Sex and Proximity to Reproductive Maturity Influence the Survival, Final Maturation, and Blood Physiology of Pacific Salmon When Exposed to High Temperature during a Simulated Migration

Author(s): Ken M. Jeffries, Scott G. Hinch, Eduardo G. Martins, Timothy D. Clark, Andrew G. Lotto, David A. Patterson, Steven J. Cooke, Anthony P. Farrell, Kristina M. Miller

Reviewed work(s):
Source: *Physiological and Biochemical Zoology*, Vol. 85, No. 1 (January/February 2012), pp. 62-73
Published by: The University of Chicago Press
Stable URL: http://www.jstor.org/stable/10.1086/663770
Accessed: 21/01/2012 16:03

Sex and Proximity to Reproductive Maturity Influence the Survival, Final Maturation, and Blood Physiology of Pacific Salmon When Exposed to High Temperature during a Simulated Migration

Ken M. Jeffries[1,*]
Scott G. Hinch[1,2]
Eduardo G. Martins[1]
Timothy D. Clark[1,3]
Andrew G. Lotto[1]
David A. Patterson[4]
Steven J. Cooke[5]
Anthony P. Farrell[3]
Kristina M. Miller[6]

[1]Centre for Applied Conservation Research and Department of Forest Sciences, University of British Columbia, 2424 Main Mall, Vancouver, British Columbia V6T 1Z4, Canada; [2]Institute for Resources, Environment and Sustainability, University of British Columbia, 2202 Main Mall, Vancouver, British Columbia V6T 1Z4, Canada; [3]Faculty of Land and Food Systems, University of British Columbia, 2357 Main Mall, Vancouver, British Columbia V6T 1Z4, Canada; [4]Fisheries and Oceans Canada, Cooperative Resource Management Institute, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, Canada; [5]Fish Ecology and Conservation Physiology Laboratory, Institute of Environmental Science and Department of Biology, Carleton University, Ottawa, Ontario K1S 5B6, Canada; [6]Fisheries and Oceans Canada, Molecular Genetics Section, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, British Columbia V9T 6N7, Canada

Accepted 11/10/2011; Electronically Published 12/15/2011

ABSTRACT

Some Pacific salmon populations have been experiencing increasingly warmer river temperatures during their once-in-a-lifetime spawning migration, which has been associated with en route and prespawn mortality. The mechanisms underlying such temperature-mediated mortality are poorly understood. Wild adult pink (*Oncorhynchus gorbuscha*) and sockeye (*Oncorhynchus nerka*) salmon were used in this study. The objectives were to investigate the effects of elevated water temperature on mortality, final maturation, and blood properties under controlled conditions that simulated a "cool" (13°C) and "warm" (19°C) freshwater spawning migration. After 10 d at 13°C, observed mortality was 50%–80% in all groups, which suggested that there was likely some mortality associated with handling and confinement. Observed mortality after 10 d at 19°C was higher, reaching ≥98% in male pink salmon and female pink and sockeye salmon. Thus, male sockeye salmon were the most thermally tolerant (54% observed mortality). Model selection supported the temperature- and sex-specific mortality patterns. The pink salmon were closer to reproductive maturation and farther along the senescence trajectory than sockeye salmon, which likely influenced their survival and physiological responses throughout the experiment. Females of both species held at 19°C had reduced plasma sex steroids compared with those held at 13°C, and female pink salmon were less likely to become fully mature at 19° than at 13°C. Male and female sockeye salmon held at 19°C had higher plasma chloride and osmolality than those held at 13°C, indicative of a thermally related stress response. These findings suggest that sex differences and proximity to reproductive maturity must be considered when predicting thermal tolerance and the magnitude of en route and prespawn mortality for Pacific salmon.

