

Fitness and behavioral correlates of pre-stress and stress-induced plasma cortisol titers in pink salmon (*Oncorhynchus gorbuscha*) upon arrival at spawning grounds

K.V. Cook^{a,*}, S.H. McConnachie^a, K.M. Gilmour^b, S.G. Hinch^c, S.J. Cooke^{a,d}

^a Fish Ecology and Conservation Physiology Laboratory, Biology Department, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6

^b Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON, Canada K1N 6N5

^c Forest Sciences Department, University of British Columbia, 2424 Main Mall, Vancouver, BC, Canada V6T 1Z4

^d Institute of Environmental Science, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6

ARTICLE INFO

Article history:

Received 25 March 2011

Revised 26 July 2011

Accepted 26 July 2011

Available online 2 August 2011

Keywords:

Stress response

Cortisol

Fish

Cort-fitness hypothesis

Pacific salmon

Spawning behavior

Longevity

Reproductive success

Glucocorticoid

ABSTRACT

Semelparous Pacific salmon (*Oncorhynchus* spp.) serve as an excellent model for examining the relationships between life history, behavior and individual variation in glucocorticoid (GC) stress hormone levels because reproductive behaviors are highly variable between individuals and failure to reproduce results in zero fitness. Pink salmon (*O. gorbuscha*) were intercepted upon arrival at the spawning grounds across three time periods. Pre-stress and stress-induced plasma cortisol concentrations were assessed in relation to behavior, longevity and reproductive success. Results revealed differences between sexes and with arrival time. The study period marked a year of high reproductive success and only nine females (12% of sample) failed to spawn. Female pre-spawn mortalities were characterized by significantly elevated stress-induced cortisol concentrations and decreased longevity as well as pre-stress cortisol above the normal range in pink salmon from the study area. Interestingly, reproductive behaviors were only associated with pre-stress cortisol levels. For females, aggression and mate interaction time were reduced in individuals with elevated pre-stress cortisol concentrations. In males, a similar negative relationship between pre-stress cortisol concentration and mate interaction time was detected. The observed behavioral correlations are likely a factor of social status where dominant individuals, known to have higher reproductive success, are characterized by lower cortisol levels relative to subordinate conspecifics. Findings show both elevated pre-stress and stress-induced cortisol concentrations at arrival to the spawning grounds to be associated with reduced survival.

© 2011 Elsevier Inc. All rights reserved.

Introduction

Individual variation in glucocorticoid (GC) stress hormones are thought to mediate life history trade-offs (Ricklefs and Wikelski, 2002) and behavior (Koolhaas et al., 1999). However, fish remain an underused model to assess these relationships (Mommensen et al., 1999; Wingfield, 2003) despite wide variation in reproductive strategies and behavior as well as a thorough understanding of their stress physiology (see Barton, 2002; Mommensen et al., 1999; Wendelaar Bonga, 1997). When teleost fish are exposed to an acute stressor, the hypothalamic-pituitary-interrenal (HPI) axis is activated resulting in an increase in circulating cortisol concentrations. Cortisol, the primary GC hormone in fish, acts to mobilize energy stores allowing the individual to cope with the stressor (Barton, 2002; Wendelaar Bonga, 1997). As

this steroid is synthesized and then secreted upon demand, levels begin to rise several minutes after exposure to a stressor. It is therefore possible to measure pre-stress (baseline) concentrations as well as stress-induced values (the absolute post-stress value) and the total response (change from pre-stress to stress-induced concentrations) within an individual (Romero, 2004). These measures exhibit considerable variability that is shown to be consistent within an individual (Pottinger et al., 1992; Romero and Reed, 2008).

Recent interest has emerged in linking variation in GC titers with measures of individual fitness across a range of taxa (Bonier et al., 2009a; Breuner et al., 2008). The “Cort-Fitness hypothesis” has been proposed whereby elevated pre-stress cortisol levels are assumed to indicate an individual or population of reduced relative fitness (Bonier et al., 2009a). The physiological response to acute stress is also an adaptive mechanism that facilitates escape from challenging situations (Wingfield et al., 1998). Therefore, intuitively, it would seem that a greater stress response would have positive fitness effects. However, launching a stress response is energetically demanding. Given the energy available to an animal is finite, this stress response results in the reallocation of energy away from costly activities (i.e. territoriality, courtship and immune defense) to self-maintenance

* Corresponding author.

E-mail addresses: katrina.vcook@gmail.com (K.V. Cook),

s.h.mconnachie@gmail.com (S.H. McConnachie), Kathleen.Gilmour@uottawa.ca (K.M. Gilmour), scott.hinch@ubc.ca (S.G. Hinch), scooke@connect.carleton.ca (S.J. Cooke).

and current survival (Ricklefs and Wikelski, 2002; Wingfield et al., 1998). Studies correlating GC concentrations with direct measures of fitness have yielded inconsistent results (Bonier et al., 2009a; Breuner et al., 2008). A common thread throughout the literature is that these relationships are highly context dependant, being influenced by a variety of factors including life history stage (Bonier et al., 2009b), environmental stability (Angelier et al., 2009) and energetic constraints (Angelier et al., 2010; Cote et al., 2010). For example, as animals adopt strategies that maximize individual fitness, short-lived animals with a greater investment in reproduction will likely not exhibit the same trade-offs between survival and reproduction (i.e. Ricklefs and Wikelski, 2002) as long-lived animals with future breeding opportunities (Wingfield and Sapolsky, 2003). However, physiological diversity is maintained in all cases and very little is known regarding the consequences of this diversity in semelparous species.

Semelparous Pacific salmon (*Oncorhynchus* spp.) serve as an interesting and practical model for exploring performance correlates of GCs. Fitness metrics such as spawning behavior and reproductive success can be easily quantified (Healey et al., 2003; Mehranvar et al., 2004; Quinn, 1999) and lifetime reproductive output is compressed into a single breeding effort allowing for direct quantification of fitness. Cortisol has both inhibitory and stimulatory effects in Pacific salmon. Stress indicators (e.g. osmolality, lactate, plasma cortisol and glucose) markedly increase in the transition to freshwater (Cooke et al., 2006a; Cooke et al., 2006b; Crossin et al., 2009) and throughout the migration (Crossin et al., 2003; Hendry and Berg, 1999). These elevated concentrations assist with natal stream homing (Carruth et al., 2002) and can accelerate ovulation (Schreck, 2010) but also cause tissue degeneration (Hendry and Berg, 1999; Maldonado et al., 2000) and suppress immune function (Maule et al., 1996). The physiological profiles of spawning Pacific salmon are unique. Most notably, cortisol levels are exceptionally high. The HPI axis becomes hyperactivated and hyperadrenocorticism occurs as a result of hyperplasia of the interrenal cells (Robertson et al., 1961). In fact, post-spawning mortality is largely due to the deleterious effects of excessive levels of cortisol. High concentrations are linked to a systematic degeneration of glands and organs that culminates in programmed death (McQuillan et al., 2003; Robertson and Wexler, 1959).

