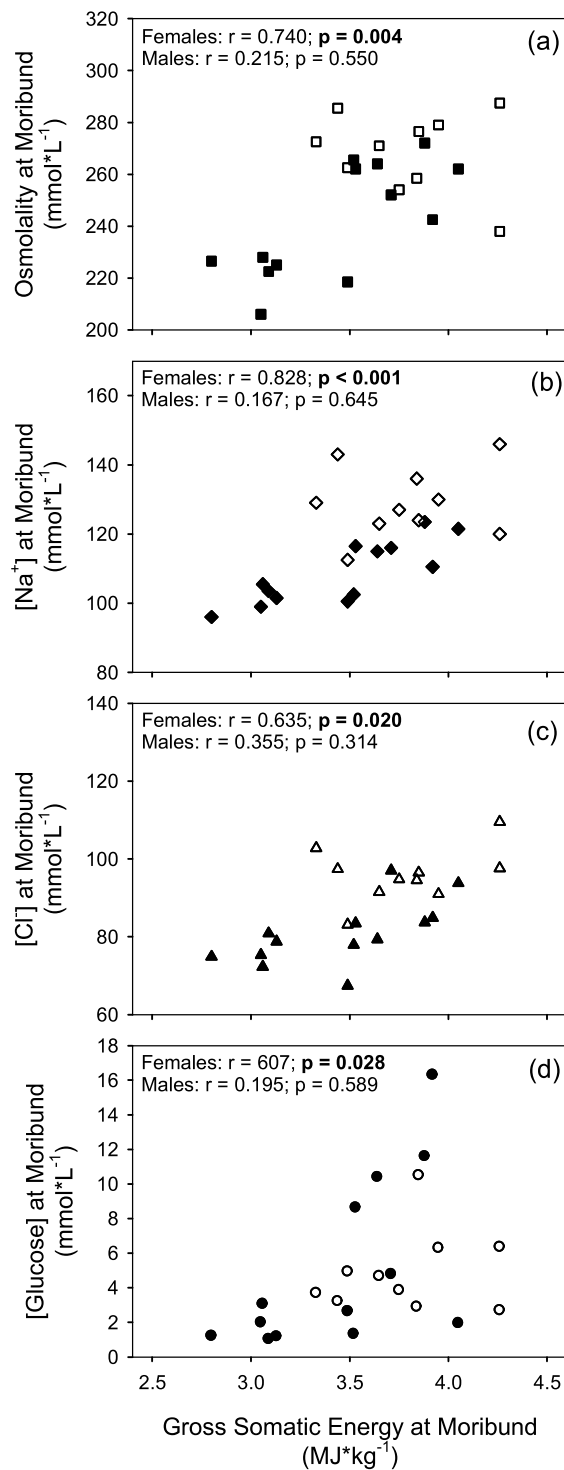
Figure 3.1: Correlations between gross somatic energy concentration and plasma osmolality (a), Na^+ (b), Cl^- (c), and glucose (d) concentrations in moribund female (black symbols) and male (open symbols) sockeye salmon (*Oncorhynchus nerka*) at the Weaver Creek Spawning Channel in October, 2004. N values can be found in Table 3.1.
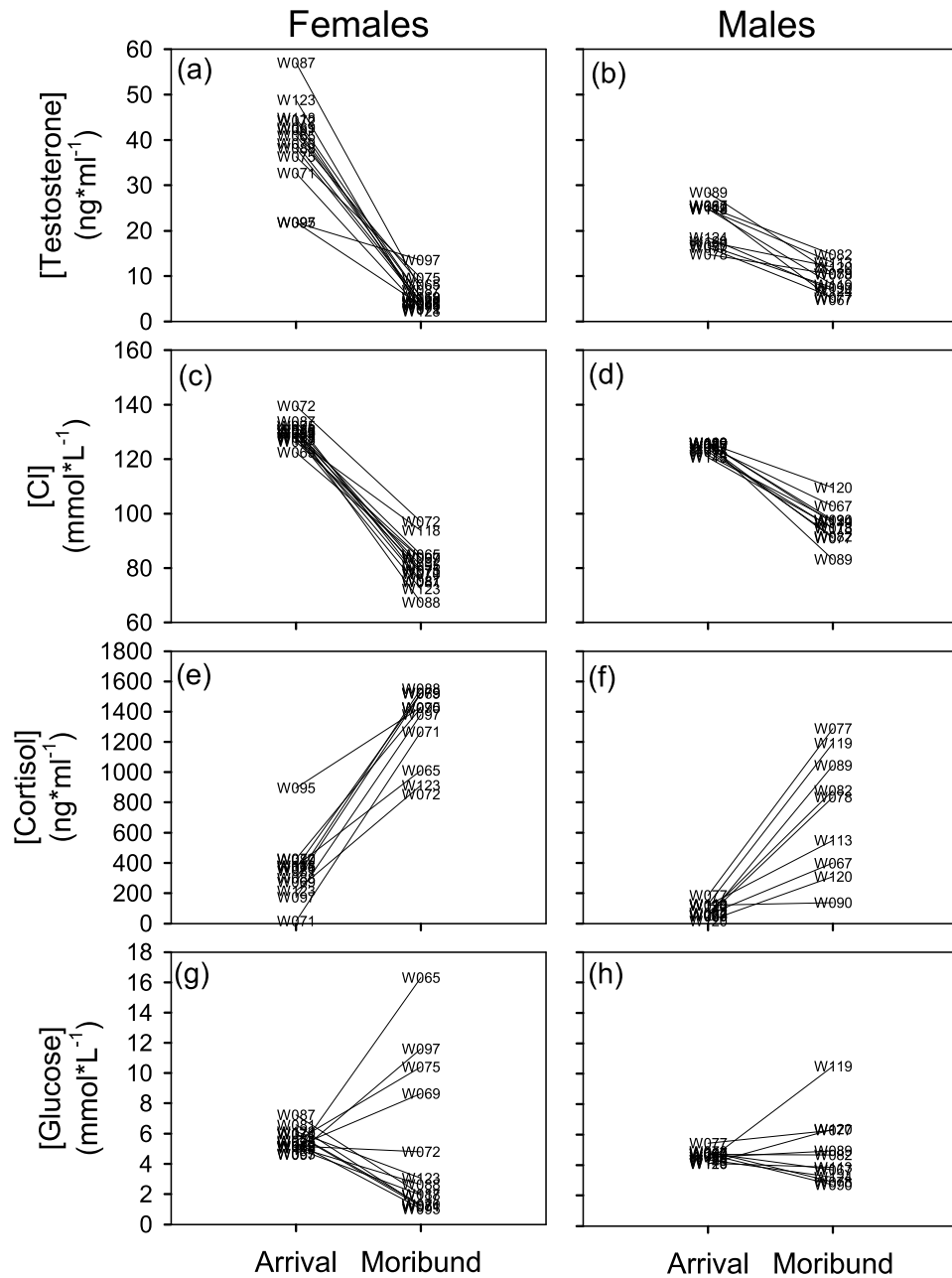
Figure 3.2: Individual patterns of blood physiology of sockeye salmon (*Oncorhynchus nerka*) during senescence. Arrival and moribund values of plasma testosterone (a-b), Cl⁻ (c-d), cortisol (e-f), and glucose (g-h) concentrations in individual females (a, c, e, and g) and males (b, d, f, and h) at the Weaver Creek Spawning Channel in October, 2004 are shown. Each individual fish is labelled with its identification number. N values can be found in Table 3.1.
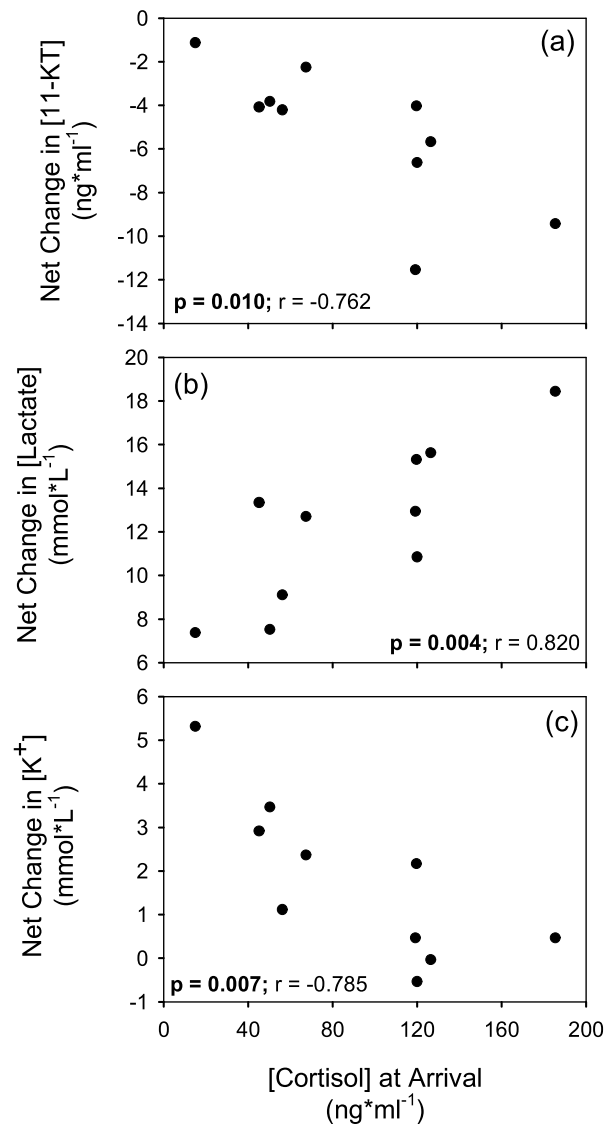
Figure 3.3: Correlations between plasma cortisol concentrations at spawning ground arrival and net changes in plasma 11-ketotestosterone (11-KT; a), lactate (b), and K^+ (c) concentrations during time on the spawning grounds in male sockeye salmon (*Oncorhynchus nerka*) at the Weaver Creek Spawning Channel in October, 2004. N values can be found in Table 3.1.

Literature Cited

Ackerman PA, Forsyth RB, Mazur CF, and Iwama GK. 2000. Stress hormones and the cellular stress response in salmonids. Fish Physiology and Biochemistry 23:327-336.

Barry TP, Unwin MJ, Malison JA, and Quinn TP. 2001. Free and total cortisol levels in semelparous and iteroparous chinook salmon. Journal of Fish Biology 59:1673-1676.

Barton BA. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517-525.

Burgner RL. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). Pages 1-117 in Groot C and Margolis L (eds). Pacific salmon life histories. UBC Press, Vancouver.

Cabin RJ and Mitchell RJ. 2000. To Bonferroni or not to Bonferroni: when and how are the questions. Bulletin of the Ecological Society of America 81:246-248.

Cardwell JR, Sorensen PW, van der Kraak GJ, and Liley NR. 1996. Effect of dominance status on sex hormone levels in laboratory and wild-spawning male trout. General and Comparative Endocrinology 101:333-341.

Carruth LL, Dores RM, Maldonado TA, Norris DO, Ruth T, and Jones RE. 2000. Elevation of plasma cortisol during the spawning migration of landlocked kokanee salmon (*Oncorhynchus nerka kennerlyi*). Comparative and Biochemical Physiology, C: Toxicology and Pharmacology 127:123-131.

Cooke SJ, Hinch SG, Farrell AP, Lapointe MF, Jones SRM, MacDonald JS, Patterson DA, Healey MC, and van der Kraak G. 2004. Abnormal migration timing and high en route mortality of sockeye salmon in the Fraser River, British Columbia. Fisheries 29:22-33.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Healey MC, Macdonald JS, Shrimpton JM, van der Kraak G, and Farrell AP. 2006a. Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. Ecology 87:1575-1586.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Shrimpton JM, van der Kraak G, and Farrell AP. 2006b. Physiology of individual Late-run Fraser River sockeye salmon (*Oncorhynchus nerka*) sampled in the ocean correlates with fate during spawning migration. Canadian Journal of Fisheries and Aquatic Sciences 63:1469-1480.

Cooke SJ, Hinch SG, Farrell AP, Patterson DA, Miller-Saunders K, Welch DW, Donaldson MR, Hanson KC, Crossin GT, Mathes MT, Lotto AG, Cooperman MS, Hruska KA, Olsson IC, Wagner GN, Thomson R, Hourston R, English KK, Larsson S, Shrimpton JM, and van der Kraak G. 2008. Developing a mechanistic understanding of fish

migrations by linking telemetry with physiology, behavior, genomics and experimental biology: an interdisciplinary case study on adult Fraser River sockeye salmon. Fisheries 33:321-338.

Crossin GT, Hinch SG, Farrell AP, Whelley MP, and Healey MC. 2003. Pink salmon (*Oncorhynchus gorbuscha*) migratory energetics: response to migratory difficulty and comparisons with sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Zoology 81:1986-1995.

Crossin GT, Hinch SG, Farrell AP, Higgs DA, Lotto AG, Oakes JD, and Healey MC. 2004. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. Journal of Fish Biology 65:788-810.

Crossin GT and Hinch SG. 2005. A nonlethal, rapid method for assessing the somatic energy content of migrating adult Pacific salmon. Transactions of the American Fisheries Society 134:184-191.

Crossin GT. 2008. Factors affecting the timing and success of sockeye salmon spawning migrations. PhD Thesis, University of British Columbia, Vancouver, BC.

Crossin GT, Hinch SG, Cooke SJ, Welch DW, Lotto AG, Patterson DA, Jones SRM, Leggatt RA, Mathes MT, Shrimpton JM, van der Kraak G, and Farrell AP. 2008. Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migrations. Canadian Journal of Zoology 86:127-140.

Crossin GT, Hinch SG, Welch DW, Cooke SJ, Patterson DA, Hills JA, Zohar Y, Klenke U, Jacobs MC, Pon LB, Winchell PM, and Farrell AP. 2009. Physiological profiles of sockeye salmon in the Northeastern Pacific Ocean and the effects of exogenous GnRH and testosterone on rates of homeward migration. Marine and Freshwater Behaviour and Physiology 42:89-108.

Dickhoff WW. 1989. Salmonids and annual fishes: death after sex. Pages 253-266 in Schreibman MP and Scanes CG (eds). Development, Maturation, and Senescence of Neuroendocrine Systems: A Comparative Approach. Academic Press, New York.

Elofsson UOE, Mayer I, Damsgård B, and Winberg S. 2000. Intermale competition in sexually mature Arctic charr: effects on brain monoamines, endocrine stress responses, sex hormone levels, and behavior. General and Comparative Endocrinology 118:450-460.