Introduction

Many populations of Pacific salmon (*Oncorhynchus* spp.) now encounter warmer rivers during their spawning migration than at any time since records have been kept. For example, average summer water temperatures experienced by migratory salmon in one of Canada's largest rivers, the Fraser River in British Columbia, has increased by >1.8°C over the past ~60 yr, with 13 of the warmest years on record occurring over the past 20 summers (Patterson et al. 2007; eWatch 2010). Similarly, Pacific salmon in the Columbia River in the United States now migrate through waters that can be ~2.5°C above historical levels (Quinn and Adams 1996). Spawning migrations during high-water-temperature episodes are associated with increased levels of en route mortality (mortality during migration; Macdonald et al. 2010; Martins et al. 2011). Because the semelparous life history of Pacific salmon allows for only one opportunity to complete their spawning migration and reproduce, en route mortality results in a lifetime fitness of zero for those individuals. Even the survivors of migrations during warm-water episodes may experience profound negative consequences, such

* Corresponding author; e-mail: kenmjeffries@gmail.com.

Physiological and Biochemical Zoology 85(1):62–73. 2012. 1522-2152/2012/8501-1082$15.00. DOI: 10.1086/663770

as delayed final maturation—a common response to elevated water temperature in salmonids (Pankhurst and King 2010)—or elevated levels of premature mortality on spawning grounds (termed "prespawn mortality"; Gilhousen 1990). Climate models predict a continued warming of approximately 0.12°–0.14°C per decade over the next century for summer water temperatures in the Fraser River (Ferrari et al. 2007). Thus, temperature-related mortality in Pacific salmon is predicted to occur more frequently and will likely result in a reduced number of salmon reaching spawning grounds and successfully reproducing in the future (Morrison et al. 2002; Mantua et al. 2010; Hague et al. 2011; Martins et al. 2011); temperature-related mortality is also likely to alter suitable habitat availability in the southern periphery of these species' distributions (Eaton and Scheller 1996; Beechie et al. 2006).

Several field-based studies have shown a relationship between water temperature and en route mortality in various Pacific salmon species throughout their natural range (Keefer et al. 2008, 2010; Taylor 2008; Martins et al. 2011). There are several proposed mechanisms to explain why elevated water temperatures may influence Pacific salmon survival, which include (1) more rapid depletion of endogenous energy reserves (Rand et al. 2006), which provide the sole fuel source for in-river migration and gonadal development because Pacific salmon cease feeding in the ocean; (2) a collapse in aerobic scope (Farrell et al. 2008) and impairment of cardiorespiratory function (Clark et al. 2008); and (3) a temperature-dependent increase in disease and parasite progression (Servizi and Jensen 1977; Gilhousen 1990; Crossin et al. 2008; Bradford et al. 2010). These mechanisms may act solely or synergistically to ultimately affect spawning migration success. Regardless, the physiological mechanisms underlying the mortality remain speculative, and there are few controlled laboratory-based studies that have examined the effects of water temperature on wild adult Pacific salmon. Of those that do exist, elevated water temperature has been shown to increase mortality (Jensen et al. 2004; Crossin et al. 2008), but physiological analyses have been sparse and physiological disturbances due to temperature treatments have not been detected after prolonged thermal exposure (i.e., 24 d in Crossin et al. 2008).

In light of these knowledge gaps, this study sought to quantify the physiological and survival consequences of continuous exposure to elevated water temperature in Pacific salmon. We held wild-caught adult pink (*Oncorhynchus gorbuscha* Walbaum) and sockeye (*Oncorhynchus nerka* Walbaum) salmon under thermal conditions that simulated a "cool" (13°C) or "warm" (19°C) freshwater migration. We hypothesized that if the 19°C water treatment induced chronic stress, it would result in increased mortality, elevated indices of stress in the blood plasma, and delayed or inhibited final maturation. Because sex-based differences in survival have been reported previously for Pacific salmon (Patterson et al. 2004; Crossin et al. 2008; Keefer et al. 2010), we also contrasted thermally driven mortality patterns and blood plasma responses between males and females. Furthermore, because Fraser River pink salmon appear to be more thermally tolerant (Clark et al. 2011) than sockeye salmon populations (Macdonald et al. 2010; Eliason et al. 2011), we expected pink salmon to be less affected by the warm-temperature treatment.