In addition to the emergence of disease and the onset of somatic degeneration, Pacific salmon are also limited by energy on the spawning grounds. Once in freshwater, they cease feeding and depend entirely on endogenous energy reserves. Over 50% of body energy is required to reach the spawning grounds (Crossin et al., 2004) and upon arrival, highly aggressive behavior and territoriality pose additional stress and energetic costs (Healey et al., 2003). Females compete for access to quality nest sites and defend their territories (Essington et al., 2000) and in males, aggression determines access to mates (Healey et al., 2003). Spawning behaviors in Pacific salmon have been quantified in relation to energy allocation (Healey et al., 2003), reproductive success (Mehranvar et al., 2004) and pre-stress stress indicators (Hruska et al., 2010), but not to our knowledge in relation to acute GC concentrations.

In this study we characterized pre-stress and stress-induced levels of circulating cortisol concentrations in pink salmon (*O. gorbuscha*) on the spawning grounds across three different arrival times in relation to behavior, longevity (total time in the channel) and reproductive success (percentage of eggs released, in females only). Longevity has fitness consequences for both sexes. For males, the longer they sustain reproductive maturity in the channel, the more likely they are to mate with multiple females and the longer a female defends a nest, the less likely it will be dug up by subsequent females (Quinn and Foote, 1994). We hypothesized that cortisol concentrations would differ between sexes and with arrival date. Given previous comparisons of cortisol concentrations between sexes of mature Pacific salmon (Donaldson

and Fagerlund, 1972), we predicted concentrations would be higher in females. With respect to arrival date, later-arriving fish arrive in an advanced state of maturity and cortisol concentrations increase as Pacific salmon mature and approach senescence (McQuillan et al., 2003). However, animals with high reproductive investment are expected to have an attenuated stress response (Wingfield and Sapolsky, 2003). Therefore, we predicted pre-stress cortisol levels would increase but stress-induced cortisol would decrease with arrival date. Our second hypothesis was that cortisol concentrations would differ between reproductively successful and unsuccessful females. Maintaining elevated GCs is energetically costly (Wingfield and Sapolsky, 2003) and spawning fish have very limited energy resources (Crossin et al., 2004). We predicted that those fish with elevated levels of pre-stress and stress-induced cortisol would have decreased longevity and spend fewer days on a redd. Lastly, we hypothesized that cortisol plays a role in defining reproductive behaviors within an individual. In juvenile salmonids, subordinate social status is a chronic stressor characterized by elevated baseline cortisol levels (Gilmour et al., 2005) and in populations of rainbow trout (*O. mykiss*), individuals with a consistently low cortisol response to standardized stressors become socially dominant (Øverli et al., 2000). We therefore predicted more aggressive, dominant individuals to have decreased pre-stress and stress-induced cortisol. However, we also predicted for an effect of size, as larger fish are known to hold dominant positions in some species of Pacific salmon (Foote, 1990).

Methods

All work was conducted in accordance with CCAC guidelines under an animal care permit obtained from Carleton University and Fisheries and Oceans Canada. Research was carried out in October 2009 at the Weaver Creek Spawning Channel, located in the lower Fraser valley in British Columbia, Canada (Fig. 1). The 2930 m long and 6.1 m wide artificial spawning channel is approximately 100 km from the mouth of the Fraser River and 2 km downstream of Harrison Lake (Quinn, 1999; Fig. 1). The spawning channel was constructed from a small tributary of the Fraser River to enhance salmon production, particularly sockeye (*O. nerka*), but chum (*O. keta*) and pink salmon are also present and abundant. In this closed system, gravel substrate is of appropriate size for spawning (1.2–7.6 cm), depth is consistent (25–30 cm), flow conditions are controlled and stable (0.4 m/s), and fish densities are monitored (Quinn, 1999).

Physiological sampling and determination of reproductive success

Upon leaving the natural river system, fish ascend a pool and weir fishway (distance of 15 m) to enter a concrete holding area from which they are passed through a counting fence into the spawning channel (see Fig. 1). This holding area is approximately 1.5 deep, 2.5 wide and 100 m long and has relatively high densities of all 3 species. Water quality is monitored by Fisheries and Oceans Canada and densities are adjusted accordingly to maintain adequate levels of dissolved oxygen. How long a fish was resident in the holding area is a function of arrival time and spawning channel management practices (i.e. the number of each species in the channel at the time). Fish were collected from this holding area in the fall of 2009 in three different groups. Sampling groups were spread across the run representing early (Oct. 5th), middle (Oct. 9th) and late (Oct. 13th) timing groups from the population and all sampling occurred in the morning of each day. The spawning arrival period for this species at Weaver Creek was between ~Oct. 1st and 18th and based on external sexual characteristics, level of maturity increased with arrival date. Many early arriving fish entered in the channel but were not yet ready to spawn and did not immediately form territories. It was assumed that all fish at one sampling time were of similar maturity and had an

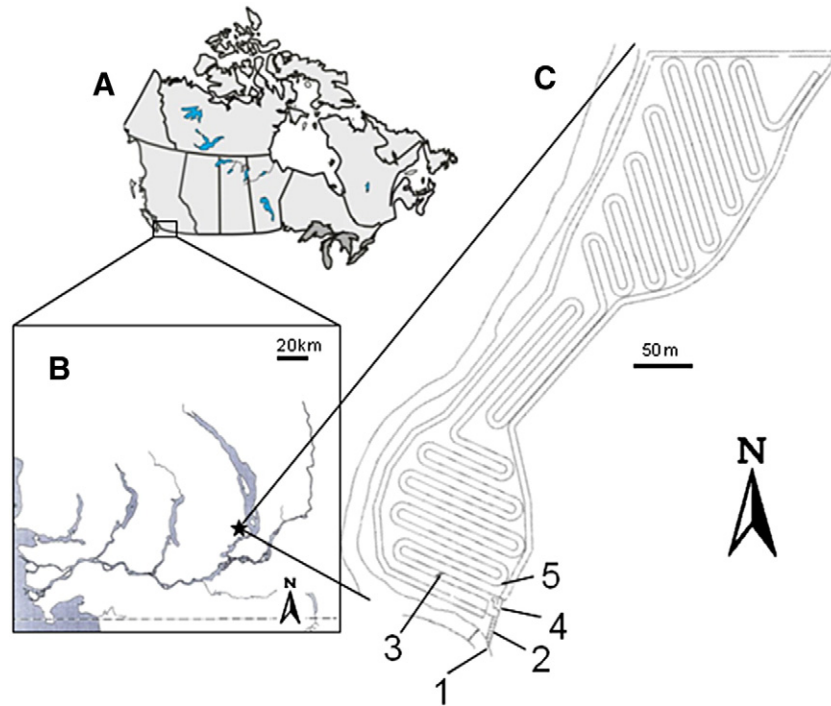


Fig. 1. Map detailing the study site. The Weaver Creek Spawning Channel (C) is located in the lower Fraser River (B) in British Columbia, Canada (A). The artificial channel is part of a natural creek system (1). Salmon leave the creek, swim up a fish ladder (2) and hold below the splitter shed (3) before entering the holding pool from which fish were sampled (4). After sampling, fish were released to the channel to spawn (5). Figure extracted with permission from Hruska et al. (2011).

equal likelihood of passing through the counting fence and entering the channel.