Essington TE, Quinn TP, and Ewert VE. 2000. Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences 57:205-213.

Fagerlund UHM, McBride JR, and Williams IV. 1995. Stress and tolerance. Pages 461-503 in Groot C, Margolis L, and Clarke WC (eds). Physiological Ecology of Pacific Salmon. UBC Press, Vancouver, BC.

Farrell AP, Gallaugher P, Clarke C, DeLury N, Kreiberg H, Parkhouse W, and Routledge R. 2000. Physiological status of coho salmon (*Oncorhynchus kisutch*) captured in commercial nonretention fisheries. Canadian Journal of Fisheries and Aquatic Sciences 57:1668-1678.

Farrell AP, Hinch SG, Cooke SJ, Patterson DA, Crossin GT, Lapointe M, and Mathes MT. 2008. Pacific salmon in hot water: applying metabolic scope models and biotemetry to predict the success of spawning migrations. Physiological and Biochemical Zoology 81:697-708.

Finch CE. 1990. Longevity, Senescence, and the Genome. University of Chicago Press, Chicago, USA.

Foote CJ. 1990. An experimental comparison of males and female spawning territoriality in a Pacific salmon. Behaviour 115:283-314.

French CJ, Hochachka PW, and Mommsen TP. 1983. Metabolic organization of liver during spawning migration of sockeye salmon. American Journal of Physiology – Regulatory, Integrative, Comparative Physiology 245:R827-R830.

Gilmour KM, Wilson RW, and Sloman KA. 2005. The integration of behaviour into comparative physiology. Physiological and Biochemical Zoology 78:669-678.

Healey MC, Lake R, and Hinch SG. 2003. Energy expenditures during reproduction by sockeye salmon (*Oncorhynchus nerka*). Behaviour 140:161-182.

Hendry AP, Berg OK, and Quinn TP. 2001. Breeding location choice in salmon: causes (habitat, competition, body size, energy stores) and consequences (life span, energy stores). Oikos. 93:407-418.

Hendry AP and Beall E. 2004. Energy use in spawning Atlantic salmon. Ecology of Freshwater Fish. 13:185-196.

Hendry AP, Morbey YE, Berg OK, and Wenburg JK. 2004. Adaptive variation in senescence: reproductive lifespan in a wild salmon population. Proceedings of the Royal Society of London Series B. 271:259-266.

Hinch SG, Diewert RE, Lissimore TJ, Prince AMJ, Healey MC, and Henderson MA. 1996. Use of electromyogram telemetry to assess difficult passage areas for river migrating adult sockeye salmon. Transactions of the American Fisheries Society 125:253-260.

Hinch SG, Cooke SJ, Healey MC, and Farrell AP. 2006. Behavioural physiology of fish migrations: salmon as a model approach. Pages 240-296 in Sloman KA, Wilson RW, and Balshine S (eds). Behaviour and physiology of fish. Elsevier, Amsterdam.

Houston AH. 1990. Blood and circulation. Pages 273-334 in Schreck CB and Moyle PB (eds). Methods for Fish Biology. American Fisheries Society, Bethesda.

Idler DR, Ronald AP, and Schmidt PJ. 1959. Biochemical studies on sockeye salmon during spawning migration. 7. Steroid hormones in plasma. Canadian Journal of Biochemistry and Physiology 37:1227-1238.

Kubokawa K, Watanabe T, Yoshioka M, and Iwata M. 1999. Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breeding season. Aquaculture 172:335-349.

Mathes MT, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Lotto AG, and Farrell AP. 2010. Effect of water temperature, timing, physiological condition and lake thermal refugia on migrating adult Weaver Creek sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 67:70-84.

Maule AG, Schrock R, Slater C, Fitzpatrick MS, and Schreck CB. 1996. Immune and endocrine responses of adult chinook salmon during freshwater immigration and sexual maturation. Fish and Shellfish Immunology 6:221-233.

McBride JR, Fagerlund UHM, Smith M, and Tomlinson N. 1965. Post-spawning death of Pacific salmon: Sockeye salmon (*Oncorhynchus nerka*) maturing and spawning in captivity. Journal of the Fisheries Research Board of Canada 22:775-782.

McDonald DG and Milligan CL. 1992. Chemical properties of blood. Pages 55-133 in Hoar WS, Randall DJ, and Farrell AP (eds). The Cardiovascular System. Academic Press, San Diego, CA.

McDonald DG and Robinson JG. 1993. Physiological responses to stress in lake trout: effects of water hardness and genotype. Transactions of the American Fisheries Society 122:1146-1155.

McDonald G and Milligan L. 1997. Ionic, osmotic, and acid-base regulation in stress. Pages 119-144 in Iwama GK (ed). Fish Stress and Health in Aquaculture. Cambridge University Press, Cambridge.

McMaster M, Munkittrick K, and van der Kraak G. 1992. Protocol for measuring circulating levels of gonadal sex steroids in fish. Canadian Technical Report for Fisheries and Aquatic Sciences 1836:1-29.

Mehranvar L. 2002. A genetic analysis of the mating system of sockeye salmon. MSc Thesis, University of British Columbia, Vancouver.

Mehranvar L, Healey M, Farrell A, and Hinch S. 2004. Social versus genetic measures of reproductive success in sockeye salmon, *Oncorhynchus nerka*. Evolutionary Ecology Research 6:1167-1181.

Miller KM, Schulze AD, Ginther N, Li S, Patterson DA, Farrell AP, and Hinch SG. 2009. Salmon spawning migration: Metabolic shifts and environmental triggers. Comparative Biochemistry and Physiology, D Genomics and Proteomics 4:75-89.

Molinero A and Gonzalez J. 1995. Comparative effects of MS222 and 2-phenoxyethanol on gilthead sea bream (*Sparus aurata* L.) during confinement. Comparative Biochemistry and Physiology, A Comparative Physiology 111:405-414.

Morbey YE and Ydenberg RC. 2003. Timing games in the reproductive phenology of female Pacific salmon (*Oncorhynchus* spp.). The American Naturalist 161:284-298.

Nadeau PS, Hinch SG, Hruska KA, Pon LB, and Patterson DA. 2010. The effects of experimental energy depletion on the physiological condition and survival of adult sockeye salmon (*Oncorhynchus nerka*) during spawning migration. Environmental Biology of Fish 88:241-251.

Patterson DA, Macdonald JS, Hinch SG, Healey MC, and Farrell AP. 2004. The effect of exercise and captivity on energy partitioning, reproductive maturation, and fertilization success in adult sockeye salmon. Journal of Fish Biology 64:1039-1059.

Pickering AD and Pottinger TG. 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiology and Biochemistry 7:253-258.

Pickering AD and Pottinger TG. 1995. Biochemical effects of stress. Pages 349-379 in Hochachka PW and Mommsen TP (eds). Biochemistry and Molecular Biology of Fishes. Vol. 5. Environmental and Ecological Biochemistry. Elsevier, Amsterdam.

Pottinger TG, Balm PHM, and Pickering AD. 1995. Sexual maturity modifies the responsiveness of the pituitary-interrenal axis to stress in male rainbow trout. General and Comparative Endocrinology 98:311-320.

Quinn TP. 1999. Variation in Pacific salmon reproductive behaviour associated with species, sex, and levels of competition. Behaviour 136:179-204.

Rice WR. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Robertson OH and Wexler BC. 1957. Pituitary degeneration and adrenal tissue hyperplasia in spawning Pacific salmon. Science 125:1295-1296.

Robertson OH, Wexler BC, and Miller BF. 1961. Degenerative changes in the cardiovascular system of the spawning Pacific salmon (*Oncorhynchus tshawytscha*). Circulation Research 9:826-834.

Schreck CB, Contreras-Sanchez W, and Fitzpatrick MS. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197:3-24.

Shrimpton JM, Patterson DA, Richards JG, Cooke SJ, Schulte PM, Hinch SG, and Farrell AP. 2005. Ionoregulatory changes in different populations of maturing sockeye salmon (*Oncorhynchus nerka*) during ocean and river migration. Journal of Experimental Biology 208:4069-4078.

Slater CH and Schreck CB. 1993. Testosterone alters the immune response of chinook salmon, *Oncorhynchus tshawytscha*. General and Comparative Endocrinology 89:291-298.

Stein-Behrens BA and Saplosky RM. 1992. Stress, glucocorticoids, and aging. Aging Clinical and Experimental Research 4:197–210.

Truscott B, Idler DR, So YP, and Walsh JM. 1986. Maturational steroids and gonadotropin in upstream migratory sockeye salmon. General and Comparative Endocrinology 62:99-110.

van den Berghe EP and Gross MR. 1986. Length of breeding life of coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Zoology 64:1482-1486.

van der Kraak G and Chang J. 1990. Arachidonic acid stimulates steroidogenesis in goldfish preovulatory ovarian follicles. General and Comparative Endocrinology 77:221–228.

van Overbeeke AP and McBride JR. 1971. Histological effects of 11-ketotestosterone, 17α-methyltestosterone, estradiol cyprionate, and cortisol on the interrenal tissue, thyroid gland, and pituitary gland of gonadectomized sockeye salmon (*Oncorhynchus nerka*). Journal of the Fisheries Research Board of Canada 28:477-484.

Wagner GN, Lotto AG, Kuchel L, Jones SRM, Patterson DA, Cooke SJ, Macdonald JS, van der Kraak G, Healey MC, Shrimpton M, English K, Hinch SG, and Farrell AP. 2005. Metabolic rates and swimming performance of adult Fraser River sockeye salmon (*Oncorhynchus nerka*) after a controlled infection with *Parvicapsula minibicornis*. Canadian Journal of Fisheries and Aquatic Sciences 62:2124–2133.

Wedemeyer GA, Barton BA, and McLeay DJ. 1990. Stress and acclimation. Pages 451-489 in Schreck CB and Moyle PB (eds). Methods for Fish Biology, American Fisheries Society, Bethesda, MD.

Wendelaar Bonga SE. 1997. The stress response in fish. Physiological Reviews 77:591-625.

Young JL, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Farrell AP, van der Kraak G, Lotto AG, Lister A, Healey MC, and English KK. 2006. Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 63:1067-1077.

Chapter 4: Blood physiology as a predictor of reproductive longevity and egg retention in female sockeye salmon (*Oncorhynchus nerka*)[3]

Introduction

Egg retention, wherein females fail to completely spawn (i.e., unable to completely extrude all eggs from the body cavity during spawning), has been observed across a range of salmon species (reviewed by Groot and Margolis, 1991; de Gaudemar and Beall, 1998). Prevalence of egg retention is generally low (<5% pre-spawning mortality). However, in some years prevalence of egg retention can be quite high (>25% pre-spawning mortality), resulting in the loss of millions of unspawned eggs (Gilhousen, 1990; Fukushima and Smoker, 1997; Quinn et al., 2007). In a previous study (Chapter 2) I found that egg retention was correlated to reproductive longevity in sockeye salmon (*Oncorhynchus nerka*); females that died earlier after arrival retained a higher proportion of their eggs at death. However, why some fish live longer on spawning grounds than others is still unclear. By evaluating individual-level characteristics associated with reproductive longevity and egg retention, it may be possible to elucidate why some individuals succeed while others do not.

Previous studies have found putative links between environmental conditions encountered during migration / spawning and egg retention. For example, Quinn et al. (2007) found a positive correlation between egg retention (23% and 44% of potential egg deposition lost from the population) and spawner density in sockeye salmon (*Oncorhynchus nerka*) from the Alagnak River, Alaska. Gilhousen (1990) evaluated factors correlated with pre-spawning mortality (i.e., 'death prior to complete extrusion of the eggs') across stocks of sockeye salmon in the Fraser River watershed; high levels of pre-spawning mortality were correlated with high water temperatures during migration and / or spawning and high incidence of disease. These studies provided valuable insight into the environmental conditions that may be associated with egg retention; however, the physiological and behavioural mechanisms associated with this phenomenon are still unknown.

Recent studies on Fraser River sockeye that combine physiological biopsies with telemetry have revealed strong links between physiological condition and survival during spawning migration (Cooke et al., 2006a,b, 2008; Young et al., 2006; Crossin et al., 2008). Fish that

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[3] A version of this chapter will be submitted for publication. Hruska KA, Hinch SG, Healey MC, Patterson DA, and Farrell AP. 2010. Blood physiology as a predictor of reproductive longevity and egg retention in female sockeye salmon.

perished during river migration and, hence, died earlier than those that reached spawning grounds, exhibited signs of physiological stress and ionoregulatory imbalance (e.g., had high plasma concentrations of Na^+, osmolality, and lactate), were reproductively advanced (e.g., had high concentrations of plasma 17β-estradiol or testosterone), were migrating inefficiently (e.g., had high plasma lactate), and / or had low somatic energy reserves when they were tagged either at river entry or at other locales during their river migration (Cooke et al., 2006a,b; Young et al., 2006; Crossin et al., 2008; Mathes et al. 2010). The implication from this previous work is that physiological condition affects when and where adults perish. It is unknown whether the physiological processes responsible for migration mortality are the same as, or related to, those responsible for when fish die on spawning grounds.

During the freshwater migration, adult Pacific salmon begin to senesce, culminating in rapid deteriorative changes involving a loss of homeostasis (Chapter 3). Physiological changes, which may reflect the early stages of senescence, have been observed prior to freshwater entry. For example, symptoms of immuno-suppression and disease are already evident in sockeye salmon captured > 700 km from the Fraser River mouth (Miller et al., 2009). As sockeye salmon get closer to the Fraser River, increases in plasma sex steroid levels advance reproductive maturity (Crossin et al., 2009) and gill function changes in preparation for freshwater entry (Shrimpton et al., 2005; Hinch et al., 2006). Differences among individuals in their migration experience could lead to individual variability in rate of senescence and hence reproductive longevity or success. For example, fish that encounter high flows during migration can lose homeostatic balance and experience reduced energy reserves (Hinch et al., 2006; Nadeau et al., 2010). Moreover, high encountered flows during the migration can lead to higher physiological stress (Nadeau et al., 2010) and stress has been shown to depress the rate of reproductive maturity (Schreck et al., 2001). Laboratory studies have demonstrated that levels of stress responses and swimming and cardiac performance vary among Pacific salmon populations (Lee et al., 2003; Nadeau et al., 2010; E Eliason, University of British Columbia, unpublished data). Thus, whether related to genotypic and / or environmental effects, individuals arrive on spawning grounds in different physiological states (Chapter 3) and these differences could drive variability among fish in reproductive longevity and egg retention.

