Influences of Sex and Activity Level on Physiological Changes in Individual Adult Sockeye Salmon during Rapid Senescence

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ABSTRACT

A noninvasive biopsy protocol was used to sample plasma and gill tissue in individual sockeye salmon (Oncorhynchus nerka) during the critical life stage associated with spawning—arrival at a spawning channel through senescence to death several days later. Our main objective was to characterize the physiological changes associated with rapid senescence in terms of the physiological stress/cortisol hypersecretion model and the energy exhaustion model. Salmon lived an average of 5 d in the spawning channel, during which time there were three major physiological trends that were independent of sexual status: a large increase in plasma indicators of stress and exercise (i.e., lactate and cortisol), a decrease in the major plasma ions (i.e., Cl− and Na+) and osmolality, and a decrease in gross somatic energy reserves. Contrary to a generalized stress response, plasma glucose decreased in approximately 2/3 of the fish after arrival, as opposed to increasing. Furthermore, plasma cortisol levels at spawning-ground arrival were not correlated with the degree of ionoregulatory changes during rapid senescence. One mechanism of mortality in some fish may involve the exhaustion of energy reserves, resulting in the inability to mobilize plasma glucose. Sex had a significant modulating effect on the degree of physiological change. Females exhibited a greater magnitude of change for gross somatic energy, osmolality, and plasma concentrations of Cl−, Na+, cortisol, testosterone, 11-ketotestosterone, 17,20β-progesterone, and estradiol. The activity level of an individual on the spawning grounds appeared to influence the degree of some physiological changes during senescence. For example, males that received a greater frequency of attacks exhibited larger net decreases in plasma 11-ketotestosterone while on the spawning grounds. These results suggest that rapid senescence on spawning grounds is influenced by multiple physiological processes and perhaps behavior. This study provides some of the first data to look at sex differences in senescence in Pacific salmon.

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 that live longer on the spawning grounds not only have lower levels of egg retention at death (K. Hruska unpublished) but also are able to guard their reds longer in order to prevent superimposition by later-arriving females (Morbey and Ydenberg 2003; Hendry et al. 2004). Therefore, there is considerable interest in the mechanisms that cause and prolong the senescence process. This study examined individual sockeye salmon (Oncorhynchus nerka) during residence on the spawning grounds in order to characterize the physiological changes associated with senescence.

Through the use of physiological telemetry, adult Fraser River sockeye salmon have become one of the best-studied fishes in terms of understanding the behavioral physiology of migrations (Cooke et al. 2008). Reproductive hormone and osmoregulatory indexes suggest that fish are preparing for entry into freshwater and spawning >700 km from the Fraser River mouth (Crossin et al. 2009). Symptoms of immunosuppression and disease are also becoming evident at this phase of their migra-
tion (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 gall 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, 2006b; Crossin et al. 2009) indicate that this is a particularly stressful and active phase of their migration. Extreme freshwater conditions (e.g., high temperatures or flows) cause additional physiological stress 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 immunocompromised, 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, 2006b; 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 is due to 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 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 Overbeke 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 not only to high plasma cortisol concentrations but also to a loss of homeostatic balance during migration (Hinch et al. 2006; Nadeau 2007). Marine and river biopsy telemetry research has found that migrating adult Pacific salmon were less likely to reach spawning grounds if fish displayed indexes of ionoregulatory or metabolic stress (e.g., high plasma concentrations of Na⁺, osmolality, and lactate; Cooke et al. 2006a, 2006b; 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 (K. Hruska, unpublished data). 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 are 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 fueled by endogenous energy reserves (Hinch et al. 2006). Measurements of whole-body energy reserves in adult sockeye salmon and pink salmon (Oncorhynchus gorbuscha) have found, across several different populations, that moribund adults on the spawning grounds all have similar gross somatic energy levels (∼4 MJ/kg), 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 longevity on the spawning grounds (Mehranvar 2002). McBride et al. (1965) actually extended the life span of maturing or spawned sockeye salmon by up to 10 wk (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 its migration fate; females with relatively low levels of gross somatic energy tended to perish before reaching spawning areas (Cooke et al. 2006a, 2006b; Young et al. 2006; Crossin et al. 2008).