Given defined sexual characteristics, as soon as a fish was identified in the holding area, it could be dip-netted very rapidly with limited disturbance to other fish. This allowed us to easily obtain equal numbers of each sex. Immediately after capture, a standard stressor of two minutes air exposure was applied. This stressor is known to increase cortisol concentrations (Arends et al., 1999), and ensured that all individuals were exposed to an equivalent magnitude of stress. During air exposure, fish were held in a moistened, foam-lined, V-shaped trough, sampled for approximately 1.5 mL of blood by caudal puncture using 3 mL lithium-heparinized vacutainers (B.D. Vacutainer, Franklin Lakes, NJ) with 21-gauge, 1.5" needles and tagged with a Peterson disk or cinch tag. Post sample collection, pressure was applied to the sampling site to facilitate clotting. A second blood sample was collected using the same procedure after 25 min to measure stress-induced cortisol. Between sampling times, individuals were placed in black nylon fish bags (1 m × 0.2 m) with mesh ends oriented into the water flow in the channel. The second blood sample was collected while fish remained in the fish bags and fish were released into the channel immediately afterwards. Blood samples were held in a water-ice slurry for no more than 45 min and centrifuged for 5 min at 10,000 g (Compact II Centrifuge, Clay Adams, Parsippany, NJ). Plasma samples were flash frozen in liquid nitrogen and stored at -80°C until analysis. Plasma cortisol concentration was determined using a commercial radioimmunoassay kit (Immuno-Chem Cortisol ^{125}I RIA; MP Biomedicals, Orangeburg, NY) and a Cobra Auto-Gamma counter (Hewlett-Packard, Palo Alto, CA), a procedure commonly used to measure cortisol in fish plasma (Gamperl et al., 1994). All samples were assayed together. Assay detectability is 3 ng mL^{-1} and intra-assay variability was $9.3 \pm 0.7\%$.

Thirty fish of each sex were processed from each timing group resulting in a total of 180 fish. The early and late fish were tagged with Peterson disk tags, and individuals were located in the channel by walking its length twice-daily and reading the tags using binoculars.

Each fish was observed for $\sim 30\text{ s}$ to determine the status of redd establishment and calculate the total number of days on a redd (for females). The point at which each fish left pooling aggregations to create or defend territories was noted. As detailed behavioral observations were carried out at the same time on a separate group of fish (see below), fish from the middle group were not tagged to be located in the channel due to time constraints. Instead these fish were tagged with cinch tags from which ID could be read upon retrieval but not from shore. Dead fish were collected daily and longevity was calculated as the number of days between initial sample and death. Following the methods of Hruska et al. (2010), reproductive success was determined (on carcasses) as the percentage of eggs remaining relative to the expected number of eggs as determined by a fork-length-to-egg-mass relationship constructed from ripe and non-spawned females. It was not uncommon for small numbers of eggs to be lost during processing or transportation to the lab. Therefore, females releasing between 51 and 89% of their eggs ($n=10$) were excluded from analyses of reproductive success as we could not determine if they were partial spawners or had lost a portion of eggs in transit and were actually non-spawners. For analyses, females that released $>90\%$ of eggs were considered spawners ($n=77$), and those that released $<50\%$ of eggs, non-spawners ($n=9$). According to this method, we are able to definitely separate females that successfully released most eggs from those that did not. Additionally, fish were excluded that were never recovered from the channel ($n=5$), were entirely scavenged by gulls (*Larus* spp.; $n=2$) or from which we were unable to obtain either a pre-stress or stress-induced blood sample ($n=4$).

Behavioral assessments

An additional group of 13 fish of each sex from the middle timing group was used to conduct detailed behavioral observations in an experimental arena. These fish were held in the spawning channel in an area enclosed with Vexar rigid mesh netting (approximately

5 m × 2 m; Masternet, Mississauga, Ontario). Each individual was observed from shore for 10 min daily until death and the order of observation was randomly chosen each day. For both sexes, behavioral metrics included aggression score and mate interaction time. Aggressive behaviors included chases, charges, and bites as described by Healey et al. (2003). Aggression score was calculated for each fish using an approach adapted from Mehranvar et al. (2004) as the total number of attacks given minus the number of attacks received, divided by the total observation time for each individual. Mate interaction time was quantified for both sexes. For males it was the time spent courting a female while either in a dominant or satellite position. Foote (1990) identified four distinct social positions males occupy on the spawning channel. The dominant male is that individual closest to a females flank, competitor males are satellites competing for access, sneak males hold position behind a mating pair and occasionally enter the territory and loner males are not involved with any female (Foote, 1990). Timing interaction times is a means to quantify dominance as the dominant male would receive the highest time, competitors the second highest as one male would often leave as a result of aggressive attacks and sneak males would only receive time when they approached the female. For females, mate interaction time included the time courted by males and if courted by multiple partners during one observation period, times with all mates were included in the score. For both sexes, mate interaction time was calculated as total time spent interacting with the opposite sex divided by total observation time and multiplied by 100 to represent the percentage of total observation time. For females, number of days on a redd were also quantified.

Statistical analyses

Statistical analyses were conducted using SPSS Statistics 19.0 (2010). Residuals were examined for normal distributions using the Shapiro–Wilk goodness-of-fit test and heterogeneity of variance was assessed using Levene's test. Variables were log-transformed to meet assumptions of normality where necessary. If log-transformation did not yield normality, non-parametric statistical tests were used. All regression models were conducted with a stepwise forward selection procedure (P to enter = 0.05, P to remove = 0.10) and tested for multicollinearity by assessing variable variance proportions. If predictor variables share high variance proportions on the same eigenvalue, the assumption of multicollinearity is broken. Accuracy of the logistic regression model was determined by analyzing residual statistics according to Field (2009). The level of significance for all tests was assessed at 0.05 (α). Means are reported \pm standard error of the mean (SEM).

Two-way ANOVAs was used to determine the effect of sampling period and gender on transformed pre-stress cortisol, stress-induced cortisol and fish size (FL). As longevity could not be transformed to meet assumptions of normality, separate non-parametric tests assessed differences between sex (Mann–Whitney U) and across sampling periods (Jonckheere–Terpstra). Regression analyses were conducted to determine predictors of spawning success (binary logistic; spawners vs. non-spawners), days on a redd and longevity (multiple regression). Preliminary analyses revealed longevity to decrease with arrival time, likely representing individual maturity upon arrival, and was thus included as a predictor of reproductive success and days on a redd along with cortisol measures in females. However, longevity is the only fitness metric for males and is known to have fitness consequences in both sexes. To use longevity as a fitness metric, we assumed fish from each sampling group were of equal maturity and conducted multiple regressions within each sampling group with pre-stress and stress-induced cortisol as predictors along with sex (binary-coded). In the enclosure experiment, multiple regression was also used to determine behavioral predictors (aggression score, mate interaction time) of success (number of days on a

redd, females; longevity, males) and physiological predictors (pre-stress and stress-induced cortisol) of behavior (aggression score, mate interaction time) in both sexes while including size (FL) as a predictor in all cases.

We excluded the cortisol response (change from pre-stress to stress-induced) from statistical analyses. In mature Pacific salmon, as exceptionally high circulating cortisol concentrations are maintained, stress-induced cortisol rather than the calculated response may be more comparable to analyses of the response to iteroparous species with near-zero baselines. Furthermore, preliminary exploratory analyses revealed no trends with the cortisol response and a recent review has suggested that absolute stress-induced concentrations of GCs are more likely to provide correct interpretations of data (Romero, 2004).