How physiological state at spawning ground arrival affects reproductive longevity and egg retention is the focus of this research. As fish rapidly senesce on spawning grounds, physiological stress increases and ionoregulation begins to fail (Chapter 3). Thus, I would

predict that fish that arrive on spawning grounds with high levels of physiological stress (e.g., high levels of plasma cortisol, glucose, and lactate) and homeostatic imbalance (e.g., low plasma osmolality) may die shortly after arrival and, thus, retain more eggs at death. The degree of reproductive maturity at arrival on spawning grounds can influence an individual's spawning success. Following ovulation, female Atlantic salmon (*Salmo salar*) had greater egg retention and lower fertilization success the longer spawning was delayed (de Gaudemar and Beall, 1998). Therefore, I would predict that females with lower plasma concentrations of 17β-estradiol, testosterone, and 17,20β-progesterone (indicators of more advanced reproductive maturity – Truscott et al., 1986; see Chapter 3) would start spawning sooner, have shorter reproductive longevity, and have higher egg retention at death. Lastly, because Pacific salmon stop eating before leaving the marine environment and must complete migration, develop gonads and spawn solely using energy reserves (Burgner, 1991), fish that arrive at spawning grounds with low energy reserves and / or deplete their energy reserves quickly after arrival may live only a short period on spawning grounds. Indeed, energy limitation has been associated with rates of senescence in Pacific salmon (Dickhoff, 1989; see Chapter 3). If energy reserves are a primary limiting factor then I would predict that all females would die with similarly low energy levels, regardless of reproductive longevity or levels of egg retention.

The pattern and intensity of an individual's behaviour can influence physiological stress responses (Sloman et al., 2004) and energy expenditure (Healey et al., 2003). In Pacific salmon, activity levels have been shown to influence reproductive longevity (van den Berghe and Gross, 1986); however, other authors have not found relationships between activity levels and either energy use or reproductive longevity (Foote, 1990; Hendry et al., 2001; Healey et al., 2003; Morbey and Ydenberg, 2003). The type and frequency of aggressive activity also varies with the reproductive status of an individual (Healey and Prince, 1998; Hendry et al., 2001; Healey et al., 2003), thus a characterization of the spawning status of individual females was considered integral to this study.

The purpose of this study was to assess how physiological state at spawning ground arrival related to reproductive longevity and egg retention. I biopsied female sockeye salmon as they arrived at the spawning grounds and obtained information on key ionoregulatory and stress indicators (e.g., plasma ions, cortisol, lactate, and glucose concentrations, and hematocrit), energy indicators (plasma glucose concentrations and tissue energy content), and reproductive maturation indicators (e.g., plasma hormones). I examined the strength

and direction of the numerical relationships between physiological measures and reproductive longevity and egg retention and used this information to assess the relative importance of ionoregulatory / stress, reproductive maturation and energy limitation factors as determinants of reproductive longevity and egg retention.

Methods

The study was carried out in the fall of 2006 at the Weaver Creek Spawning Channel. See Chapter 2 for a complete description of the spawning channel. In 2006, the spawning channel was opened on October 5 and spawning took place until the first week of November. To assess overall and seasonal changes in reproductive longevity and pre-spawning mortality I tagged (details below) and released 250 female sockeye salmon during three sampling periods (Oct. 5-6, Oct. 13, and Oct. 19), which spanned the main period of salmon entry into the channel.

Salmon were collected for tagging from the holding pool below the entrance to the spawning channel (Figure 1.2). Fish were captured by dip net and immediately placed, ventral side up, in a flow-through trough for processing. I used all fish that were captured, regardless of condition, so that the data were representative of the population at arrival. I assumed that all fish in the holding pool had an equal likelihood of entering the spawning channel when the gates were open. Fish were bio-sampled (i.e., 3 ml blood sample and 0.03 g gill biopsy; 30-90 s duration), measured (fork length, snout length, POH length, and body depth) and tagged with individually marked Peterson discs. This bio-sampling procedure has been validated and used in several recent adult sockeye studies and has minimal impacts on the survival and behaviour of the fish (Cooke et al., 2008; Crossin et al., 2008). The belly of each female was palpated to determine maturity: a female was considered mature if the eggs felt loose within the abdomen and / or eggs were extruded from the vent with the application of gentle pressure; a female was considered immature if the eggs felt tight within the abdomen and eggs were not extruded with the application of gentle pressure. Each fish was released into the spawning channel immediately after processing. Total handling time from capture to release in seconds was recorded for each fish. Handling procedures were completed in accordance with animal care protocols (protocol number A05-0424; Appendix

2). On October 5, 2009, ten additional females were tagged and released with no biopsy sampling as a control for the bio-sampling technique.

Both sides of the spawning channel were surveyed daily to map the locations of tagged individuals in the channel. Each fish was observed for approximately 20-30 s to assess the status of redd establishment and spawning activity. For each female, I calculated two behavioural indices, time to establish redd and time on redd following the procedures described in Chapter 2.

Dead fish were collected daily. Fork length, total mass and gonad mass were measured for all fish. A piece of dorsal musculature extending from the operculum to the dorsal insertion and down from the dorsal midline was removed from the left side of the fish for estimation of gross somatic energy reserves at death. This tissue was stored in an air-tight plastic bag at -20°C until further processing. Reproductive longevity and egg retention were calculated for each fish following the procedures in Chapter 2. Eleven of the 250 tagged females were never recovered from the channel and were excluded from reproductive longevity calculations. There was evidence of scavenging by gulls (*Larus* spp.) on eight additional females so these fish were excluded from egg retention analyses due to the potential bias in final egg retention values.

The pieces of dorsal muscle were homogenized and proximate constituent analysis performed on a sub-sample of tissue homogenate following procedures of Crossin et al. (2004) to determine gross somatic energy. Blood samples were spun in a centrifuge for 5 min to separate plasma from the cellular components. Three 0.5 ml samples of plasma were collected and immediately stored on dry ice till the samples could be transported to and stored in a -80°C freezer pending further processing. Plasma ions, cortisol, and osmolality were measured following the procedures described by Farrell et al. (2000). Testosterone, 17β-estradiol, 17,20β-progesterone, and 11-ketotestosterone were measured by radioimmunoassay (van der Kraak and Chang, 1990; McMaster et al., 1992).

Plasma ion, lactate, and glucose concentrations and osmolality were measured for all fish. Plasma concentrations of testosterone, 17β-estradiol, 17,20β-progesterone, cortisol, and K^+ concentrations and gross somatic energy were measured in 60 of the females; these females were chosen to include females that were short-lived (lived 2 to 4 d), medium-lived (lived 7 to 8 d), or long-lived (lived 11 to 14 d) and had low egg retention (<25% egg

retention), medium egg retention (25 to 75% egg retention), or high egg retention (>75% egg retention) at death. The medium-lived category was chosen to include females that lived the median reproductive longevity in the spawning channel. The short-lived category was chosen to include 15% of females with the shortest reproductive longevity in the spawning channel, while excluding females that died within the first day in the spawning channel due to the potential influence of handling effects on these females. The long-lived category was chosen to include the 5% of females with the longest reproductive longevity; a lower percentage of females were selected for the long-lived category due to the positive skew of the data. The egg retention categories were chosen to align with management categorization of pre-spawning mortality in Fraser River salmon populations (Gilhousen, 1990).

All statistics were performed using either SAS 9.1 or JMPIN 4.0.4 (SAS Institute, USA). Effects were considered significant at $\alpha = 0.05$. Values are given as mean $\pm$ SEM unless otherwise indicated. Due to multiple comparisons, I applied Bonferroni corrections to minimize the chance of a Type II error (Rice, 1989). However, Bonferroni corrections are highly conservative, so I indicated effects at $\alpha = 0.05$ to allow the reader to define for him/herself effects that are biologically meaningful (Cabin and Mitchell, 2000). Residuals for each variable were assessed for normality. Physiological variables were transformed to achieve normality: plasma lactate, Cl^-, Na^+, and osmolality were square root transformed; plasma glucose was fourth root transformed; plasma 17β-estradiol was fifth root transformed. Egg retention could not be normalized due to the large number of zero values in the dataset; similarly, time to redd data could not be normalized due to the large number of unity values.

ANOVAs were used to test for differences in physiological and morphological parameters among sampling periods, reproductive longevity categories and spawning success categories. Linear regressions were used to examine the influence of individual physiological parameters on reproductive longevity and egg retention. Reproductive longevity, sample period, and fork length were included as covariates in the regressions on egg retention

Results

The blood chemistry of females arriving at the spawning channel differed with arrival time. Osmolality was higher in females that arrived during the start (i.e., Oct. 5-6, 2006) and middle (i.e., Oct. 13, 2006) of the spawning season relative to the latest arriving (i.e., Oct. 19, 2006) females (Table 4.1; F = 22.91; p < 0.001). Plasma Na^+ and Cl^- concentrations were higher and plasma lactate concentrations were lower in the first arrival group compared to the middle and late arrival groups. Testosterone and 17,20β-progesterone were higher in the first arrival group relative to the second arrival group. Females that arrived in the first sampling session were shorter in length than later-arriving females (Table 4.1).

Medium-lived (died within 7 to 8 d of arrival) and long-lived (died within 11 to 14 d of arrival) females died with lower tissue energy reserves than short-lived females (died within 2 to 4 d of arrival) at the spawning grounds (F = 4.83; p = 0.012; Table 4.2). Similarly, low egg retention females died with lower energy reserves than high egg retention females (F = 6.18; p = 0.004; Table 4.3).

Short-lived females had lower levels of plasma testosterone (F = 11.53; p < 0.001) and 17β-estradiol (F = 4.64; p = 0.014) than medium-lived and long-lived females (Table 4.2; Figure 4.1). In addition, short-lived and medium-lived females had lower plasma 17,20β-progesterone concentrations at arrival than the long-lived females (Table 4.2; Figure 4.1; F = 7.93; p = 0.001). However, there was no relationship between plasma reproductive hormone concentrations at arrival and either egg retention (Table 4.3) or the time to establish a redd (at α= 0.05).

Significant regressions were evident (all p < 0.001) for reproductive longevity with each of the plasma ions and metabolites measured (i.e., lactate, glucose, Cl^-, Na^+, and osmolality; Figure 4.2). High levels of plasma glucose and lactate and low levels of plasma ion concentrations and osmolality were strong predictors of short-lived females.

There were also significant regressions between each of the ions and metabolites and egg retention; however these relationships were weak and physiological status could not explain very much of the variation in egg retention (R^2 ranged from 0.055 to 0.169). Overall, blood physiology predicted reproductive longevity better than egg retention. With the inclusion of reproductive longevity, sample period and fork length as covariates, the regressions to

predict egg retention explained more of the variability (Table 4.4; adjusted R^2 ranged from 0.240 to 0.321). In each case, reproductive longevity was a major variable predicting egg retention (Table 4.4; $p<0.001$ for all regressions). Plasma lactate and Cl^- (as well as plasma Na^+ and osmolality when not Bonferroni-corrected) were significant predictors of egg retention, when the models were corrected for reproductive longevity, sample period and fork length (Table 4.4). Thus, high plasma lactate concentrations and low plasma Cl^- concentrations were predictors of egg retention, even after the effects of reproductive longevity on egg retention were accounted for.

There were no significant differences in plasma ions or metabolites at arrival between females that established a redd within 2 d, established a redd between 3 and 8 d, or did not establish a redd (all $p > 0.05$). There was no difference between biopsied and control fish for either reproductive longevity (F = 1.14; p = 0.287; control = 6.44 ± 0.77 d; bio-sampled = 7.30 ± 0.20 d) or egg retention (F = 0.01; p = 0.932; control = 28 ± 13%; bio-sampled = 26 ± 2%).

Discussion

The physiological condition of sockeye salmon on arrival at the spawning ground was an indicator of an individual's egg deposition success during spawning in that shorter-lived individuals (i.e., those dying within 4 d) had lower concentrations of plasma osmolality, ions and reproductive hormones, and higher plasma concentrations of lactate at spawning ground arrival. These results suggest that short-lived fish experienced a rapid senescent decline (Chapter 3). Overall, my results support the notion that reproductive maturity and physiological stress / ionoregulatory dysfunction play significant roles in reproductive longevity. However, as I will summarize below, it is difficult to tease apart the effects of stress and maturation because these processes interact with and influence each other (Schreck et al., 2001; Schreck, 2010). My data do not support an energy limitation explanation for reproductive longevity.

While physiological status at arrival was useful in predicting mortality within a few days of arrival, it did not predict how long fish lived beyond that. This result could be viewed from two perspectives. First, it could indicate that homeostasis of blood physiology is maintained until approximately 4 d before death, at which time fish start to lose their ability to maintain

homeostasis leading to rapid senescent change. This idea is supported by data collected from adult Harrison River sockeye salmon that were held in tanks to simulate migration – these fish were sampled up to five times during the holding period and levels of plasma osmolality, Cl^-, and Na^+ were observed to decrease a minimum of 2 d before the fish died (K Jeffries, University of British Columbia, unpublished data). Second, it is equally likely that that short-lived females were already physiologically stressed or experiencing osmoregulatory impairment prior to arrival at the spawning grounds. When freshwater fish encounter a chronic stressor, plasma osmolality and major ion concentrations (i.e., Cl^-, and Na^+) typically decrease (McDonald and Milligan, 1992; Pickering and Pottinger, 1995; Ackerman et al., 2000) and plasma lactate and glucose concentrations increase (McDonald and Milligan, 1992). Thus, the migration environment could have played a role. For example, migrating adult Weaver Creek sockeye salmon held in high flow raceways (~0.4 $m \cdot s^{-1}$) for 3 weeks exhibited high levels of plasma lactate and low levels of plasma ions and osmolality relative to individuals held in low flow raceways (~0.1 $m \cdot s^{-1}$) or to 'control' wild fish (Nadeau et al., 2010).