Overlying these physiological mechanisms are individual animal behaviors, 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 behavior has been shown to affect blood physiology, such as plasma hormone (e.g., testosterone, 11-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 spawning-ground longevity of an individual (Van den Berghe and Gross 1986); however, evidence to support this theory has been contradictory (Foote 1990; Hendry et al. 2001; Healey et al. 2003; Morrey and Ydenberg 2003).

Our main objective was to examine the rapid-senescence
phenomenon in terms of the physiological stress/cortisol hypersecretion and energy exhaustion hypotheses. We also wanted to determine whether activity patterns of individual fish affect the physiological changes observed while on the spawning grounds. 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). We biopsied sockeye salmon on arrival at the spawning grounds and when they became moribund, and we observed their activity patterns, longevity, and egg deposition success during residence on the spawning grounds.

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 that 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/kg. We predict that fish that die due to energy exhaustion would exhibit hypoglycemia (i.e., plasma glucose concentrations <4 mmol/L) due to an inability to mobilize energy reserves. We 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.

Material 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 (Fig. 1). 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 (for a complete description of the channel, see Quinn 1999). 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 m wide × 7.5 m long) were constructed in the channel. The walls of the enclosures were constructed of wooden frames covered with Vexar (Masternet, Mississauga, Ontario). 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 × 50-mm Vexar, which maximized through-flow of water relative to strength, whereas the sides were covered with 20 × 20-mm Vexar, to prevent fish from snagging their tags on the side. Vexar (20 mm × 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 (Hous- ton 1990), using a heparanized vacutainer (1.5 inch, 21 gauge). The blood sample was placed in an ice-water slurry for a max-
(2004) for use on fish) can have significant effects on plasma cortisol, glucose, and lactate concentrations (Moliner and Gonzalez 1995).

Fish were anesthetized in 60 mg/L MS-222 for 120–150 s to obtain an anesthesia level of 5 (i.e., loss of gross body movements and cessation of opercular movements) for the remaining measurements and/or for the electromyogram 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, West Lothian, Scotland), following the procedures of Crossin and Hinch (2005).

Electromyogram radio transmitters (cylindrically shaped, 53 mm length, 16 mm diameter, 18.5 g mass; Lotek Wireless, Newmarket, Ontario) 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 of 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 we implanted transmitters between the skin and the 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 that in the males. The surgeries to implant the transmitters took approximately 5 min.

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 treatment (nine males and nine females) and a low-density treatment (five males and five females); fish were allocated to the two densities for another study (K. Hruska, S. Hinch, and M. Healey, unpublished data). 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 indexes of social reproductive success and link prespawning energy levels with reproductive behavior. We used similar procedures, study site, and enclosure design to study the same stock of sockeye salmon as used by 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 behavioral interaction observed. Number of attacks given was 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., 0–4 in the low-density enclosures and 0–8 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 resampled 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; the 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 slab 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 airtight plastic bag at −20°C until further processing.

Longevity for each fish was calculated as the number of days between initial sample and death. Gonadosomatic index at death (GSI<sub>d</sub>) 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<sub>e</sub>) 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<sub>e</sub>/GSI<sub>d</sub> × 100%.

The pieces of dorsal muscle were homogenized and proximate constituent analysis was performed on a subsample of tissue homogenate, following procedures of Crossin et al. (2004). 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
Physiological Changes during Senescence in Sockeye Salmon

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, Chicago). We used α = 0.05 for all tests. Because of multiple comparisons, we applied Bonferroni corrections to minimize the chance of a Type II error (Rice 1989). However, Bonferroni corrections are highly conservative, so we indicated effects at α = 0.05 to allow the readers to define for themselves effects that are biologically meaningful (Cabin and Mitchell 2000).

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

Plasma testosterone, chloride, cortisol, and glucose were selected to illustrate individual patterns of physiological changes during senescence. We chose chloride because it is one of the major ions found in the plasma and is expected to change in response to stressors. We 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 displayed because it is an indicator of physiological stress in fish and tends to increase in response to stimulation of the hypothalamo-chromaffin cell axis (Wendelaar Bonga 1997). Cortisol was selected because it is the major stress hormone in fish, it has been linked to the senescent changes in Pacific salmon, and its release is triggered by activation of the hypothalamus-pituitary-interrenal axis (McDonald and Milligan 1992; Wendelaar Bonga 1997).