Results

Of 103 fish processed of each sex, we retrieved 98 females and 91 males (Table 1). Fish size (fork length; FL) did not differ across the sampling periods (one-way ANOVA: $F_{3,93} = 1.088$, $p = 0.358$; Table 1) and was greater in males (52.6 ± 0.38) than females (50.7 ± 0.20 ; Mann–Whitney U test: $U = 5696.0$, $z = 4.279$, $P < 0.0001$). FL was not correlated with any measure of cortisol or reproductive success in either sex. Two-way ANOVAs revealed an effect of gender (pre-stress $F_{1,181} = 100.1$, $p < 0.0001$; stress-induced $F_{1,178} = 51.9$, $p < 0.0001$) and sampling period (pre-stress $F_{2,181} = 7.7$, $p < 0.001$; stress-induced $F_{2,178} = 14.8$, $p < 0.0001$) on cortisol concentrations but no significant interaction effect (pre-stress $F_{2,181} = 1.9$, $p = 0.143$; stress-induced $F_{2,181} = 0.6$, $p = 0.549$). In both measures and in both sexes, cortisol did not differ between the early and middle run but was decreased in the late run and females had higher cortisol concentrations than males (Pre-stress: males 147.56 ± 11.2 , females 333.78 ± 17.7 ; Stress-induced: males 249.69 ± 13.9 , females 496.93 ± 22.0 ; Fig. 2).

Fitness metrics and cortisol

Fitness metrics for females included percentage of eggs dropped, number of days on a redd and longevity. Percentage of eggs dropped did not differ between sampling groups (Kruskal–Wallis: $H_3 = 37.2$, $p < 0.0001$). Females were grouped into spawners ($n = 73$) or non-spawners ($n = 9$). Mean percentage of eggs dropped for spawners was 98.6 ± 0.2 and 41.6% of sample released 100% all eggs ($n = 32$). Mean percentage of eggs dropped for non-spawners was 8.7 ± 5.7 with 67.7% of fish releasing no eggs ($n = 6$). A significant binary logistic model ($\chi^2_2 = 21.99$, $P < 0.0001$) revealed stress-induced cortisol ($Z_1 = 9.61$, $P = 0.002$) and longevity ($Z_1 = 6.94$, $P = 0.008$) to predict spawning success (Table 2). Number of days on a redd was significantly greater in successful fish (Mann–Whitney U test: $U = 358.5$, $z = 3.71$, $P < 0.0001$; Table 3), confirming this variable as an indirect fitness metric. Number of days on a redd ranged from 0 to 10 days and was similar between the early (4.0 ± 0.5 days) and late (4.2 ± 0.3 days) groups (Student's t -test: $t_{42} = 0.424$, $p = 0.674$). Longevity was the only significant predictor of number of days on a redd (Multiple regression: $R^2 = 0.160$, $F_{1,66} = 12.599$, $P = 0.001$). There was a positive relationship between these

Table 1

Fork lengths (FL; mean \pm SE) and sample sizes for pink salmon (*Onchorynchus gorbusha*) sampled across 3 time periods from the Weaver Creek spawning channel.

	Early (Oct. 5th)		Middle (Oct. 9th)		Late (Oct. 13th)		Enclosure (Oct. 9th)	
	FL	n	FL	n	FL	n	FL	n
Males	52.7 \pm 0.7	28	50.2 \pm 1.8	22	53.4 \pm 0.7	28	51.8 \pm 0.7	13
Females	50.7 \pm 0.36	30	50.5 \pm 0.4	26	50.8 \pm 0.3	29	50.1 \pm 0.5	13

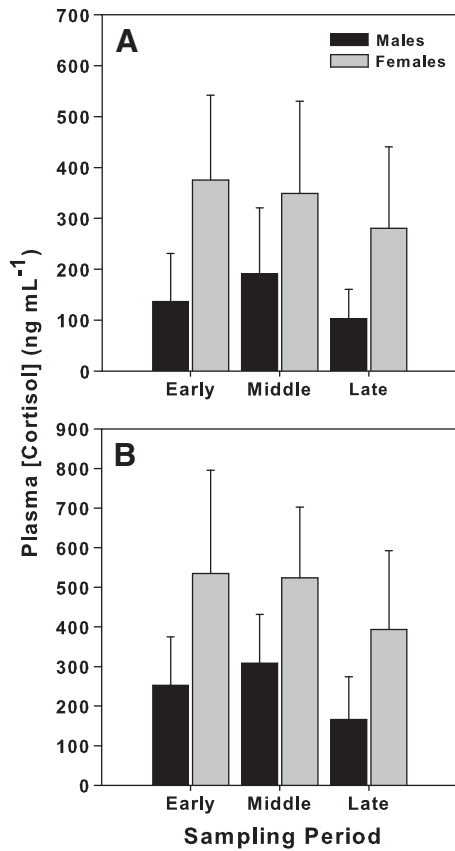


Fig. 2. Baseline (A) and stress-induced (B) cortisol concentrations between sexes and as a function of arrival time for pink salmon (*Onchorynchus gorbuscha*) sampled from the Weaver Creek Spawning Channel in 2009. Both measures of cortisol significantly differed between sexes and across sampling times ($P < 0.05$) being greater in females and dropping in the late sampling period in both sexes.

variables. Logically, the more days a female spends in the channel overall, the longer she protects a redd (data not shown).

Longevity did not differ between sexes (Mann–Whitney U test: $U = 4861.0$, $z = 1.360$, $P = 0.17$). In both sexes there was a significant decreasing trend with arrival date (Jonckheere–Terpstra: males $J = 477.0$, $z = -6.5$, $r = -.67$, $p < 0.0001$; females $J = 712.0$, $z = -5.8$, $r = -.59$, $p < 0.0001$; Fig. 3), emphasizing its probable relationship with maturity upon arrival. Assuming fish from each sampling period were of similar maturity, multiple regression analyses were conducted within each group separately to use longevity as a fitness metric but no model was significant. That longevity did not differ between spawners and non-spawners suggests that this is not a reliable measure of fitness in this system (Mann–Whitney U test: $U = 470.5$, $z = 1.76$, $P = 0.078$).

Table 2

Results from a binary logistic regression model assessing predictors of reproductive success in female pink salmon (*Onchorynchus gorbuscha*) sampled at three different times upon arrival to the Weaver Creek Spawning Channel. Significant differences between spawners (released >90% of eggs) and non-spawners (released <50% of eggs) are indicated in bold type.

		B ± SE	P-value
Step 1	Constant	5.26 ± 1.5	<0.0001
	Stress-induced Cortisol (ng mL ⁻¹)	-.05 ± .02	0.01
	$R^2 = 0.11$ (Cox and Snell), 0.23 (Nagelkerke), $X^2_{(1)} = 9.78$, $P = 0.002$		
Step 2	Constant	2.92 ± 1.7	0.08
	Stress-induced cortisol (ng mL ⁻¹)	-.078 ± .03	0.002
	Longevity (days)	.49 ± 0.19	0.008
	Baseline cortisol (ng mL ⁻¹)		0.135
$R^2 = 0.24$ (Cox & Snell), 0.47 (Nagelkerke), $X^2_{(2)} = 21.99$, $P < 0.0001$			

Table 3

Means of physiological parameters (baseline and stress-induced plasma cortisol concentrations) and fitness proxies (longevity and number of days on a redd) for reproductively successful (spawners that released >90% of eggs) and unsuccessful (non-spawners that release <50% of eggs) female pink salmon (*Onchorynchus gorbuscha*) sampled from the Weaver Creek Spawning Channel.