In the present study, short-lived females had higher plasma lactate and lower plasma Cl^- levels than were found in females that arrived at the spawning channel in 2004 (Chapter 3). This difference in results between years may reflect differences in migration conditions. In 2004, extreme temperatures (e.g., > 19°C) were acutely lethal to early migrating Weaver Creek sockeye – fish that arrived on spawning grounds were those that migrated later in the season when migration temperatures were much cooler and similar to long-term averages (e.g., 14-16°C; Mathes et al. 2010). In September 2006, Fraser River temperatures ranged from ~13.5 to 18°C, temperatures that were above the 25-year average (mean temperature decreases from 16°C to 13°C during September; Fraser River Environmental Watch, 2006), yet not acutely lethal to migrants. Because migration into the Fraser River occurs over several weeks and some individuals can take thermal refuge in Harrison Lake (Mathes et al. 2010), spawners could have experienced a large range in migration temperatures. Whether the shorter-lived spawners in my study experienced the warmest migration temperatures is unknown. Thermal holding studies have confirmed that migrating Weaver Creek sockeye held for three weeks until maturity at 18°C had twice the mortality, with survivors exhibiting higher levels of physiological stress and impaired osmoregulatory systems compared to fish held at 10°C (Crossin et al. 2008).

Physiological stress can affect reproduction in vertebrates in numerous ways; effects of stress can include a down-regulation in reproductive hormone levels, impairment of reproductive behaviour, and a reduction in gamete provisioning (Shreck et al., 2001; Schreck, 2010). It has been theorized that Pacific salmon have adaptive mechanisms to resist the effects of stress on reproduction, as they only have one opportunity for spawning (Wingfield and Sapolsky, 2003). While these theorized mechanisms may allow an individual to resist the effects of moderate levels of physiological stress, there appears to be a stressor threshold, beyond which a fish can no longer maintain homeostasis and may start to experience premature senescence. Reproductive longevity beyond the first 4 d was not related to physiological metrics at spawning ground arrival, suggesting that the migration experience and associated stressors may only influence spawning success early in the spawning period and that other biological and / or environmental factors on the spawning grounds may be more important for determining spawning success in longer-lived individuals.

I found that females that arrived with lower reproductive hormone levels were more likely to die sooner after spawning ground arrival, suggesting a link between reproductive maturity and reproductive longevity. However, I found no differences in plasma reproductive hormone levels between females considered mature versus immature at arrival. Because physiological stress can down-regulate the expression of reproductive hormones (Schreck et al., 2001), plasma reproductive hormone levels may reflect the degree of physiological stress exhibited by individuals.

It is difficult to elucidate the respective effects of reproductive maturity and physiological stress on reproductive longevity. In this study, stress variables and reproductive hormones explained similar amounts of variability in reproductive longevity (~10-30%). Further studies designed to experimentally manipulate one or both of these pathways and observe effects on reproductive longevity, behaviour and egg retention are needed to resolve this issue.

Several previous studies suggest that energy might limit reproductive longevity. Mehranvar (2002) observed that sockeye salmon that arrived at the spawning grounds with greater amounts of gross somatic energy lived longer on the spawning grounds. In addition, McBride et al. (1965) was able to extend the lifespan of maturing and spawned sockeye salmon through force feeding. Moribund females with gross somatic energy reserves < 3.5 MJ•kg^{-1} exhibited a decrease in plasma glucose concentrations between spawning ground

arrival and becoming moribund, purportedly due to an inability to mobilize energy stores (Chapter 3). Finally, Crossin et al. (2003, 2004) observed a similar energetic threshold for death of ~4 MJ•kg^{-1} across several stocks of both sockeye and pink salmon (*O. gorbuscha*). However, in my study, somatic energy density did not appear to be a factor limiting a female's ability to complete spawning. The females that died earlier after arrival and with more eggs had greater energy reserves at death, and more successful females were able to exhaust their energy reserves to a greater extent before death.

Egg retention was not related directly to physiological metrics but instead was most strongly related to reproductive longevity. Nonetheless, physiological stress may have had indirect effects on egg retention. Females with high plasma lactate and low Cl$^-$ concentrations not only lived for a shorter time in the spawning channel, but they also had more eggs retained at death for a given reproductive longevity. Migratory stress can impair gonad development (Schreck et al., 2001) by altering 17β-estradiol and vitellogenin levels and thus changing reproductive development trajectories (Pickering et al., 1987; Tyler and Sumpter, 1996; Macdonald, 2000). In my study, females that were more reproductively advanced at spawning ground arrival did not retain more eggs at death. Although not quantified directly here, many individuals that died within days of arrival expelled eggs during sampling (K Hruska, personal observation), indicating they were over-mature at arrival. In Atlantic salmon (*Salmo salar*) that were experimentally prevented from spawning, females that were delayed longest after maturity exhibited the highest egg retention and lowest fertilization success (de Gaudemar and Beall, 1998).

In summary, this study provides evidence of links between physiological status of female sockeye salmon at spawning ground arrival and indicators of egg retention. Specifically, reproductive longevity appears to be affected by physiological stress indicating that migration environments can play a key role in spawning success. Egg retention appears to be less influenced by physiological condition at arrival. There were no relationships between physiological condition and the time taken to establish a redd, indicating that physiological condition may have little influence on the motivation to begin spawning activities. In addition, the physiological condition at arrival did not predict any success indicators beyond 4 d on the spawning channel. Thus, more information is still needed to explain why some individuals arrive at the spawning grounds, spend several days waiting and then fail to spawn before death (e.g., Chapter 2). Further studies are needed to discern the interactions between individual physiology / behaviour patterns and environmental conditions on the

spawning grounds as putative explanations for differences in egg retention beyond the first 4 d.

Table 4.1: Mean (± SEM) of physiological and morphological variables in female sockeye salmon (*Oncorhynchus nerka*) that arrived during the start (Oct. 5-6), the middle (Oct. 13), and the end (Oct. 19) of the arrival period at the Weaver Creek Spawning Channel in October, 2006. Plasma hormones and potassium concentrations and gross somatic energy were only analyzed for 60 females over the first two sampling periods. Bold p-values indicate significant effects with Bonferroni correction[1]. * indicates effect significant at α = 0.05.

Variable	Oct. 5-6, 2006	n	Oct. 13, 2006	n	Oct. 19, 2006	n	F-value	p-value
GSE (MJ•kg^{-1})	3.281 ± 0.053 a	40	3.374 ± 0.068 a	19	nd[2]	0	1.08	0.303
[Testosterone] (ng•ml^{-1})	110.7 ± 6.6 a	42	85.7 ± 10.8 b	18	nd	0	4.08	0.048*
[17β-estradiol] (ng•ml^{-1})	0.266 ± 0.031 a	42	0.261 ± 0.052 a	18	nd	0	0.04	0.834
[17,20β-P][3] (ng•ml^{-1})	385 ± 33 a	36	256 ± 37 b	19	nd	0	5.84	0.019*
Osmolality (mmol•L^{-1})	310.68 ± 0.57 a	136	309.39 ± 0.98 a	70	301.33 ± 1.30 b	39	22.91	**<0.001**
[Cl$^-$] (mmol•L^{-1})	129.83 ± 0.32 a	135	126.54 ± 0.55 b	70	126.95 ± 0.74 b	39	17.82	**<0.001**
[Na$^+$] (mmol•L^{-1})	158.88 ± 0.44 a	136	154.71 ± 0.67 b	70	153.97 ± 0.93 b	39	20.42	**<0.001**
[K$^+$] (mmol•L^{-1})	1.755 ± 0.059 a	42	1.850 ± 0.061 a	18	nd	0	0.93	0.340
[Lactate] (mmol•L^{-1})	1.05 ± 0.04 a	136	1.40 ± 0.13 b	70	1.50 ± 0.15 b	39	8.84	**<0.001**
[Glucose] (mmol•L^{-1})	5.327 ± 0.075 a	136	5.255 ± 0.095 a	70	5.420 ± 0.168 a	39	0.37	0.693
[Cortisol] (ng•ml^{-1})	184.6 ± 9.3 a	42	174.6 ± 12.4 a	18	nd	0	0.37	0.543
Fork Length (cm)	58.52 ± 0.27 a	129	59.68 ± 0.42 b	66	60.44 ± 0.67 b	31	5.76	0.004*
POH Length (cm)	48.55 ± 0.25 a	129	49.92 ± 0.39 b	66	49.58 ± 0.52 ab	31	5.33	0.006*
Snout Length (cm)	5.058 ± 0.050 a	129	5.273 ± 0.072 a	66	5.210 ± 0.115 a	31	3.22	0.042*
Girth (cm)	31.43 ± 0.17 a	137	31.07 ± 0.30 a	69	31.38 ± 0.37 a	39	0.62	0.538

[1] Bonferroni correction α = 0.003
[2] nd = no data
[3] 17,20β-P = 17,20β-Progesterone

Table 4.2: Mean (± SEM) of physiological and morphological variables in female sockeye salmon (*Oncorhynchus nerka*) that were classified as short-lived (died after 2 to 4 days), medium-lived (died after 7 to 8 days), or long-lived (died after 11 to 14 days) in the Weaver Creek Spawning Channel in October, 2006. Bold p-values indicate significant effects with Bonferroni correction[1]. * indicates effect significant at α = 0.05.

Variable	Short-lived	n	Medium-lived	n	Long-lived	n	F-value	p-value
GSE (MJ•kg^{-1})	3.496 ± 0.077 a	17	3.265 ± 0.048 b	27	3.184 ± 0.094 b	15	4.83	0.012*
[Testosterone] (ng•ml^{-1})	64.7 ± 6.1 a	16	112.1 ± 7.8 b	29	127.0 ± 11.3 b	15	11.53	**<0.001**
[17β-estradiol] (ng•ml^{-1})	0.165 ± 0.023 a	16	0.283 ± 0.036 b	29	0.336 ± 0.070 b	15	4.64	0.014*
[17,20β-P]^3 (ng•ml^{-1})	241 ± 31 a	17	323 ± 36 a	24	491 ± 58 b	14	7.93	**0.001**
[K$^+$] (mmol•L^{-1})	1.831 ± 0.077 a	16	1.841 ± 0.067 a	29	1.620 ± 0.088 a	15	2.26	0.113
[Cortisol] (ng•ml^{-1})	174 ± 14 a	16	187 ± 10 a	29	179 ± 17 a	15	0.26	0.771
Fork Length (cm)	61.00 ± 0.89 a	17	59.10 ± 0.51 a	29	58.50 ± 0.93 a	15	2.78	0.070
POH Length (cm)	51.62 ± 0.78 a	17	48.95 ± 0.45 b	29	49.53 ± 1.05 ab	15	4.02	**0.023**
Snout Length (cm)	5.38 ± 0.13 a	17	4.97 ± 0.10 b	29	5.20 ± 0.14 ab	15	3.37	**0.041**
Girth (cm)	31.53 ± 0.71 a	16	31.31 ± 0.38 a	29	31.97 ± 0.53 a	15	0.41	0.666

[1] Bonferroni correction α = 0.003
[2] 17,20β-P = 17,20β-Progesterone

Table 4.3: Mean (± SEM) of physiological and morphological variables in female sockeye salmon (*Oncorhynchus nerka*) classified as being low egg retention (<25% egg retention), medium egg retention (25 to 75% egg retention), or high egg retention (>75% egg retention) after residence on the Weaver Creek Spawning Channel in October, 2006. Bold p-values indicate significant effects with Bonferroni correction[1]. * indicates effect significant at α = 0.05.

Variable	Low Egg Retention	n	Medium Egg Retention	n	High Egg Retention	n	F-value	p-value
GSE (MJ•kg^{-1})	3.213 ± 0.049 a	37	3.391 ± 0.095 ab	10	3.547 ± 0.080 b	12	6.18	0.004*
[Testosterone] (ng•ml^{-1})	113 ± 7 a	38	92 ± 14 a	10	83 ± 13 a	12	2.52	0.089
[17β-estradiol] (ng•ml^{-1})	0.282 ± 0.037 a	38	0.219 ± 0.030 a	10	0.249 ± 0.054 a	12	0.34	0.717
[17,20β-P][3] (ng•ml^{-1})	378 ± 36 a	34	253 ± 53 a	9	298 ± 48 a	12	1.86	0.166
[K$^+$] (mmol•L^{-1})	1.726 ± 0.054 a	38	1.940 ± 0.098 a	10	1.833 ± 0.118 a	12	1.66	0.200
[Cortisol] (ng•ml^{-1})	185 ± 10 a	38	174 ± 16 a	10	178 ± 17	12	0.16	0.851
Fork Length (cm)	58.89 ± 0.49 a	38	61.45 ± 1.23 a	10	59.69 ± 0.94 a	13	2.50	0.091
POH Length (cm)	49.16 ± 0.50 a	38	51.90 ± 1.02 b	10	50.23 ± 0.93 ab	13	3.10	0.053
Snout Length (cm)	5.09 ± 0.08 a	38	5.30 ± 0.20 a	10	5.15 ± 0.19 a	13	0.55	0.580
Girth (cm)	31.46 ± 0.33 a	37	32.15 ± 1.27 a	10	31.27 ± 0.49 a	13	0.47	0.626

[1] Bonferroni correction α = 0.003
[2] 17,20β-P = 17,20β-Progesterone

Table 4.4: Regression output for egg retention as a function of each of the major plasma ion and metabolite concentrations with sample period, fork length, and reproductive longevity used as covariates. Blood plasma samples were collected from female sockeye salmon (*Oncorhynchus nerka*) as they arrived at the Weaver Creek Spawning Channel over three sample periods in October, 2006. Reproductive longevity and egg retention were obtained from observational data for each female and carcass collection at death. Bold p-values indicate significant effects with Bonferroni correction[1]. * indicates effect significant at α = 0.05.