To explore the individual-level patterns in senescence, we correlated parameter values at arrival and at moribund, we correlated gross somatic energy reserves with plasma ion concentrations in moribund fish, we correlated plasma cortisol concentrations at spawning-ground arrival with net changes in all other parameters, and we correlated activity level rankings with net changes in physiology.

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 rebiopsied. 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 1). Plasma concentrations of K+, and especially lactate and cortisol, increased in both sexes, and 17,20β-progesterone increased in males (Table 1). In contrast, plasma osmolality and plasma concentrations of Cl−, Na+, and especially testosterone, 11-ketotestosterone, and estradiol decreased significantly in both sexes; 17,20β-progesterone decreased significantly in females (Table 1).

Females exhibited larger decreases in ions and energy during senescence than did 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 1), irrespective of whether they arrived with plasma concentrations higher (i.e., testosterone, 17,20β-progesterone, estradiol) or lower (i.e., 11-ketotestosterone) than those of males. In fact, 17,20β-progesterone increased in males during senescence.

Longevity was similar for females and males: 5.3 ± 0.5 and 5.2 ± 0.4 d, respectively. (For all 56 salmon, longevity was 5.9 ± 0.4 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%-75%); only one female retained more than 25% of eggs at death. Median GSI, for males was 1.4% (range = 0.6%-2.6%). The males that underwent the electromyogram (EMG) surgery had shorter longevity (F = 20.8, P = 0.002; EMG = 4.33 ± 0.30; no EMG = 6.50 ± 0.37) and had lower plasma osmolality (F = 8.70, P = 0.019; EMG = −24.83 ± 4.66; no EMG = −3.12 ± 5.70). There were no significant differences in other blood physiological parameters in males or in any endpoint in females between individuals with EMG transmitters and those without at α = 0.05. There were no significant differences in longevity, egg retention, or net change in plasma physiology between high- and low-density enclosures for either males or females at α = 0.05.

When we 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, we found four significant correlations for females (i.e., glucose, estradiol, 17,20β-progesterone, and gill Na+/K+ ATPase activity; Table 2). However, with the application of Bonferroni corrections, only one of these correlations was still significant (females: estradiol, P = 0.005). Glucose was the only parameter for which a significant negative correlation between arrival and moribund values was detected (Table 2; P = 0.026, r = −0.612); females that arrived at the spawning channel with relatively high plasma glucose (≥5.5 mmol/L) concentrations tended to exhibit a decrease in plasma glucose concentrations when they were moribund (Table 2).

In females, there was a positive correlation between gross somatic energy reserves and plasma glucose concentrations in the moribund state (Fig. 2; P = 0.028, r = 0.607). Females with final energy reserves less than ~3.5 MJ/kg had final plasma glucose concentrations less than 4 mmol/L. All of the females with plasma glucose concentrations less than 4 mmol/L at moribund exhibited a net decrease in plasma glucose concentration.
Table 1: Mean (SE and \( n \)) of all physiological measures from male and female sockeye salmon (\textit{Oncorhynchus nerka}) at spawning-ground arrival and in moribund state in the Weaver Creek Spawning Channel in October 2004