	Means ± SE	
	Spawners (n = 9)	Non-spawners (n = 73)
Stress-induced Cortisol (ng mL ⁻¹)	481.59 ± 21.2	712.85 ± 68.8
Baseline Cortisol (ng mL ⁻¹)	317.19 ± 18.0	436.12 ± 78.1
Longevity (days)	9.10 ± 0.4	6.56 ± 1.2
Days on a Redd (days)	4.65 ± 0.29	0.93 ± 0.5

Behavioral Metrics and Cortisol

Behavioral metrics included aggression score and mate interaction time. In females, behavior predicted number of days on a redd (Multiple regression: $R^2 = 0.59$, $F_{1,11} = 15.71$, $P = 0.002$) where more aggressive females spent more days on a redd. There was also a non-significant positive association with mate interaction time but no effect of size (FL). In males, there was a negative relationship between mate interaction time and longevity (Multiple Regression: model $R^2 = 0.41$, $F_{1,11} = 7.55$, $P = 0.019$) but no effect of aggression or FL. This relationship can be attributed to the observation of mature fish immediately taking position with a female and dying soon after and less mature males that began courting females a few days after arrival. Pre-stress cortisol predicted individual behavior in both sexes (Fig. 4). In females, pre-stress cortisol concentrations upon arrival predicted both aggression and mate interaction time (Multiple regression: $R^2 = 0.33$, $F_{(1,10)} = 5.02$, $P = 0.049$ and $R^2 = 0.54$, $F_{(1,10)} = 11.51$, $P = 0.007$, respectively). Neither stress-induced cortisol nor FL were significant factors. Similarly in males, there was a negative relationship between pre-stress cortisol and mate interaction time (Multiple regression: $R^2 = 0.59$, $F_{(1,11)} = 5.99$, $P = 0.032$). There were no significant associations between aggression and cortisol concentrations, or between aggression and FL.

Discussion

A recent review has suggested the need for more direct measures of fitness in relation to the acute stress response (Breuner et al., 2008). We accomplished this by linking reproductive success in a semelparous species to individual variability in circulating cortisol titers following exposure to acute stress. Although other studies exploring

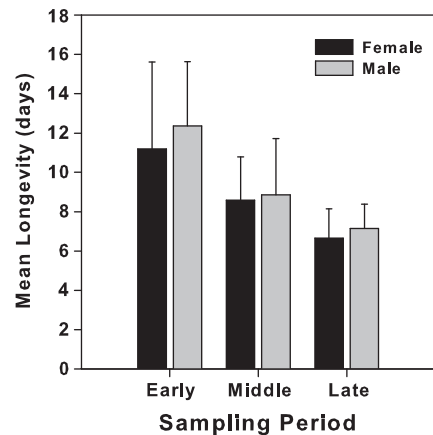


Fig. 3. Longevity, i.e. the number of days in the spawning channel from sampling to death, for both sexes of pink salmon (*Onchorynchus gorbuscha*) as a function of sampling time. There were no differences between sexes ($P > 0.05$) and longevity significantly decreased with sampling period ($P < 0.05$). Fish were sampled from the Weaver Creek Spawning Channel.

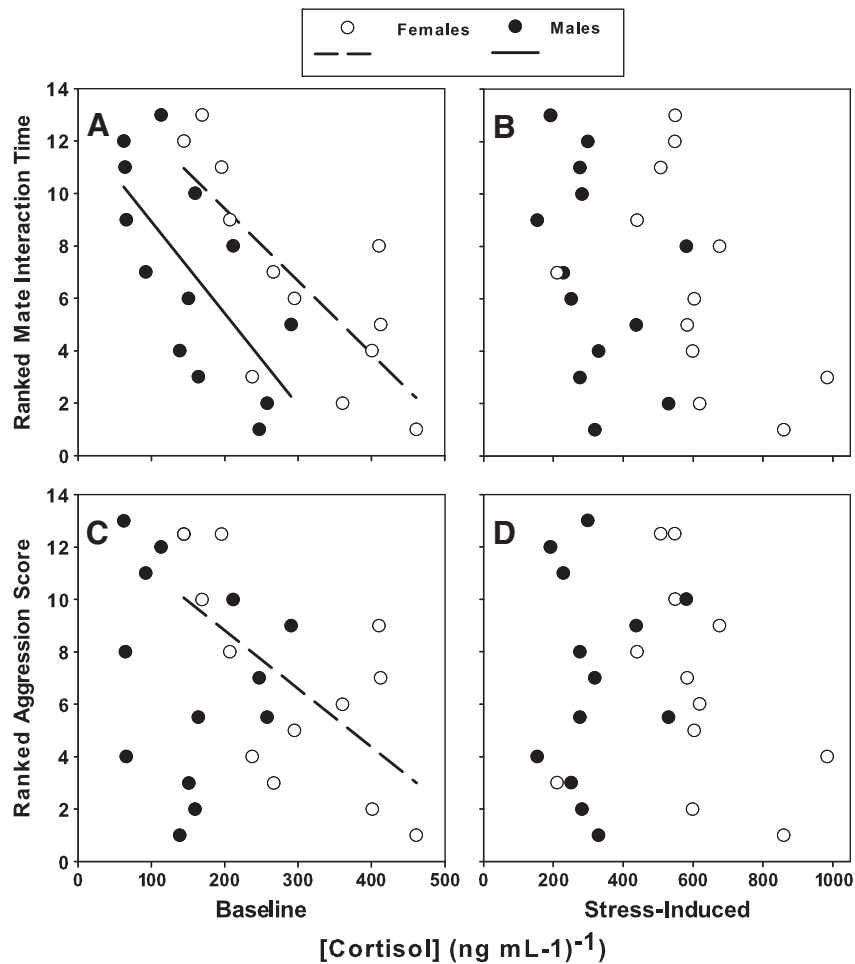


Fig. 4. Correlations between ranked behavioral metrics and cortisol concentrations for both sexes of pink salmon (*Onchorynchus gorbuscha*) sampled from the Weaver Creek Spawning Channel and held in an experimental area within the channel. Panels are regressions of the following in both sexes: mate interaction time and baseline [cortisol] (A), mate interaction and stress-induced [cortisol] (B), aggression score and baseline [cortisol] (C), aggression and stress-induced [cortisol] (D). Greater mate interaction time and aggression are characteristics of dominant fish.

fitness correlates of the stress response have emerged since the review (i.e. Angelier et al., 2009; MacDougall-Shackleton et al., 2009), conclusions remain inconsistent and the present study is unique as individuals are fully invested in reproduction and pre-stress cortisol values are exceptionally high. While stress-induced cortisol predicted spawning success, behavioral metrics were correlated with pre-stress cortisol.