Variable	Osmolality				Chloride				Sodium			
	df	SS	F-value	p-value	df	SS	F-value	p-value	df	SS	F-value	p-value
Full Model	6	64891	15.01	**<0.001**	6	74775	18.47	**<0.001**	6	58271	12.93	**<0.001**
Sample Period	2	8249	5.79	**0.004**	2	7913	5.68	**0.003**	2	8083	5.38	**0.005**
Fork Length	1	2107	2.92	0.089	1	2278	3.38	0.068	1	2244	2.99	0.085
Longevity	1	14031	19.47	**<0.001**	1	13341	19.77	**<0.001**	1	23752	31.63	**<0.001**
Variable	1	2887	4.01	*0.047*	1	5522	8.18	**0.005**	1	3425	4.69	0.031
Longevity x Var.[2]	1	11221	15.57	**<0.001**	1	14054	20.83	**<0.001**	1	4713	6.28	0.013
Error	217	156341			216	145765			217	162961		
Total	223	221233			222	220539			223	221233		
Adjusted R^2				0.274				0.321				0.243

Variable	Glucose				Lactate			
	df	SS	F-value	p-value	df	SS	F-value	p-value
Full Model	6	57612	12.74	**<0.001**	5	59797	16.15	**<0.001**
Sample Period	2	4892	3.24	*0.041*	2	7715	5.21	**0.006**
Fork Length	1	2446	3.24	0.073	1	3495	4.72	*0.031*
Longevity	1	32165	42.66	**<0.001**	1	29396	39.70	**<0.001**
Variable	1	367	0.49	0.486	1	10032	13.55	**<0.001**
Longevity x Var.	1	5426	7.20	**0.008**				NS[3]
Error	217	163621			218	161435		
Total	223	221233			223	221233		
Adjusted R^2				0.240				0.270

[1] Bonferroni correction α = 0.010
[2] Var. = Variable
[3] NS = interaction not significant, so interaction term was removed from analysis.
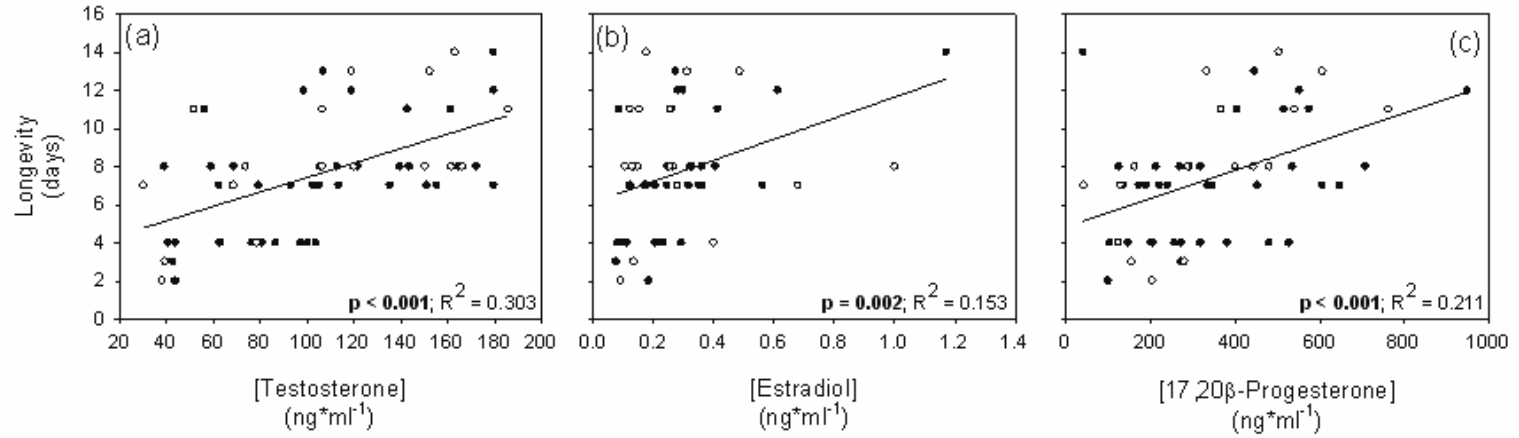
Figure 4.1: Linear regression of longevity on the spawning grounds as a function of plasma testosterone (a), 17β-estradiol (b), and 17,20β-progesterone (c) concentrations in females at arrival at the Weaver Creek Spawning Channel in October, 2006. There were no differences in the relationships for females which were considered mature (black circles) or immature (open circles) at spawning ground arrival.
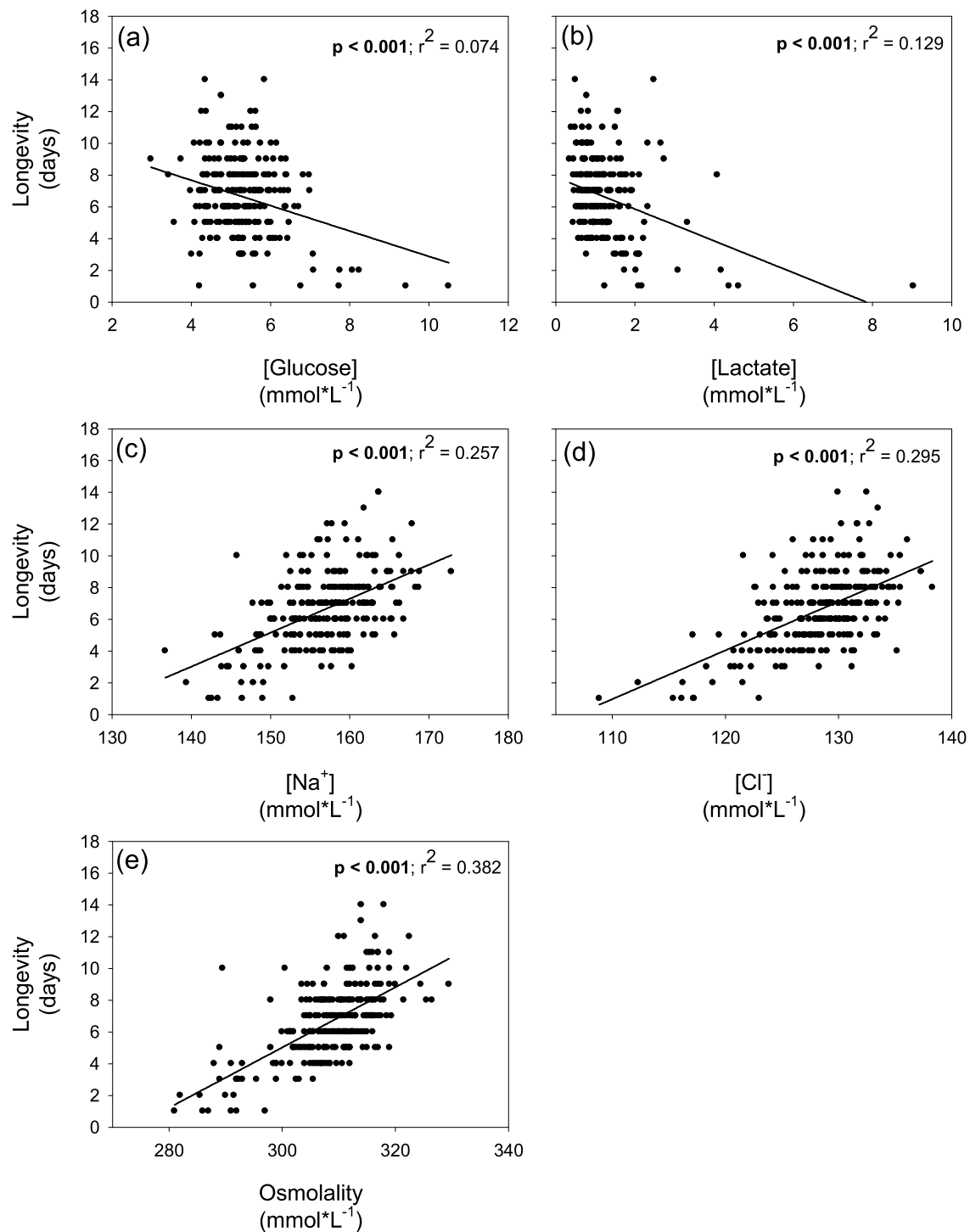
Figure 4.2: Linear regressions of reproductive longevity on the spawning grounds versus plasma glucose (a), lactate (b), Na^+ (c), and Cl^- (d) concentrations and osmolality (e) in female sockeye salmon (*Oncorhynchus nerka*) at arrival at the Weaver Creek Spawning Channel in October, 2006.

Literature Cited

Ackerman PA, Forsyth RB, Mazur CF, and Iwama GK. 2000. Stress hormones and the cellular stress response in salmonids. Fish Physiology and Biochemistry 23:327-336.

Burgner RL. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). Pages 1-117 in Groot C and Margolis L (eds). Pacific salmon life histories. UBC Press, Vancouver, BC.

Cabin RJ and Mitchell RJ. 2000. To Bonferroni or not to Bonferroni: when and how are the questions. Bulletin of the Ecological Society of America 81:246-248.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Healey MC, Macdonald JS, Shrimpton JM, van der Kraak G, and Farrell AP. 2006a. Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. Ecology 87:1575-1586.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Shrimpton JM, van der Kraak G, and Farrell AP. 2006b. Physiology of individual Late-run Fraser River sockeye salmon (*Oncorhynchus nerka*) sampled in the ocean correlates with fate during spawning migration. Canadian Journal of Fisheries and Aquatic Sciences 63:1469-1480.

Cooke SJ, Hinch SG, Farrell AP, Patterson DA, Miller-Saunders K, Welch DW, Donaldson MR, Hanson KC, Crossin GT, Mathes MT, Lotto AG, Cooperman MS, Hruska KA, Olsson IC, Wagner GN, Thomson R, Hourston R, English KK, Larsson S, Shrimpton JM, and van der Kraak G. 2008. Developing a mechanistic understanding of fish migrations by linking telemetry with physiology, behavior, genomics and experimental biology: an interdisciplinary case study on adult Fraser River sockeye salmon. Fisheries 33:321-338.

Crossin GT, Hinch SG, Farrell AP, Whelley MP, and Healey MC. 2003. Pink salmon (*Oncorhynchus gorbuscha*) migratory energetics: response to migratory difficulty and comparisons with sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Zoology 81:1986-1995.

Crossin GT, Hinch SG, Farrell AP, Higgs DA, Lotto AG, Oakes JD, and Healey MC. 2004. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. Journal of Fish Biology 65:788-810.

Crossin GT, Hinch SG, Cooke SJ, Welch DW, Lotto AG, Patterson DA, Jones SRM, Leggatt RA, Mathes MT, Shrimpton JM, van der Kraak G, and Farrell AP. 2008. Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migrations. Canadian Journal of Zoology 86:127-140.

Crossin GT, Hinch SG, Cooke SJ, Cooperman MS, Patterson DA, Welch DW, Hanson KC, Olsson I, English KK, and Farrell AP. 2009. Mechanisms influencing the timing and success of reproductive migration in a capital breeding semelparous fish species, the sockeye salmon. Physiological and Biochemical Zoology 82:635-652.

de Gaudemar B and Beall E. 1998. Effects of overripening on spawning behaviour and reproductive success of Atlantic salmon females spawning in a controlled flow channel. Journal of Fish Biology 53:434-446.

Dickhoff WW. 1989. Salmonids and annual fishes: death after sex. Pages 253-266 in Schreibman MP and Scanes CG (eds). Development, Maturation, and Senescence of Neuroendocrine Systems: A Comparative Approach. Academic Press, New York, NY.

Farrell AP, Gallaugher P, Clarke C, DeLury N, Kreiberg H, Parkhouse W, and Routledge R. 2000. Physiological status of coho salmon (*Oncorhynchus kisutch*) captured in commercial nonretention fisheries. Canadian Journal of Fisheries and Aquatic Sciences 57:1668-1678.

Foote CJ. 1990. An experimental comparison of males and female spawning territoriality in a Pacific salmon. Behaviour 115:283-314.

Fraser River Environmental Watch Report. October 3, 2006. http://www.pac.dfo-mpo.gc.ca/science/habitat/frw-rfo/reports-rapports/2006/03Oct06/03oct06-eng.htm. Fisheries and Oceans, Canada.

Fukushima M and Smoker WW. 1997. Determinants of stream life, spawning efficiency, and spawning habitat in pink salmon in the Auke Lake system, Alaska. Canadian Journal of Fisheries and Aquatic Sciences 54:96-104.

Gilhousen P. 1990. Prespawning mortalities of sockeye salmon in the Fraser River system and possible causal factors. International Pacific Salmon Fisheries Commission Bulletin 26.

Groot C and Margolis L. 1991. Pacific salmon life histories. UBC Press, Vancouver, BC.

Healey MC and Prince A. 1998. Alternative tactics in the breeding behaviour of male coho salmon. Behaviour 135:1099-1024

Healey MC, Lake R, and Hinch SG. 2003. Energy expenditures during reproduction by sockeye salmon (*Oncorhynchus nerka*). Behaviour 140:161-182.

Hendry AP, Berg OK, and Quinn TP. 2001. Breeding location choice in salmon: causes (habitat, competition, body size, energy stores) and consequences (life span, energy stores). Oikos. 93:407-418.

Hinch SG, Cooke SJ, Healey MC, and Farrell AP. 2006. Behavioural physiology of fish migrations: salmon as a model approach. Pages 240-296 in Sloman KA, Wilson RW, and Balshine S (eds). Behaviour and physiology of fish. Elsevier, Amsterdam.

Lee CG, Farrell AP, Lotto A, MacNutt MJ, Hinch SG, and Healey MC. 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) coho (*O. kisutch*) salmon stocks. Journal of Experimental Biology 206:3239-3251.

Macdonald JS. 2000. Mortality during migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): a study of the effect of ocean and river environmental conditions in 1997. Canadian Technical Report of Fisheries and Aquatic Sciences 2315.

Mathes MT, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Lotto AG, and Farrell AP. 2010. Effect of water temperature, timing, physiological condition and lake thermal refugia on migrating adult Weaver Creek sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 67: 70-84.

McBride JR, Fagerlund UHM, Smith M, and Tomlinson N. 1965. Post-spawning death of Pacific salmon: Sockeye salmon (*Oncorhynchus nerka*) maturing and spawning in captivity. Journal of the Fisheries Research Board of Canada 22:775-782.

McDonald DG and Milligan CL. 1992. Chemical properties of blood. Pages 55-133 in Hoar WS, Randall DJ, and Farrell AP (eds). The Cardiovascular System. Academic Press, San Diego, CA.

McMaster M, Munkittrick K, and van der Kraak G. 1992. Protocol for measuring circulating levels of gonadal sex steroids in fish. Canadian Technical Report for Fisheries and Aquatic Sciences 1836:1-29.

Mehranvar L. 2002. A genetic analysis of the mating system of sockeye salmon. MSc thesis, University of British Columbia, Vancouver, BC.

Miller KM, Schulze AD, Ginther N, Li S, Patterson DA, Farrell AP, and Hinch SG. 2009. Salmon spawning migration: Metabolic shifts and environmental triggers. Comparative Biochemistry and Physiology, D Genomics and Proteomics 4:75-89.

Morbey YE and Ydenberg RC. 2003. Timing games in the reproductive phenology of female Pacific salmon (*Oncorhynchus* spp.). The American Naturalist 161:284-298.

Nadeau PS, Hinch SG, Hruska KA, Pon LB, and Patterson DA. 2010. The effects of experimental energy depletion on the physiological condition and survival of adult sockeye salmon (*Oncorhynchus nerka*) during spawning migration. Environmental Biology of Fish 88:241-251.