Material and Methods

Adult sockeye salmon ($n = 128$) were collected from the Harrison River (a major tributary of the Fraser River), British Columbia, Canada, from September 15 to 18, 2008 (fig. 1). DNA stock identification (Beacham et al. 2005) confirmed that all sockeye salmon were from the Harrison Rapids population (hereafter referred to as Harrison sockeye salmon). Pink salmon ($n = 156$) were collected from the same location from September 22 to 24, 2009. Fraser River pink salmon are generally divided into Lower and Upper Fraser River populations, which can be distinguished on the basis of migration timing and capture location (Groot and Margolis 1991; Crossin et al. 2003) but not by DNA identification (T. D. Beacham, personal communication). The pink salmon that spawn in the Harrison River (used in this study) belong to the Lower Fraser River stock complex. Harrison sockeye salmon typically spawn from early to mid-November in the Harrison River, while Lower Fraser River pink salmon in the Harrison River system typically spawn from early to mid-October.

In 2008 and 2009, fish were collected by beach seine from the Harrison River (water temperatures ranged from 15° to 18°C in 2008 and from 13° to 16°C in 2009) near Agassiz, British Columbia, and transported in aerated ~12°C water monitored continuously for dissolved oxygen levels to the Fisheries and Oceans Canada Cultus Lake Laboratory (CLL; fig. 1), where they were held in large tanks containing 10°–11°C water that had been sand filtered and UV sterilized. Fish recovered from transport for 2–5 d before the experimental temperature treatment period. At this time, all fish appeared vigorous, and there were no external signs of disease. A pretreatment blood sample was obtained from the caudal vasculature using a heparinized vacutainer to determine initial values for plasma variables (all sockeye salmon in 2008: $n = 22$ males and 20 females destined for the 13°C treatment, 46 males and 40 females destined for the 19°C treatment; a subset of pink salmon in 2009: $n = 16$ males and 16 females destined for the 13°C treatment, 31 males and 33 females destined for the 19°C treatment). Immediately afterward, fish were randomly distributed among six 8,000-L aerated tanks at 10°–11°C using equal fish densities and sex ratios. Each tank contained a submersible pump that created a water flow of approximately 0.3 m s^{-1} in which the fish were able to orient and maintain position by continuously swimming. The tank water temperatures were then raised at a rate of ~3°C per day until the test temperatures of 13° and 19°C were reached (four tanks for the 19°C treatment and two tanks for the 13°C treatment in each year). The 60-yr average river water temperature encountered by Harrison sockeye salmon and Lower Fraser River pink salmon during late September and early October is approximately 13°–14°C (Patterson et al. 2007). Thus, the 13°C temperature treatment closely resembled the historical thermal conditions experienced in the