Characteristics of the stress response

Both sexes exhibited pre-stress and stress-induced cortisol levels that were well above values reported for non-reproductive salmonids (see Barton and Iwama, 1991). The higher cortisol concentrations of females compared to males parallels previous research on several species of Pacific salmon (e.g. Donaldson and Fagerlund, 1972; Hane et al., 1966; Kubokawa et al., 1999) and is consistent with *in vitro* experiments on HPI axis reactivity in sexually mature chinook salmon (*O. tshawytscha*; McQuillan et al., 2003). As predicted and consistent with evolutionary theory (Wingfield and Sapolsky, 2003), stress-induced cortisol levels declined with sexual maturity. Pottinger et al. (1995) observed a similar pattern in rainbow trout and suggested that as rates of increase were similar in mature and immature fish, the cortisol/ACTH feedback equilibrium was modified in mature fish to a lower “set point”. Although the stress response is a mechanism allowing for increased individual survival, it is also energetically consuming (Ricklefs and Wikelski, 2002). An attenuated stress response is therefore expected

in animals with limited opportunities to reproduce (Wingfield and Sapolsky, 2003). When reproduction is of primary value, fitness benefits of the stress response are lost and suppression acts to conserve energy.

As condition deteriorates in mature Pacific salmon, cortisol metabolism is inhibited and concentrations peak, ultimately leading to senescence (Barry et al., 2010). Therefore, the finding of decreased pre-stress cortisol concentrations in later arriving and consequentially more mature fish was surprising and contrary to our predictions. As these fish were sampled at arrival and several days before spawning, these unexpected results are likely a function of study specimens not yet being of sufficient maturity that cortisol metabolism is inhibited. We therefore did not witness the characteristic peak that accompanies spawning and senescence. Reasons for the decrease in cortisol concentrations in the late run remain speculative but indicate that at this stage, fish are still able to metabolize cortisol. Perhaps the stress response has become muted due to excess exposure to stress (see Romero, 2004).

Fitness correlates of cortisol concentrations

Pre-spawn mortality is common across all species of Pacific salmon and the causes of this phenomenon remain unclear (Cooke et al., 2004; Quinn et al., 2007). Several theories exist. The physiological stress hypersecretion hypothesis suggests that fish with higher levels of cortisol die earlier (Barry et al., 2010; Hruska et al., 2010) and the energy exhaustion hypothesis suggests that fish die once a critical

minimal energy threshold is breached (Hruska et al., 2010). Finally, it has been predicted that successful fish will arrive early and have greater longevity (Hruska et al., 2011). These theories do not however extend to consequences of stress-induced cortisol or the stress response, known to negatively affect survival in birds and reptiles (e.g. Blas et al., 2007; MacDougall-Shackleton et al., 2009; Romero and Wikelski, 2001). Our results reveal unsuccessful females to be characterized by elevated stress-induced cortisol, fewer days on a redd and decreased longevity. Correspondingly, Hruska et al. (2011) reported that spawned fish lived approximately 2 days longer than non-spawners. Although our results of reduced fitness being associated with elevated stress-induced cortisol levels are consistent with previous findings, given the unique physiological characteristics of Pacific salmon on the spawning grounds and an over stimulated HPI axis, the implications and underlying mechanisms of this relationship may not be comparable. Perhaps failure to spawn is a stressor itself or that other factors underlying this inability are the cause of elevated cortisol concentrations.

Differences in pre-stress cortisol between spawners and non-spawners were not statistically significant but we would argue for biological significance. Average pre-stress cortisol for spawners was within the range of levels typical of female pink salmon at Weaver Creek (250 to 350 ng mL⁻¹; McConnachie, 2010) whereas those for non-spawners were considerably higher (from 413.1 to 546.2 ng mL⁻¹), providing support for the cortisol hypersecretion hypothesis. However, with only 12% of sample failing to spawn, the power of our results is low. The Weaver Creek Spawning channel is an artificial habitat that has been optimized for successful spawning. Densities are much lower than in the wild, and both substrate and flow are ideal. More variability in reproductive success might occur in a natural setting.

Reproductive success was only detectable in females. As males continually generate sperm, we lacked a direct measure of fitness. Males also adopt several different reproductive strategies which further obscures quantifying fitness. Longevity is thought to provide an indirect measure of fitness for both sexes (Quinn and Foote, 1994). However, based on our observations of early fish arriving in a less mature state and not immediately forming territories (females) or courting females (males) along with a significant decreasing trend of longevity with arrival group, longevity is perhaps more indicative of maturity upon arrival. Furthermore, we observed no behavioral or physiological predictors of longevity, as with other fitness measures (females only). To use longevity as a measure of fitness, level of maturity between individuals must be controlled for by, for example, measuring gonadal steroid hormones.

Behavioral correlates of cortisol concentrations

In both sexes, behavior was related to pre-stress but not stress-induced cortisol. Females with elevated pre-stress cortisol concentrations upon arrival had lower aggression scores and aggression predicted number of days on a redd. These results are consistent with previous literature (e.g. Gilmour et al., 2005). However, there was no relationship between aggression and cortisol in males. In fish, androgens, and especially 11-keto testosterone in males is the primary predictor of behavior, including aggression (Borg, 1994). Alternatively, behavioral correlates of cortisol occur as a result of agnostic interactions in hierarchal fish (Gilmour et al., 2005). Male aggression is also highly dependent on overall density and the operational sex ratio (OSR). The even OSR used in this study was much lower than in natural settings as usually the proportion of males to females is greater than 50:50 (Quinn et al., 1996). The dominant male attempts to monopolize access to females and subordinate males take on satellite positions surrounding the pair (Foote, 1990). According to Quinn (1996), this is achieved easier at low OSRs as competition between males is decreased. At high OSRs, the male may need to be more aggressive to attain a dominant status with a female. Indeed, we

observed the dominant male to hold position with a female and the majority of aggression occurring amongst satellite males. This observation provides a potential explanation for the lack of relationship between aggression score and pre-stress cortisol as predicted for social salmonids. The significant negative relationship between pre-stress cortisol at arrival and mate interaction time also supports this explanation and implies a role for pre-stress cortisol in determining social status in spawning males. Consistent with previous research on physiological characteristics of dominance hierarchies in juvenile salmonids where subordinate fish show increased pre-stress cortisol concentrations (Gilmour et al., 2005), the dominant individual (greater mate interaction time in males, aggression in females) has decreased pre-stress cortisol levels upon arrival.

In contrast to our predictions and contrary to previous work (Foote, 1990), we saw no associations between aggression and fork length. However, most research of physiology and behavior in mature Pacific salmon has focused on sockeye salmon. Contrary to other Pacific salmon, pink salmon have a fixed two-year life-cycle making all spawning individuals exactly 2 years of age (Heard, 1991). Therefore, lack of size variation and species-specific differences in behavior and physiology could account for observed inconsistencies with previous research. We also predicted behavior to be correlated with stress-induced cortisol. However, these predictions were based on findings from iteroparous salmonids (see review by Øverli et al., 2005). With semelparity, it has been theorized that reproductive behavior would be decoupled from the stress response (Breuner et al., 2008) as reproduction must occur despite environmental or social stressors (Wingfield and Sapolsky, 2003).