Pickering AD, Pottinger TG, Carragher J, and Sumpter JP. 1987. The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. General and Comparative Endocrinology 68:249-259.

Pickering AD and Pottinger TG. 1995. Biochemical effects of stress. Pages 349-379 in Hochachka PW and Mommsen TP (eds). Biochemistry and Molecular Biology of Fishes. Vol. 5. Environmental and Ecological Biochemistry. Elsevier, Amsterdam.

Quinn TP, Eggers DM, Clark JH, and Rich HB Jr. 2007. Density, climate, and the processes of prespawning mortality and egg retention in Pacific salmon (*Oncorhynchus* spp.). Canadian Journal of Fisheries and Aquatic Sciences 64:574-582.

Rice WR. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Schreck CB, Contreras-Sanchez W, and Fitzpatrick MS. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197:3-24.

Schreck CB. 2010. Stress and fish reproduction: the roles of allostasis and hormesis. General and Comparative Endocrinology 165:549-556.

Shrimpton JM, Patterson DA, Richards JG, Cooke SJ, Schulte PM, Hinch SG, and Farrell AP. 2005. Ionoregulatory changes in different populations of maturing sockeye salmon (*Oncorhynchus nerka*) during ocean and river migration. Journal of Experimental Biology 208:4069-4078.

Sloman KA, Scott, GR, McDonald DG, and Wood CM. 2004. Diminished social status affects ionoregulation at the gills and kidney in rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 61:618-626.

Truscott B, Idler DR, So YP, and Walsh JM. 1986. Maturational steroids and gonadotropin in upstream migratory sockeye salmon. General and Comparative Endocrinology 62:99-110.

Tyler CR and Sumpter JP. 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6:287-318.

van den Berghe EP and Gross MR. 1986. Length of breeding life of coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Zoology 64:1482-1486.

van der Kraak G and Chang J. 1990. Arachidonic acid stimulates steroidogenesis in goldfish preovulatory ovarian follicles. General and Comparative Endocrinology 77:221-228.

Wingfield JC and Sapolsky RM. 2003. Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology 15:711-724.

Young JL, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Farrell AP, van der Kraak G, Lotto AG, Lister A, Healey MC, and English KK. 2006. Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 63:1067-1077.

Chapter 5: Conclusion

Fraser River sockeye salmon (*Oncorhynchus nerka*) populations are facing threats throughout much of the watershed. While factors affecting successful upriver migration of adults are becoming increasingly well-studied (Cooke et al., 2006a,b; Young et al., 2006; Crossin et al., 2009; Mathes et al., 2010), the issue of egg retention on the spawning grounds has received little attention. This thesis provided new insights into physiology and behaviour during spawning and senescence in individual female sockeye salmon in order to bridge several gaps in our knowledge about the relationships between physiological condition, behaviour and spawning success on the spawning grounds.

Throughout this dissertation I observed a negative relationship between reproductive longevity and egg retention for females in the spawning channel. This trend was evident in both 2004 (Appendix 1) and 2006 (Chapter 2). These results provide some of the first evidence that successful egg deposition is correlated to length of life on the spawning grounds. This benefit is in addition to the redd-guarding benefit of greater reproductive longevity on the spawning grounds (Quinn and Foote, 1994; McPhee and Quinn, 1998; Steen and Quinn, 1999; Morbey and Ydenberg, 2003). It was impossible, however, to discriminate between a correlation and a causal effect of reproductive longevity on egg retention with the design of these studies. A causal relationship between these two factors would have implied that short-lived females did not have sufficient time on the spawning grounds to complete their spawning activities. An alternative interpretation is that these females were just poor individuals that lacked the means and / or motivation to obtain a redd and to complete spawning before death. There is evidence to support both of these interpretations. First, there were a large number of females that were unable to complete spawning before death. Second, there were a small number of females that lived in the spawning channel for almost two weeks but did not complete spawning; this latter result indicates that incomplete spawning was not due to time limitations for at least some females. Thus, while long life in the spawning channel is important, it is not the only factor necessary for completion of spawning prior to death. Future work on the behaviour of females between spawning ground arrival and redd establishment is needed to help determine the nature of the relationship between reproductive longevity and egg retention in spawning female sockeye salmon.

Many of the long-lived females, particularly those that had a large portion of eggs remaining at death, spent several days waiting in deeper areas of the spawning channel before establishing a redd. This waiting behaviour, which has long been observed in Pacific salmon (Morbey and Ydenberg, 2003), may have been a mechanism to avoid conflict with established females and redd superimposition by later arriving females (Blanchfield and Ridgway, 1997; Morbey and Ydenberg, 2003), or it may have resulted from competitive exclusion from preferred spawning sites. When spawning female kokanee salmon (*O. nerka*) were experimentally removed from an area, their territories were rapidly filled by waiting females (Foote, 1990), suggesting that high quality spawning territories are the limiting resource for spawning female salmonids. However, while spawner density influenced the frequency of aggressive behaviours in female spawning sockeye salmon, there was no effect of density on either the length of time to establish a redd or levels of egg retention (Appendix 1). Sockeye salmon have historically spawned at very high densities so it is not surprising that there would be mechanisms to preserve egg deposition, particularly at the moderate densities observed in the Weaver Creek population.

Living for at least 4 d in the spawning channel appeared to be crucial for the completion of spawning in Weaver Creek sockeye salmon females. In Chapter 2, I found that there were no completely spawned females that lived for fewer than 4 d in the spawning channel; this finding was supported by the data from Appendix 1. Thus, while there is evidence from other sockeye salmon populations that spawning can be completed in a shorter time frame (Clark, 1959) living for approximately 4 d after arrival appears to be a critical threshold for the completion of spawning in the Weaver Creek sockeye salmon population. The average spawning duration in this population may result from low levels of predation encountered on the spawning grounds (K Hruska, personal observation), reducing selection for rapid spawning. Females that died within 4 d of arrival at the spawning channel also had a distinct blood physiology profile compared to the females that lived longer in the spawning channel.

The distinct plasma profiles of the short-lived females are indicative of physiological changes associated with senescence. Females that died within 4 d of arrival at the spawning channel had elevated plasma lactate and glucose concentrations, and low levels of plasma reproductive hormones, ions and osmolality (Chapter 4). These physiological indicators (with the exception of glucose) were in the same direction as the physiological changes that occurred in fish between spawning ground arrival and senescence (Chapter 3). Thus, the females that died soon after arrival appeared to already be undergoing senescence by the

time they arrived at the spawning channel. Death shortly after arrival may have resulted from premature senescence, wherein individuals have already begun the rapid physiological senescent changes by the time they reached the spawning grounds. Premature senescence may be triggered by environmental conditions encountered during migration (Wagner et al. 2005; Hinch et al. 2006; Farrell et al. 2008; Crossin et al. 2008; Mathes et al., 2010). For example, sockeye salmon that were captured during migration and held in raceways to simulate migration exhibited signs of physiological stress during the holding period (Nadeau et al., 2010). When some of these individuals were released into enclosures in the spawning channel, I observed that females with more elevated plasma lactate levels and lower Cl^- and Na^+ concentrations and males with more elevated plasma lactate and glucose concentrations, and depressed plasma levels of Cl^-, Na^+, and K^+ ions and osmolality had reduced reproductive longevity (K Hruska, unpublished data).

Alternatively, these individuals may have arrived at the spawning grounds too late, leaving insufficient time to complete spawning. Delayed arrival at the spawning grounds is unlikely to be a major factor contributing to reduced reproductive longevity in this population. Weaver Creek sockeye salmon have a short and easy migration relative to all other stocks of sockeye salmon in the Fraser River (Crossin et al., 2004) and they are unlikely to encounter many barriers that would delay spawning ground arrival during their upriver migration. If Weaver Creek sockeye salmon were delayed in their arrival then I would expect the short-lived fish to be arriving later in the season, which is opposite of the observed trend (Chapter 2).

Despite the relationships between blood physiology and reproductive longevity over the first 4 d in the spawning channel, there was no evidence that blood physiology could predict reproductive longevity beyond this initial period of time. One likely explanation is that sockeye salmon only begin to experience significant levels of homeostatic imbalance at about 4 d prior to death. This explanation is consistent with the findings in a simulated migration study in which adult sockeye salmon started to exhibit decreased concentrations of plasma ions and osmolality a minimum of 2 d before they died (K Jeffries, University of British Columbia, unpublished data). Conversely, environmental conditions, such as density, on the spawning grounds can also influence reproductive longevity (Appendix 1), thereby reducing our ability to use physiological condition to predict reproductive longevity after a few days in the channel.

However, annual trends in fish physiology and spawning success do not support the predictive power of blood physiology on egg retention. I found that mean levels for blood physiology parameters varied among years (Figure 5.1). If blood physiology can predict egg retention, then I would expect to see higher levels of pre-spawning mortality in years when sockeye salmon have plasma profiles that are characteristic of senescence (i.e., low plasma ion concentrations and osmolality, higher plasma glucose and lactate) at spawning ground arrival. However the observed trends did not support my predictions (Figure 5.2). For example, in the year with the highest level of pre-spawning mortality (2006), the females had significantly higher plasma osmolality and Na^+ concentrations than in the other two years. The inter-annual variability in blood physiology may be a reflection of organismal responses to environmental stressors encountered during migration. Weaver Creek sockeye salmon encountered warm waters, during migration in all three years – with considerable portions of the run failing during migration and / or spawning in each year (Mathes et al., 2010; R Stitt, Weaver Creek Spawning Channel, FOC, personal communication). The lack of relationship across years between blood physiology and egg retention may result from premature mortality occurring at different times during migration and spawning. For example, in 2004 there was a low rate of egg retention at the spawning grounds (R Stitt, Weaver Creek Spawning Channel, FOC, personal communication) which may have resulted from many poor quality fish succumbing to premature senescence during migration. There were high levels of mortality in-river in 2004 (Mathes et al., 2010). Environmental conditions, such as high water temperatures, have been linked to mortality during the up-river migration and on the spawning grounds (Gilhousen, 1990).

Differences in plasma glucose concentrations between the sexes in sockeye salmon were evident during the latter stages of migration (Figure 5.1). One of the significant findings in Chapter 3 was the magnitude of sex differences in plasma ion concentrations during senescence. However, the data presented here indicate that sex differences may already be evident during migration. Several other studies (e.g., Young et al., 2006; Donaldson et al., 2010; Mathes et al., 2010) have not shown significant sex differences in blood physiology of migrating sockeye salmon.

In addition to blood physiology, there were several factors that were shown to affect reproductive longevity of sockeye salmon on the spawning grounds. In Chapter 2 I showed that the females that arrived at the spawning channel earliest in the season lived longer than later arriving females. This finding was consistent with several other studies that observed a

seasonal decline in reproductive longevity (van den Berghe and Gross 1989; Morbey and Ydenberg 2003; Hendry et al., 2004). Female density was shown to influence reproductive longevity and aggressive behaviour on the spawning grounds (Appendix 1). While there were links between aggressive behaviour and the degree of certain physiological changes during senescence (Chapter 3) and egg retention (Appendix 1), I found no evidence that the frequency of aggressive behaviour influenced reproductive longevity in spawning females.

Future research on the physiological behaviour of wild sockeye salmon would be valuable for developing a more comprehensive model of the factors affecting reproductive longevity and egg retention in spawning sockeye salmon females. My research has indicated that reproductive longevity can be predicted, on a short time scale, by stress indicators and reproductive hormones measured in the blood plasma at spawning ground arrival. Further experimental work is needed to distinguish between the stress / osmoregulation and the reproductive maturity hypotheses for reproductive longevity and egg retention on the spawning grounds. Additional work is necessary to explore other non-destructive physiological samples that may provide predictions of spawning success beyond 4 d on the spawning grounds. For example, microarray techniques (e.g., Miller et al., 2009) may be useful for identifying patterns of gene expression associated with premature mortality on the spawning grounds. This technique has already proved valuable for predicting migration success for adult sockeye salmon travelling through marine and river environments (Miller et al., 2009).

In conclusion, there is considerable variation in reproductive success among individual female sockeye salmon within a population due to the large range of factors that can impact spawning behaviour and reproductive longevity. This dissertation describes some of the first field-based studies designed to explore the relationships between physiological condition and behaviour and success on the spawning grounds in free-swimming sockeye salmon. Females that died shortly after spawning ground arrival had higher levels of eggs retained at death and also arrived at the spawning grounds with physiological profiles that were characteristic of reproductive maturity, stress, and osmoregulatory dysfunction relative to females that lived longer on the spawning grounds. These short-lived females appear to be experiencing altered senescence trajectories which likely resulted from stressors (e.g., warm river temperatures, disease) encountered during migration. If warm migration temperatures are accelerating the trajectory towards senescence then I expect that sockeye salmon populations will continue experiencing high levels of egg retention on the spawning grounds

as Fraser River temperatures continue to rise over the next century, as predicted in climate change models (Morrison et al., 2002; Ferrari et al., 2007).