<table>
<thead>
<tr>
<th>Category and Parameter</th>
<th>Female Arrival</th>
<th>Moribund</th>
<th>Male Arrival</th>
<th>Moribund</th>
<th>Sample Period (( t ) Value)</th>
<th>Sex Change (( F ) Value)</th>
<th>Sex Mean (( F ) Value)</th>
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<td>Osmolality (mmol/L)</td>
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<td>-6.77</td>
<td>18.56</td>
<td>3.37</td>
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<td>( P )</td>
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<td>Mean ± SE</td>
<td>291.8 ± 3.0</td>
<td>242.0 ± 6.1</td>
<td>284.7 ± 3.1</td>
<td>268.5 ± 4.9</td>
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<td>Cl(^-) (mmol/L):</td>
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<td>-14.71</td>
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<td>Mean ± SE</td>
<td>129.8 ± 1.1</td>
<td>80.7 ± 2.3</td>
<td>124.1 ± .6</td>
<td>95.9 ± 2.2</td>
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<td>Na(^+) (mmol/L)</td>
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<td>-10.22</td>
<td>16.03</td>
<td>20.57</td>
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<td>Mean ± SE</td>
<td>151.7 ± 1.9</td>
<td>108.6 ± 2.5</td>
<td>151.4 ± 1.8</td>
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<td>K(^+) (mmol/L)</td>
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<td>3.75</td>
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<td>Mean ± SE</td>
<td>2.01 ± .19</td>
<td>2.78 ± .28</td>
<td>2.59 ± .20</td>
<td>4.34 ± .60</td>
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<td>Gill Na(^+)/K(^+) ATPase activity (( \mu \text{mol ADP mg}^{-1} \text{protein h}^{-1} ))</td>
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<td>-1.11</td>
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<td>7</td>
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<td>Mean ± SE</td>
<td>2.28 ± .19</td>
<td>2.16 ± .15</td>
<td>2.44 ± .22</td>
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<td><strong>Stress metabolites:</strong></td>
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<td>Lactate (mmol/L)</td>
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<td>14.05</td>
<td>.32</td>
<td>.05</td>
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<td>( P )</td>
<td>&lt;.001</td>
<td>.577</td>
<td>.820</td>
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<tr>
<td>Mean ± SE</td>
<td>1.53 ± .13</td>
<td>12.66 ± 1.16</td>
<td>1.26 ± .17</td>
<td>13.55 ± 1.21</td>
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<tr>
<td>Glucose (mmol/L)</td>
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<td></td>
<td></td>
<td></td>
<td>-.16</td>
<td>.19</td>
<td>.55</td>
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<tr>
<td>( P )</td>
<td>.876</td>
<td>.666</td>
<td>.468</td>
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<tr>
<td>Mean ± SE</td>
<td>5.58 ± .21</td>
<td>5.08 ± 1.39</td>
<td>4.59 ± .13</td>
<td>4.91 ± .74</td>
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<td>Cortisol (ng/mL)</td>
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<td></td>
<td>9.18</td>
<td>4.54</td>
<td>20.12</td>
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<tr>
<td>( P )</td>
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<td>.049*</td>
<td>&lt;.001</td>
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<tr>
<td>Mean ± SE</td>
<td>350 ± 60</td>
<td>1,287 ± 84</td>
<td>91 ± 16</td>
<td>737 ± 136</td>
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<td>Testosterone (ng/mL)</td>
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<td>-8.22</td>
<td>31.92</td>
<td>17.04</td>
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<tr>
<td>Mean ± SE</td>
<td>39.3 ± 2.7</td>
<td>5.6 ± .9</td>
<td>21.3 ± 1.6</td>
<td>9.3 ± 1.1</td>
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<td><strong>Reproductive hormones:</strong></td>
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<td>11-KT (ng/mL)(^a)</td>
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<td>-5.29</td>
<td>17.28</td>
<td>276.99</td>
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<tr>
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<td>( n )</td>
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between arrival and the moribund state (Fig. 3). These data provide evidence for a link between low energy reserves (i.e., <3.5 MJ/kg) 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 level (4.05 MJ/kg) had a low plasma glucose concentration (i.e., 1.94 mmol/L). This female was also anomalous due to her high level of egg retention at death (egg retention = 75%; range of all other females = 0%–21%). The females that died with low gross somatic energy levels (<3.5 MJ/kg) also had significantly lower plasma concentrations of Cl− and Na+ and osmolality in the moribund state (Fig. 2). In moribund males, there were no significant relationships between energy reserves and plasma glucose, Cl−, or Na+ concentrations or plasma osmolality. Males died with energy reserves significantly higher than those of females.

In males, there were significant correlations between plasma cortisol concentrations at arrival and the net change in plasma lactate ($P = 0.004, r = 0.820$), K+ ($P = 0.007, r = 0.785$), and 11-ketotestosterone ($P = 0.010, r = 0.762$) concentrations based on Bonferroni corrections (Fig. 4). 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 ($P = 0.022, r = 0.709$); 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 ($P = 0.007, r = 0.785$). Females that participated in a greater frequency of aggressive interactions had greater increase in plasma cortisol concentrations ($P = 0.040, r = 0.598$), as well as greater decreases in plasma testosterone ($P = 0.028, r = 0.630$) and 11-ketotestosterone ($P = 0.031, r = 0.621$) during senescence, but these relationships did not reach significance after Bonferroni correction.