Conclusions

Our results find cortisol levels in pink salmon to predict both direct and indirect fitness measures. Pre-stress cortisol concentrations were associated with individual behavior. Dominant fish are known to have greater reproductive success (Chebanov et al., 1983; Quinn and Foote, 1994) and we observed individuals showing signs of dominance to have relatively lower pre-stress cortisol and spend more days protecting a redd (females only). Additionally, non-spawning females had pre-stress cortisol concentrations beyond the normal range for pink salmon of the study population. Unsuccessful female fish were also characterized by elevated stress-induced cortisol concentrations and decreased longevity. In reptiles, elevated GCs are known to increase energy expenditure (Cote et al., 2006). As pink salmon have a minimum energetic threshold (Crossin et al., 2003), increased energy expenditure attributed with this elevated cortisol in response to acute stress could likely influence longevity on the spawning grounds and reproductive success. Romero and Wikelski (2001) suggested that when in exceptionally poor condition with little chance of survival, a surge in the physiological response to stress may act to mobilize energy as a final effort of survival. The few non-spawning individuals with abnormally high stress-induced cortisol in response to acute stress may have arrived to the spawning grounds in an advanced state of deterioration, perhaps due previous stress exposure. Severe conditions act as a mechanism of natural selection (Brown and Brown, 1998), thus exerting selective pressure on physiological traits. Individual differences in responses to acute stress, especially in challenging situations or when body condition is low, could therefore be a substrate for natural selection for Pacific salmon as suggested by Romero and Wikelski (2001) for Galapagos marine iguanas during El Niño years of low food availability.

Stress-induced cortisol however did not predict indirect measures of fitness (number of days on a redd) or behavior. Our results suggest that abnormally elevated GCs indicate a poor quality individual unable to cope with the physiological demands of an extremely challenging reproductive strategy. We also observed indirect fitness consequences of elevated pre-stress cortisol concentrations. Perhaps

in a system with greater variability in reproductive success or in a year of lower survival, we would see a stronger direct effect of pre-stress cortisol thus providing support for both the cort-fitness hypothesis (Bonier et al., 2009a) and the cortisol hypersecretion hypothesis (Hruska et al., 2010). Although our findings do confirm clear negative association between cortisol concentrations and survival in pink salmon on the spawning grounds, it is unknown if this is in fact a causal relationship. There remains much to be learned regarding the evolutionary consequences of individual variability in the mechanistic capacity to respond to stress in semelparous species, especially among varying levels of reproductive success within a population.

Acknowledgments

The authors wish to thank members of the Cooke and Hinch labs for assistance with field work, particularly Connie O'Connor, Alison Colotelo, Mike Donaldson, Charlotte Whitney, Graham Raby, Juliette Mudra, and Tim Clark. We also thank Dave Patterson and his staff from DFO's Environmental Watch program for logistic and financial support. Fisheries and Oceans Canada Provided substantial in kind support. We also thank Rick Stitt and the Weaver Creek Staff, who provided housing and technical support. Financial support was provided by NSERC in the form of Discovery Grants to SJC, KMG and SGH as well as an NSERC Strategic Grant to SJC and SGH. SJC was also supported by the Canada Research Chairs Program.