Figure 5.1: Mean (± SEM) values for plasma (a) lactate, (b) glucose, (c) Na⁺, and (d) Cl⁻ concentrations and (e) osmolality in male (open symbols) and female (black symbols) Weaver Creek sockeye salmon (*Oncorhynchus nerka*) captured during migration through the Harrison River and at spawning ground arrival in 2004 (circles; Chapter 3), 2005 (triangles; K Hruska, unpublished data), and 2006 (squares; Chapter 4).
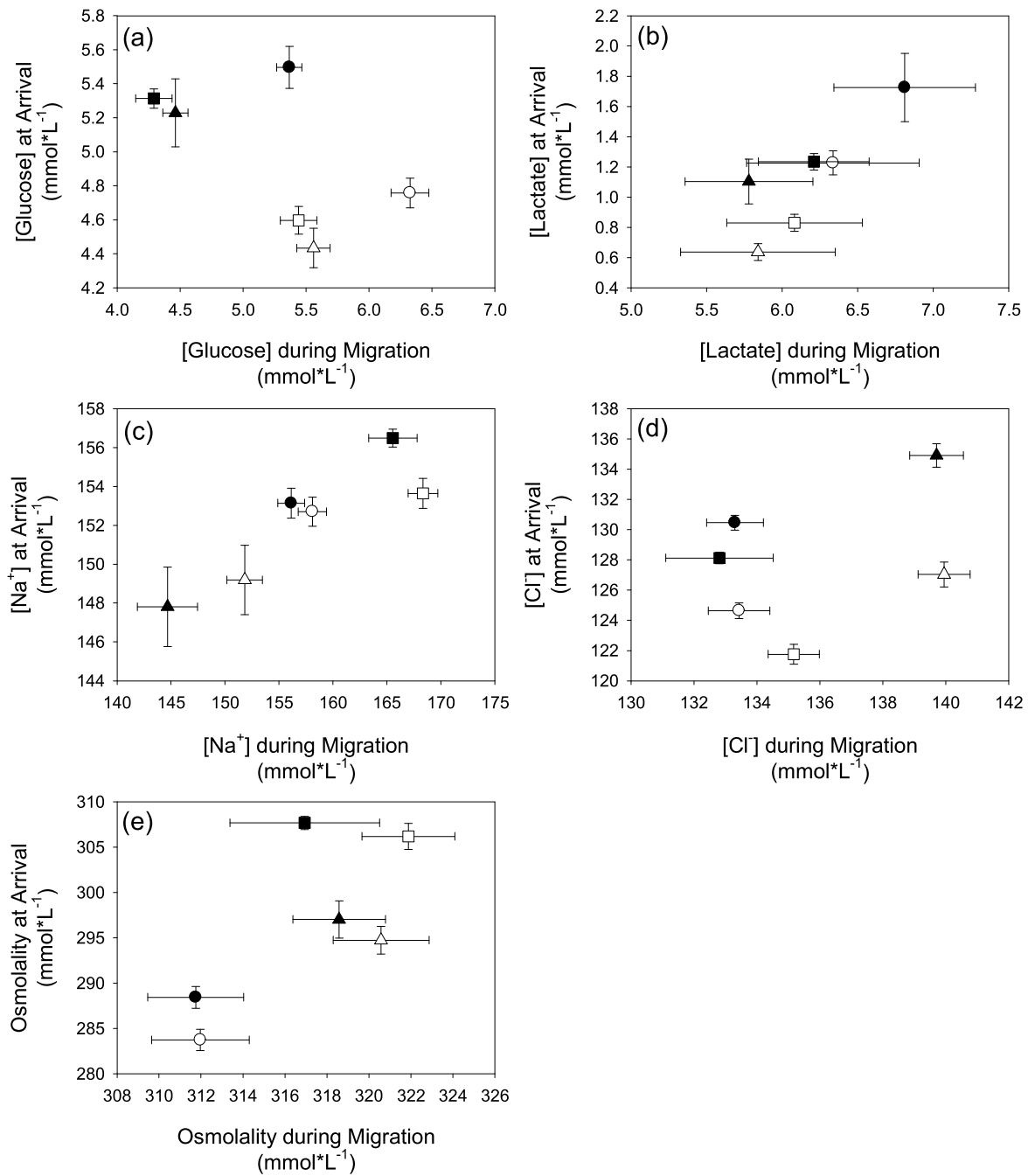
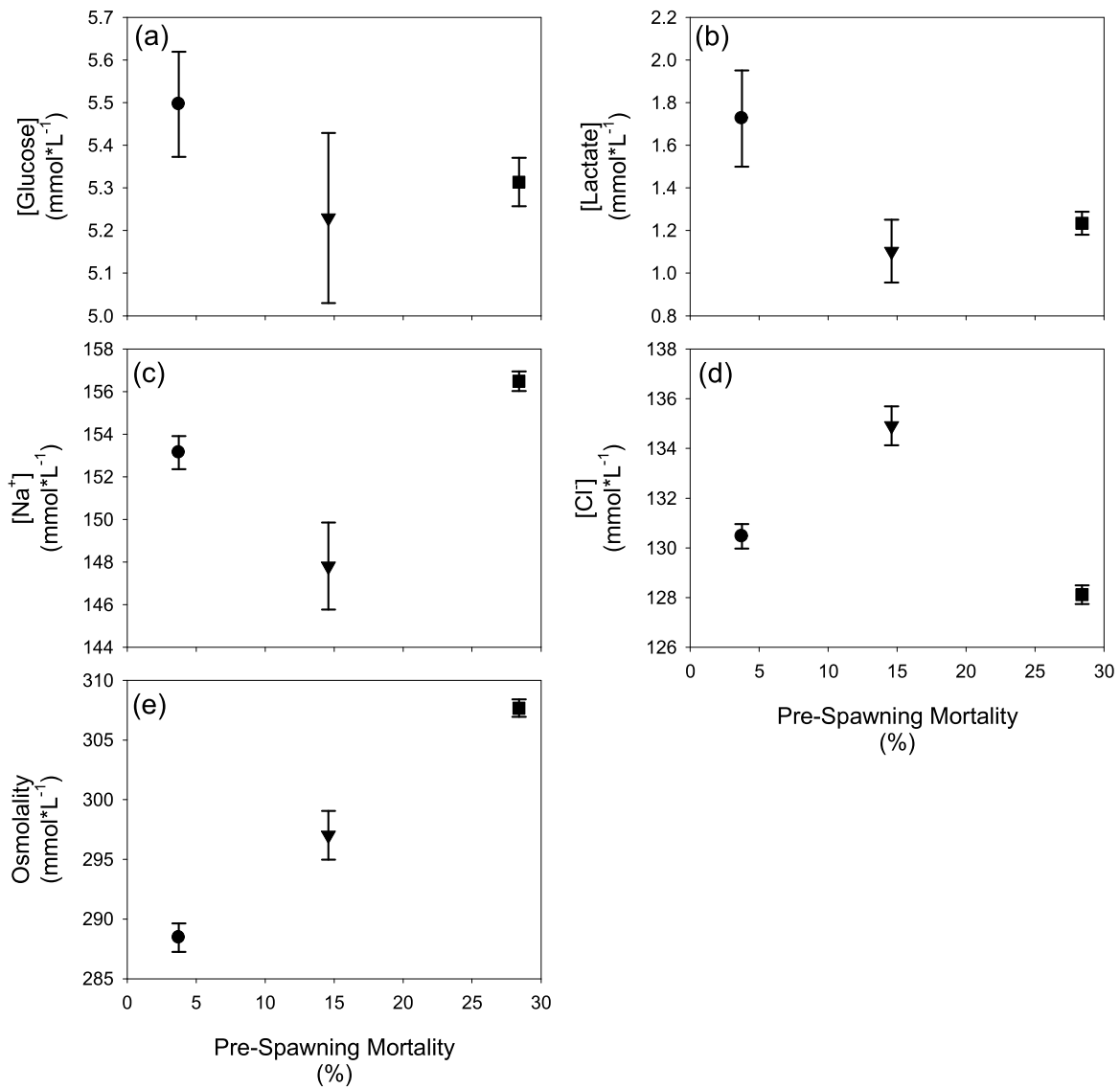Figure 5.2: Mean (± SEM) values for plasma (a) lactate, (b) glucose, (c) Na⁺, and (d) Cl⁻ concentrations and (e) osmolality in female Weaver Creek sockeye salmon (*Oncorhynchus nerka*) captured at spawning channel arrival versus the calculated pre-spawning mortality (%) in the spawning channel in 2004 (circles; Chapter 3), 2005 (triangles; K Hruska, unpublished data), and 2006 (squares; Chapter 4).

Literature Cited

Blanchfield PJ and Ridgway MS. 1997. Reproductive timing and use of redd sites by lake-spawning brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 54:747-756.

Clark WK.1959. Kodiak bear-red salmon relationships at Karluk Lake, Alaska. Transactions of the North American Wildlife Conference 24:337-345.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Healey MC, Macdonald JS, Shrimpton JM, van Der Kraak G, and Farrell AP. 2006a. Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. Ecology 87:1575-1586.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Shrimpton JM, van Der Kraak G, and Farrell AP. 2006b. Physiology of individual Late-run Fraser River sockeye salmon (*Oncorhynchus nerka*) sampled in the ocean correlates with fate during spawning migration. Canadian Journal of Fisheries Aquatic Sciences 63:1469-1480.

Crossin GT, Hinch SG, Farrell AP, Higgs DA, Lotto AG, Oakes JD, and Healey MC. 2004. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. Journal of Fish Biology 65:788-810.

Crossin GT, Hinch SG, Cooke SJ, Welch DW, Lotto AG, Patterson DA, Jones SRM, Leggatt RA, Mathes MT, Shrimpton JM, van Der Kraak G, and Farrell AP. 2008. Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migrations. Canadian Journal of Zoology 86:127-140.

Crossin GT, Hinch SG, Cooke SJ, Cooperman MS, Patterson DA, Welch DW, Hanson KC, Olsson I, English KK, and Farrell AP. 2009. Mechanisms influencing the timing and success of reproductive migration in a capital breeding semelparous fish species, the sockeye salmon. Physiological and Biochemical Zoology 82:635-652.

Donaldson MR, Hinch SG, Patterson DA, Farrell AP, Shrimpton JM, Miller-Saunders KM, Robichaud D, Hills J, Hruska KA, Hanson KC English KK, van der Kraak G, and Cooke SJ. 2009. Physiological condition differentially affects the behaviour and survival of two populations of sockeye salmon during their freshwater spawning migration. Physiological and Biochemical Zoology. in press.

Farrell AP, Hinch SG, Cooke SJ, Patterson DA, Crossin GT, Lapointe M, Mathes MT. 2008. Pacific salmon in hot water: applying metabolic scope models and biotemetry to predict the success of spawning migrations. Physiological and Biochemical Zoology 81:697-708.

Ferrari MR, Miller JR, and Russell GL. 2007. Modeling changes in summer temperature of the Fraser River during the next century. Journal of Hydrology 342:336-346.

Foote CJ. 1990. An experimental comparison of males and female spawning territoriality in a Pacific salmon. Behaviour 115:283-314.

Gilhousen P. 1990. Prespawning mortalities of sockeye salmon in the Fraser River system and possible causal factors. International Pacific Salmon Fisheries Commission Bulletin 26.

Hendry AP, Morbey YE, Berg OK, and Wenburg JK. 2004. Adaptive variation in senescence: reproductive lifespan in a wild salmon population. Proceedings of the Royal of Society of London Series B 271:259-266.

Hinch SG, Cooke SJ, Healey MC, and Farrell AP. 2006. Behavioural physiology of fish migrations: salmon as a model approach. Pages 240-296 in Sloman KA, Wilson RW, and Balshine S (eds). Behaviour and Physiology of Fish. Elsevier, Amsterdam.

Mathes MT, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Lotto AG, and Farrell AP. 2010. Effect of water temperature, timing, physiological condition and lake thermal refugia on migrating adult Weaver Creek sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 67:70-84.

McPhee MV and Quinn TP. 1998. Factors affecting the duration of nest defense and reproductive lifespan of female sockeye salmon, *Oncorhynchus nerka*. Environmental Biology of Fishes 51:369-375.

Miller KM, Schulze AD, Ginther N, Li S, Patterson DA, Farrell AP, and Hinch SG. 2009. Salmon spawning migration: Metabolic shifts and environmental triggers. Comparative Biochemistry and Physiology, D Genomics and Proteomics 4:75-89.

Morbey YE and Ydenberg RC. 2003. Timing games in the reproductive phenology of female Pacific salmon (*Oncorhynchus* spp.). The American Naturalist 161:284-298.

Morrison J, Quick M, and Foreman MGG. 2002. Climate change in the Fraser River watershed: flow and temperature projections. Journal of Hydrology 263:230-244.

Nadeau PS, Hinch SG, Hruska KA, Pon LB, and Patterson DA. 2010. The effects of experimental energy depletion on the physiological condition and survival of adult sockeye salmon (*Oncorhynchus nerka*) during spawning migration. Environmental Biology of Fish 88:241-251.

Quinn TP and Foote CJ. 1994. The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon, *Oncorhynchus nerka*. Animal Behaviour 48:751-761.

Steen RP and Quinn TP. 1999. Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. Canadian Journal of Zoology 77:836-841.

van den Berghe EP and Gross MR 1989. Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution 43:125-140.

Wagner GN, Lotto AG, Kuchel L, Jones SRM, Patterson DA, Cooke SJ, Macdonald JS, van der Kraak G, Healey MC, Shrimpton M, English K, Hinch SG, and Farrell AP. 2005. Metabolic rates and swimming performance of adult Fraser River sockeye salmon (*Oncorhynchus nerka*) after a controlled infection with *Parvicapsula minibicornis*. Canadian Journal of Fisheries and Aquatic Sciences 62:2124–2133.

Young JL, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Farrell AP, van der Kraak G, Lotto AG, Lister A, Healey MC, and English KK. 2006. Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 63:1067-1077.

Appendix 1: Relation of female density on the spawning grounds to behaviour, reproductive longevity and egg retention

Methods

The study was carried out at the Weaver Creek Spawning Channel located in south western British Columbia (Figure 1.2). For a complete description of the spawning channel see Chapter 2.

The typical stocking of female sockeye salmon in the channel ranges from 10,000 to 20,000; in 2004, approximately 14,000 sockeye females were let into the spawning channel. Densities of females alive in the spawning channel generally range from 0.2 to 0.5 females$\cdot$m^{-2} (R Stitt, Weaver Creek Spawning Channel, FOC, personal communication). Average female density in the Weaver Creek Spawning Channel (1965-1997) was calculated as 0.83 females$\cdot$m^{-2} (Essington et al., 2000); however, these values do not take mortality into account.

On October 12-13, 2004, 56 sockeye salmon were captured from the fish holding area immediately downstream of the gated entrance to the spawning channel. Fish were captured by dip net and immediately bio-sampled (30-90 s duration) for another study (Chapter 3). See Chapter 3 for details on fish sampling and tagging. Fork length was used to size match fish among enclosures so each enclosure held fish with a similar range of sizes.

Four enclosures were built in the channel (each with surface dimensions of 3 m x 7.5 m, with the long axis along one bank of the channel; Figure A1.1). See Chapter 3 for a complete description of the enclosures. Fish were assigned into either a high density (HD; 9 males and 9 females, ~0.4 females$\cdot$m^{-2}) or low density (LD; 5 males and 5 females, ~0.2 females$\cdot$m^{-2}) enclosure. These densities were chosen to be near the high and low ends, respectively, of the density range recorded for sockeye salmon in the Weaver Creek Spawning Channel (Quinn, 1999). Density treatments were randomly assigned to the four enclosures; there were 2 replicates for each density. Observations were made from Oct. 12 to Oct. 22: the start date was chosen to capture fish near peak arrival at the spawning channel (Essington et al., 2000); the end date was the last day that a female was alive in any of the enclosures.