**Discussion**

We observed major changes in the blood physiology of individual sockeye salmon during the senescent period between spawning-ground arrival and death; 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 we observed followed our predictions based on previous studies on senescence in Pacific salmon (Robertson and Wexler 1957; Dickhoff 1989; Finch 1990; Stein-Behrens and Sapolsky 1992). In addition to the expected sex differences in reproductive hormones and cortisol, we also observed differences between males and females in the changes in the major plasma ions. The repeated sampling of blood physiology in individual fish allowed us to characterize the interplay between physiological changes and activity levels and longevity on the spawning grounds.

**Physiological Stress/Cortisol Hypersecretion Hypothesis**

The physiological stress/cortisol hypersecretion model predicts that fish will show signs of physiological stress as they senesce.
This prediction is supported by our data. The sockeye salmon in our 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 when fish face a severe stressor (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 our study, the mean net change in plasma electrolyte concentrations in females was substantial (e.g., mean ΔCl\(^-\) ∼50 mmol/L 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 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 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 that was more pronounced in females (350 ng/mL) than in males (91 ng/mL). Plasma cortisol concentrations in adult Pacific salmon were well above basal plasma cortisol levels in other salmonids (e.g., 5–10 ng/mL; Pickering and Pottinger 1989) but were similar to concentrations recorded following an acute stressor (e.g., 30–300 ng/mL; Barton 2002). However, our results were consistent with values reported in the literature for migrating and sexually maturing Pacific salmon in terms of both 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 were measured in a land-locked population of kokanee salmon (Oncorhynchus 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 well after the blood samples were taken. Kubokawa et al. (1999) showed significant effects of handling stress on cortisol at 15 min after the stressor; the processing of our 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,
Physiological Changes during Senescence in Sockeye Salmon

Figure 2. Correlations between gross somatic energy concentration and plasma ion concentrations in moribund female (filled 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 1.

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 before initiating their freshwater migration (Burgner 1991). We expected that males would use more of their energy reserves on the spawning grounds than would females because males tend to be more active (Healey et al. 2003; K. Hruska, unpublished data). However, our 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 our 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 our 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 approximately 4 MJ/kg, which we
expected on the basis of the results of Crossin et al. (2003, 2004).

Many of the fish in our study experienced hypoglycemia during senescence, which would be expected if these fish had exhausted energy stores. Thus, we 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 downregulated during migration but is upregulated again during spawning, indicating that glycolysis is important for fueling activity on the spawning grounds (French et al. 1983; Miller et al. 2009). The females
Physiological Changes during Senescence in Sockeye Salmon

that had the lowest energy reserves at death also had significantly lower levels of osmolality, Cl\(^{-}\), and Na\(^{+}\) when they were moribund. Thus, while our 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 different mechanisms of mortality for some of the fish.

Other Factors

Our 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 our sockeye salmon. There are other factors that we did not specifically examine, such as disease, parasite loads, or injury, that 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 immunocompromised 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 electromyogram transmitter implantation had effects on the males but not on the females. The greater net decrease in plasma osmolality in males with transmitters supports our observations of differences between the sexes in physiological changes during senescence. When transmitter-implanted males were excluded from the analysis, greater differences between the sexes in net change in plasma osmolality were evident.

We 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 did males that received more attacks. The 11-ketotestosterone has been strongly associated with aggressive behavior 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 downregulate 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 we were 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 did 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. 2006a, 2006b; Crossin et al. 2009), the physiological causes of such mortality have been difficult to ascertain because individuals are biopsied only at the start of the observation period. Ours 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 our study should provide a baseline model of the blood physiology
changes that sockeye salmon undergo as they senesce and die. Pacific salmon are increasingly experiencing 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.

Acknowledgments

All procedures used in this study were approved by the Canadian Council on Animal Care, administered by the University of British Columbia, Simon Fraser University, and Fisheries and Oceans Canada. Tagging and physiological sampling support was provided by Andrew Lotto, Steve Cooke, Glenn Crossin, Glenn Wagner, Jeff Young, Jayme Hills, and Lucas Pon. We also acknowledge the invaluable assistance of Rick Stitt, Wayne Charlie, Fisheries and Oceans Canada, and the Weaver Creek Spawning Channel crew. Physiological assays were conducted by Jayme Davidson and Jeanette Garries. The project was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Strategic Grant to S.G.H., A.P.F., and M.C.H. and an NSERC Discovery Grant to S.G.H. K.A.H. was supported by an NSERC graduate scholarship. Sampling support was provided through the Environmental Watch Program.

Literature Cited