References

- Angelier, F., Holberton, R.L., Marra, P.P., 2009. Does stress response predict return rate in a migratory bird species? A study of American redstarts and their non-breeding habitat. *Proc. R. Soc. B* 276, 3545–3551.
- Angelier, F., Wingfield, J.C., Weimerskirch, H., Chastel, O., 2010. Hormonal correlates of individual quality in a long-lived bird: a test of the 'corticosterone–fitness hypothesis'. *Biol. Letters* 6, 846–849.
- Arends, R.J., Mancera, J.M., Munoz, J.L., Wendelaar Bonga, S.E., Flik, G., 1999. The stress response of the gilthead sea bream (*Sparus aurata* L.) to air exposure and confinement. *J. Endocrinol.* 163, 149–157.
- Barry, T.P., Marwah, A., Nunez, S., 2010. Inhibition of cortisol metabolism by 17 [alpha], 20 [beta]-P: mechanism mediating semelparity in salmon? *Gen. Comp. Endocrinol.* 165, 53–59.
- Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integr. Comp. Biol.* 42, 517–525.
- Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annu. Rev. Fish. Dis.* 1, 3–26.
- Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R., Marchant, T.A., 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. *Proc. Nat. Acad. Sci. U.S.A.* 104, 8880–8884.
- Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009a. Do pre-stress glucocorticoids predict fitness? *Trends Ecol. Evol.* 24, 634–642.
- Bonier, F., Moore, I.T., Martin, P.R., Robertson, R.J., 2009b. The relationship between fitness and pre-stress glucocorticoids in a passerine bird. *Gen. Comp. Endocrinol.* 163, 208–213.
- Borg, B., 1994. Androgens in teleost fish. *Comp. Biochem. Phys. C* 109, 219–245.
- Breuner, C.W., Patterson, S.H., Hahn, T.P., 2008. In search of relationships between the acute adrenocortical response and fitness. *Gen. Comp. Endocrinol.* 157, 288–295.
- Brown, C.R., Brown, M.B., 1998. Intense natural selection on body size and wing and tail asymmetry in cliff swallows during severe weather. *Evol.* 52, 1461–1475.
- Carruth, L.L., Jones, R.E., Norris, D.O., 2002. Cortisol and Pacific salmon: a new look at the role of stress hormones in olfaction and home-stream migration. *Integr. Comp. Biol.* 42, 574–481.
- Chebanov, N.A., Varnavskaya, N.V., Varnavskiy, V.S., 1983. Effectiveness of spawning of male sockeye salmon, *Oncorhynchus nerka* (Salmonidae), of differing hierarchical rank by means of genetic-biochemical markers. *J. Ichthyol.* 23, 51–55.
- Cooke, S.J., Hinch, S.G., Farrell, A.P., Lapointe, M.F., Jones, S.R.M., Macdonald, J.S., Patterson, D.A., Healey, M.C., 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, S.J., Hinch, S.G., Crossin, G.T., Patterson, D.A., English, K.K., Healey, M.C., Shrimpton, J.M., Van Der Kraak, G., Farrell, A.P., 2006a. Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. *Ecology* 87, 1575–1586.
- Cooke, S.J., Hinch, S.G., Crossin, G.T., Patterson, D.A., English, K.K., Shrimpton, J.M., Kraak, G.V.D., Farrell, A.P., 2006b. Physiology of individual late-run Fraser River sockeye salmon (*Oncorhynchus nerka*) sampled in the ocean correlates with fate during spawning migration. *Can. J. Fish. Aquat. Sci.* 63, 1469–1480.
- Cote, J., Clobert, J., Meylan, S., Fitze, P.S., 2006. Experimental enhancement of corticosterone levels positively affects subsequent male survival. *Horm. Behav.* 49, 320–327.
- Cote, J., Clobert, J., Montes Poloni, L., Haussy, C., Meylan, S., 2010. Food deprivation modifies corticosterone-dependent behavioural shifts in the common lizard. *Gen. Comp. Endocrinol.* 166, 142–151.
- Crossin, G.T., Whelby, M.P., Hinch, S.G., Farrell, A.P., Healey, M.C., 2003. Pink salmon (*Oncorhynchus gorbuscha*) migratory energetics: response to migratory difficulty and comparisons with sockeye salmon (*Oncorhynchus nerka*). *Can. J. Zool.* 81, 1986–1995.
- Crossin, G.T., Hinch, S.G., Farrell, A.P., Higgs, D.A., Lotto, A.G., Oakes, J.D., Healey, M.C., 2004. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. *J. Fish Biol.* 65, 788–810.
- Crossin, G.T., Hinch, S.G., Welch, D.W., Cooke, S.J., Patterson, D.A., Hills, J.A., Zohar, Y., Klenke, U., Jacobs, M.C., Pon, L.B., 2009. Physiological profiles of sockeye salmon in the Northeastern Pacific Ocean and the effects of exogenous GnRH and testosterone on rates of homeward migration. *Mar. Freshwat. Behav. Physiol.* 42, 89–108.
- Donaldson, E.M., Fagerlund, U.H.M., 1972. Corticosteroid dynamics in Pacific salmon. *Gen. Comp. Endocrinol.* 3, 254–265.
- Essington, T.E., Quinn, T.P., Ewert, V.E., 2000. Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. *Can. J. Fish. Aquat. Sci.* 57, 205–213.
- Field, A.P., 2009. *Discovering Statistics Using SPSS*. SAGE publications Ltd, 55 City Road, London England.
- Foote, C., 1990. An experimental comparison of male and female spawning territoriality in a Pacific salmon. *Behaviour* 115, 283–314.
- Gamperl, A., Vijayan, M., Boutillier, R., 1994. Experimental control of stress hormone levels in fishes: techniques and applications. *Rev. Fish Biol. Fish.* 4, 215–255.
- Gilmour, K.M., DiBattista, J.D., Thomas, J.B., 2005. Physiological causes and consequences of social status in salmonid fish. *Integr. Comp. Biol.* 45, 263–273.
- Hane, S., Robertson, O.H., Wexler, B.C., Krupp, M.A., 1966. Adrenocortical response to stress and ACTH in Pacific salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdnerii*) at successive stages in the sexual cycle. *Endocrinology* 78, 791–800.
- Healey, M., Lake, R., Hinch, S., 2003. Energy expenditures during reproduction by sockeye salmon (*Oncorhynchus nerka*). *Behavior* 140, 161–182.
- Heard, W.R., 1991. Life history of pink salmon (*Oncorhynchus gorbuscha*). In: Goot, C., Margolis, L. (Eds.), *Pacific Salmon Life Histories*. UBC Press, Vancouver, BC, pp. 119–231.
- Hendry, A.P., Berg, O.K., 1999. Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. *Can. J. Zool.* 77, 1663–1675.
- Hruska, K.A., Hinch, S.G., Healey, M.C., Patterson, D.A., Larsson, S., Farrell, A.P., 2010. Influences of sex and activity level on physiological changes in individual adult sockeye salmon during rapid senescence. *Phys. Bioch. Zool.* 83, 663–676.
- Hruska, K.A., Hinch, S.G., Patterson, D.A., Healey, M.C., 2011. Egg retention in relation to arrival timing and reproductive longevity in female sockeye salmon (*Oncorhynchus nerka*). *Can. J. Fish. Aquat. Sci.* 68, 250–259.
- Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. *Neurosci. Biobehav. Rev.* 23, 925–935.
- Kubokawa, K., Watanabe, T., Yoshioka, M., 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.
- MacDougall-Shackleton, S.A., Dindia, L., Newman, A.E.M., Potvin, D.A., Stewart, K.A., MacDougall-Shackleton, E.A., 2009. Stress, song and survival in sparrows. *Biol. Letters* 5, 746–748.
- Maldonado, T.A., Jones, R.E., Norris, D.O., 2000. Distribution of [beta]-amyloid and amyloid precursor protein in the brain of spawning (senescent) salmon: a natural, brain-aging model. *Brain Res.* 858, 237–251.
- Maule, A.G., Schrock, R., Slater, C., Fitzpatrick, M.S., Schreck, C.B., 1996. Immune and endocrine responses of adult chinook salmon during freshwater immigration and sexual maturation. *Fish Shellfish Immunol.* 6, 221–233.
- McConnachie, S.H., 2010. Understanding the ecological consequences of stress in wild fish using exogenous cortisol implants. M. Sc Thesis. Carleton University, Ottawa, Canada.
- McQuillan, H.J., Lokman, P.M., Young, G., 2003. Effects of sex steroids, sex, and sexual maturity on cortisol production: an in vitro comparison of chinook salmon and rainbow trout interrenals. *Gen. Comp. Endocrinol.* 133, 154–163.
- Mehranvar, L., Healey, M., Farrell, A., Hinch, S., 2004. Social versus genetic measures of reproductive success in sockeye salmon, *Oncorhynchus nerka*. *Evol. Ecol. Res.* 6, 1167–1181.
- Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Rev. Fish Biol. Fish.* 9, 211–268.
- Øverli, Ø., Harris, C.A., Winberg, S., 2000. Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain Behav. Evol.* 54, 263–275.
- Øverli, Ø., Winberg, S., Pottinger, T., 2005. Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout—a review. *Integr. Comp. Biol.* 45, 463–474.
- Pottinger, T.G., Pickering, A.D., Hurley, M.A., 1992. Consistency in the stress response of individuals of two strains of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 103, 275–289.
- Pottinger, T.G., Balm, P.H.M., Pickering, A.D., 1995. Sexual maturity modifies the responsiveness of the pituitary-interrenal axis to stress in male rainbow trout. *Gen. Comp. Endocrinol.* 98, 311–320.
- Quinn, T.P., 1999. Variation in Pacific salmon reproductive behaviour associated with species, sex and levels of competition. *Behaviour* 136, 179–204.

- Quinn, T., Foote, C., 1994. The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon, *Oncorhynchus nerka*. *Anim. Behav.* 48, 751–761.
- Quinn, T.P., Adkison, M.D., Ward, M.B., 1996. Behavioral tactics of male sockeye salmon (*Oncorhynchus nerka*) under varying operational sex ratios. *Ethology* 102, 304–322.
- Quinn, T.P., Eggers, D.M., Clark, J.H., Rich, J., 2007. Density, climate, and the processes of prespawning mortality and egg retention in Pacific salmon (*Oncorhynchus* spp.). *Can. J. Fish. Aquat. Sci.* 64, 574–582.
- Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. *Trends Ecol. Evo.* 17, 462–468.
- Robertson, O.H., Wexler, B.C., 1959. Hyperplasia of the adrenal and cortical tissue in Pacific salmon (genus *Oncorhynchus*) and rainbow trout (*Salmo gairdneri*) accompanying sexual maturation and spawning. *Endocrinology* 65, 225–238.
- Robertson, O.H., Wexler, B.C., Miller, B.F., 1961. Degenerative changes in the cardiovascular system of the spawning Pacific salmon (*Oncorhynchus tshawytscha*). *Circ Res* 9, 826–834.
- Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. *Trends Ecol. Evo.* 19, 249–255.
- Romero, L.M., Reed, J.M., 2008. Repeatability of pre-stress corticosterone concentrations. *Gen. Comp. Endocrinol.* 156, 27–33.
- Romero, L.M., Wikelski, M., 2001. Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Nino events. *Proc. Natl. Acad. Sci. U. S. A.* 98, 7366–7370.
- Schreck, C.B., 2010. Stress and fish reproduction: the roles of allostasis and hormones. *Gen. Comp. Endocrinol.* 165, 549–556.
- Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiol. Rev.* 77, 591–626.
- Wingfield, J.C., 2003. Control of behavioural strategies for capricious environments. *Anim. Behav.* 66, 807–816.
- Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. *J. Neuroendocrinol.* 15, 711–724.
- Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *Am. Zool.* 38, 191–206.