Each fish was observed for 5 min daily during daylight hours by a single observer (K Hruska). The order of observations was randomized daily. Observations were recorded by speaking into a digital audio recording device and later transcribed. Specific behaviours, which included charges, chases, bites, digs, quivers, and spawning events were recorded based on descriptions in Healey et al (2003). For each behaviour observed, the type of interaction, the status of the focal fish as the recipient or the aggressor, the identity of the interacting fish(es), and the time were recorded. Although observations were made on both males and females, the remainder of the methods and all results focus on female fish. I used my observations of digs, quivers, and spawning events to characterize the redd establishment and spawning status for each female. A female was considered to have established a redd when she was observed digging a redd and defending the area around the redd. Females were categorized based on the speed of redd establishment: early redd females established a redd within 2 d of arrival, late redd females took 3 d or more to establish a redd, and no redd females failed to establish a redd.

For each female, all attacks (i.e., chase, charge, or bite), regardless of whether the interacting fish was male or female, were grouped into two categories dependent on whether the focal fish gave or received the attack. The number of attacks given and the number of attacks received were then averaged over the total number of minutes that the fish was observed to obtain the number of attacks given per minute and the number of attacks received per minute, respectively.

Reproductive longevity for each fish was calculated as the number of days between placement in the enclosures and death. Gonadosomatic index at death (GSI_D) was calculated by dividing the remaining gonad mass by the somatic mass at death (i.e., total body mass minus gonad mass). Expected gonadosomatic index (GSI_E) was calculated based on the relationship between gonadosomatic index and somatic mass for unspawned, mature females in this population (K Hruska, unpublished data). Egg retention was then calculated as GSI_D / GSI_E * 100%. I classified females as completely spawned if the female had less than 0.5% egg retention at death, as a small number of eggs may occasionally become trapped behind an organ and thus be retained in the body cavity (Chapter 2).

All statistics were calculated using SAS 9.1 or JMP 4.0.4 (SAS Institute, USA). Reproductive longevity was log transformed, frequency of agonistic behaviour data were square root transformed and egg retention data were fifth root transformed to meet the assumptions of

normality and homogeneity of variances. I ran a one-way ANOVA on reproductive longevity and found a difference between mature and immature females; thus, I did not pool data across maturity classes for the remaining analyses. Effects of density on reproductive longevity and behavioural data were analyzed with a one-way ANOVA. Logistic regression was used to quantify the effects of reproductive longevity on completion of spawning. Chi-square analysis was used to determine whether completion of spawning was influenced by either density or maturity status. All means are given for untransformed data with their standard errors. Effects were considered significant at $\alpha = 0.05$.

Results

Length of time females were alive in the enclosures ranged from 3 to 10 d. Reproductive longevity was influenced by maturity status at arrival (F = 8.04; p < 0.001). Immature females lived an average of 2 d longer in the enclosures than females that arrived at the spawning channel in the mature state, and they lived longer in low density enclosures than in high density enclosures (Table A1.1; F = 6.42; p = 0.024). There was no effect of spawner density on reproductive longevity in mature females (F = 0.01; p = 0.906).

Egg retention ranged from 0 to ~100% and was not significantly related to either density or maturity status (Table A1.1). In each of the density and maturity groups, females spanned the full range of egg retention (i.e., from females with high levels of egg retention (> 70% egg retention at death) to completely spawned (< 0.5% egg retention at death)). Of the 28 females placed into the enclosures, 15 (54%) were classified as completely spawned at death. Completion of spawning was not significantly affected by either density ($\chi^2 = 1.15$; p = 0.283; HD = 39% of females completed spawning; LD = 60% of females completed spawning) or maturity status ($\chi^2 = 0.19$; p = 0.662; mature = 42% of females completed spawning; immature = 50% of females completed spawning). There was no relationship between fork length and either reproductive longevity or egg retention (p > 0.60).

There was a significant relationship between reproductive longevity and a female's ability to complete spawning ($\chi^2 = 4.42$; p = 0.036). When analyzed separately by density, I found a positive relationship between reproductive longevity and ability to complete spawning in high density enclosures (Figure A1.2; $\chi^2 = 6.19$; p = 0.013) but not in low density enclosures ($\chi^2 = 0.44$; p = 0.507).

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Among immature females, the frequency of attacks given was higher in high density enclosures than in low density enclosures (Table A1.1). Attacks given by mature females or received by immature females did not differ between high and low density. The variability in frequency of attacks given by mature females in high density enclosures was high as these females tended to be either very aggressive (i.e., two highest frequencies of attacks given) or very passive (i.e., lowest frequency of attacks given; Table A1.1). There was no difference in the frequency of attacks received for either maturity class (Table A1.1). Females that had a higher frequency of attacks as the aggressor had lower egg retention at death (Figure A1.3).

Twenty three of 28 females in the enclosures (82%) established redds within 2 d of arrival at the spawning channel. Neither density (χ^2 = 0.05; p = 0.825) nor maturity at arrival (χ^2 = 1.30; p = 0.255) had a significant effect on whether or not a female established a redd within 2 d of arrival. Fork length also had no influence on whether a female established a redd within 2 d of arrival (χ^2 = 1.04; p = 0.308). However, the females that established a redd within 2 d of arrival had lower levels of egg retention at death than females that took longer or failed to establish redds (F = 5.24; p = 0.030; redd within 2 d = 10.3 ± 3.8%; no redd within 2 d = 55.3 ± 22.8%).

Fork length of the females ranged from 55 to 67.5 cm. There was no significant difference in fork length between maturity classes (F = 3.16; p = 0.87; mature = 63.1 ± 0.8 cm; immature = 60.8 ± 0.9 cm).

Table A1.1: Mean (SE, n, and range) of reproductive longevity, egg retention, and behavioural endpoints in mature and immature female sockeye salmon spawning in 'high' (HD) and 'low' (LD) density enclosures at the Weaver Creek Spawning Channel in October, 2004. Untransformed values are shown. F (or χ^2) and p values are indicated. Values in bold indicate a significant effect at α = 0.05.

| Endpoint | Maturity | High Density | | | Low Density | | | F-value | P-value |
		Mean ± SEM	n	Range	Mean ±	n	Range		
# Attacks Given per Minute	mature	0.79 ± 0.41	6	0 – 2.2	0.44 ± 0.19	6	0.2 – 1.1	0.22	0.651
	immature	0.62 ± 0.09	12	0 – 1.2	0.25 ± 0.06	4	0.1 – 0.3	**7.26**	**0.018**
# Attacks Received per Minute	mature	0.52 ± 0.13	6	0.1 – 1.0	0.21 ± 0.07	6	0 – 0.5	4.56	0.059
	immature	0.40 ± 0.08	12	0 – 0.8	0.40 ± 0.13	4	0.1 – 0.6	<0.01	0.981
Reproductive longevity (days)	mature	4.8 ± 0.4	6	4 – 6	4.8 ± 0.5	6	3 - 6	0.01	0.906
	immature	6.0 ± 0.5	12	4 – 9	8.5 ± 0.6	4	7 - 10	**6.42**	**0.024**
Egg Retention (%)	mature	33.9 ± 18.9	6	0 – 105	12.5 ± 12.0	6	0 - 72	1.25	0.290
	immature	11.7 ± 6.1	12	0 – 75	23.5 ± 17.9	4	0 - 75	0.02	0.880

Water flow

6m

7.5m

3m

Enclosure #4
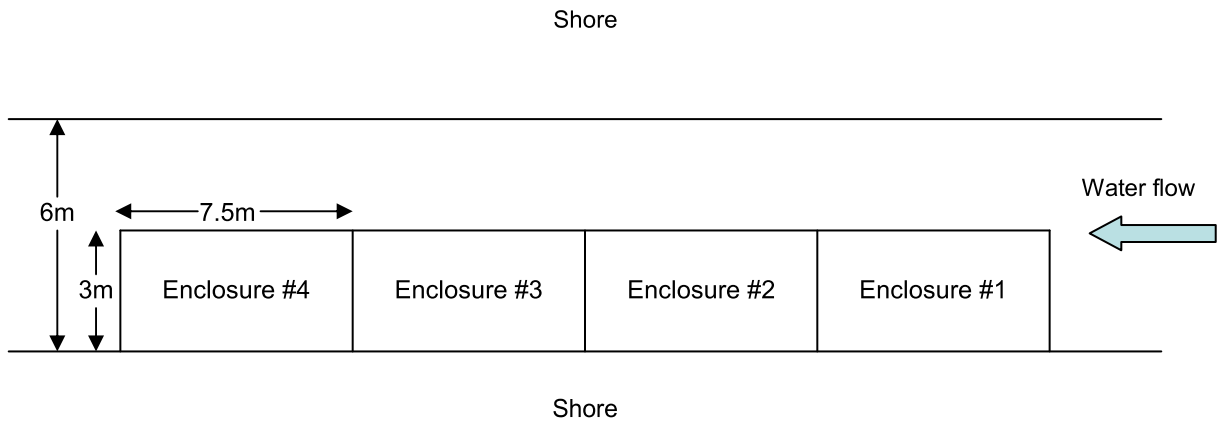
Enclosure #3

Enclosure #2

Enclosure #1

Shore

Figure A1.1: Diagrammatic representation of enclosure setup. Four enclosures (3 m wide by 7.5 m long) were lined up end to end along one bank of the spawning channel. The high density treatment enclosures were #1 and #3; the low density treatment enclosures were #2 and #4.
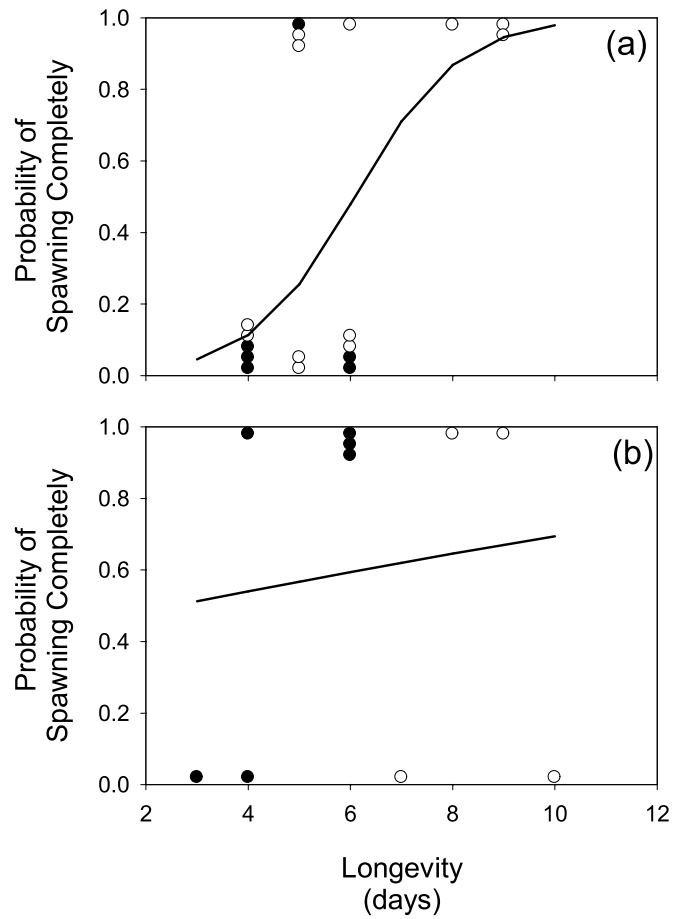
Figure A1.2: Probability of complete spawning as a function of reproductive longevity for female sockeye salmon placed in (a) high density (0.4 females•m^{-2}) and(b) low density (0.2 females•m^{-2}) (b) enclosures in Weaver Creek Spawning Channel in October, 2004. Black circles indicate females that were mature at arrival and open circles indicate females that arrived at the channel in an immature state.
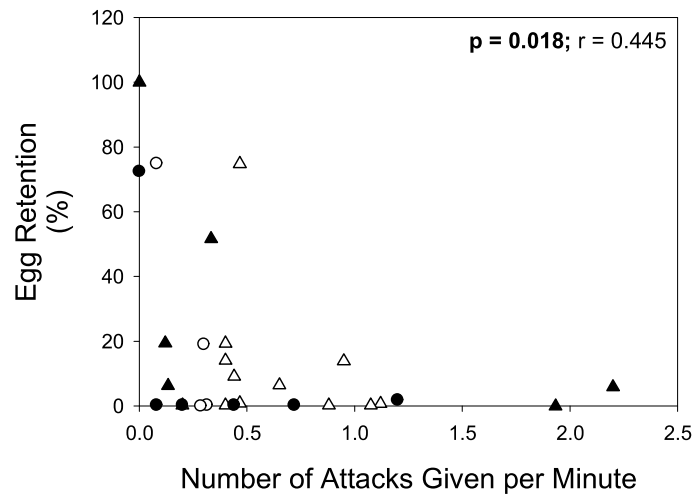
Figure A1.3: Scatterplot of egg retention vs. number of attacks given per minute for female sockeye salmon (*Oncorhynchus nerka*) placed in high density (0.4 females•m^{-2}; triangles) and low density (0.2 females•m^{-2}; circles) enclosures in Weaver Creek Spawning Channel in October, 2004. Black symbols indicate females that were mature at arrival and open symbols indicate females that arrived at the channel in an immature state.

Literature Cited

Essington TE, Quinn TP, and Ewert VE. 2000. Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences 57:205-213.

Healey MC, Lake R, and Hinch SG. 2003. Energy expenditures during reproduction by sockeye salmon (*Oncorhynchus nerka*). Behaviour 140:161-182.

Quinn TP. 1999. Variation in Pacific salmon reproductive behaviour associated with species, sex, and levels of competition. Behaviour 136:179-204.

Appendix 2: Animal Care Certificate

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), Early Stuart or Early Shuswap stock 70
> Trout wild Rainbow trout (Oncorhynchus mykiss) 1000
> Salmon Sockeye salmon (O. nerka), Late runs 500
> Salmon Sockeye salmon (O. nerka), Gates Creek stock 140
> 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:	Climate warming and high salmon migration mortality
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:	Natural Sciences and Engineering Research Council of Canada (NSERC)
Funding Title:	Abnormal migration and premature mortality in Pacific salmon
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:	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 Pacific Salmon Forum
Funding Title:	Migrations, spawning behaviours, and physiology of wild adult sockeye salmon in the Fraser River: impacts of global warming scenario
Funding Agency:	Natural Sciences and Engineering Research Council of Canada (NSERC)
Funding Title:	Energetics, behaviour and fitness of anadromous migrating fish
Unfunded title:	Climate change and high salmon mortality

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.

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