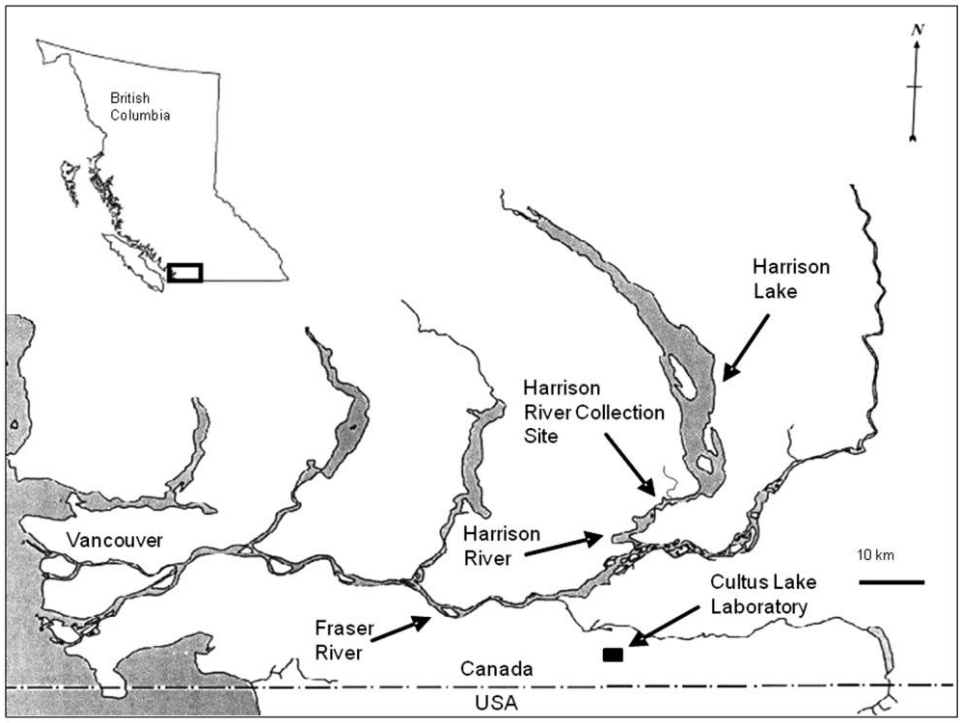

Figure 1. Locations of the Harrison River collection site and Cultus Lake Laboratory near the lower Fraser River, British Columbia, Canada.

river. In contrast, the 19°C temperature treatment represented an extreme situation encountered in the Fraser River by pink salmon and early-entry late-run sockeye salmon (see below) in five of 15 years from 1992 to 2006 (Patterson et al. 2007) and in 2008 and 2009 (eWatch 2010). Since 1995, some late-run sockeye salmon have entered the Fraser River 2–8 wk earlier than their historic norm, exposing them to warmer than normal water temperatures and for longer periods of time, leading to high incidences of en route mortality in these early migrants (exceeding 90% mortality in some instances; Cooke et al. 2004).

After 5 d of a stable test temperature for the 19°C treatment (termed "experimental day 5," but 8 d after the pretreatment samples were taken), blood was resampled from surviving fish in both temperature groups (temperature treatment samples) to determine the effect of water temperature on plasma properties (sockeye salmon in 2008: $n = 11$ males and 7 females exposed to 13°C, 16 males and 4 females exposed to 19°C; pink salmon in 2009: $n = 23$ males and 21 females exposed to 13°C, 21 males and 18 females exposed to 19°C). During the 10-d temperature treatment, dead fish were promptly removed from tanks, and the mortality data were used in the survival analysis. A 10-d temperature exposure period was considered ecologically relevant because this length of time represents a significant portion of the freshwater migration of many stocks of Fraser River Pacific salmon (English et al. 2005). On experimental day 10 in 2009, all female pink salmon in the 13°C treatment were killed to determine maturation status. Surviving female sockeye salmon from the 13°C treatment in 2008 were not killed on experimental day 10 because they were still approximately 1 mo from their historical spawning period and reproductive maturity. Instead, female sockeye salmon were assessed for maturation status on an individual basis as they were found dead in the tanks. Only female fish were examined for maturation status and were considered mature ("ripe") when eggs were released upon firmly squeezing the fish along the lateral lines or when eggs were loose within the body cavity during dissection. Female gonads were weighed (± 0.1 g) to compare gonad masses between treatment groups. Gonadosomatic indices were calculated as gonad mass/(body mass − gonad mass) × 100.

Plasma Analyses

Blood samples (~3 mL) were immediately centrifuged for 7 min, and plasma was stored at −80°C before analyses. Plasma osmolality, chloride, glucose, and lactate were measured in duplicate or triplicate using the procedures outlined in Farrell et al. (2001). Plasma cortisol, testosterone, and 17β-estradiol were measured using commercial ELISA kits (Neogen Corporation, http://www.neogen.com, catalog nos. 402710, 402110, 402510). Testosterone and 17β-estradiol samples were extracted in ethyl ether according to manufacturer's protocols. Cortisol, testosterone, and 17β-estradiol samples were run in duplicate at appropriate dilutions.

Survival Analysis

The effects of sex and temperature on cumulative mortality were assessed using parametric survival analysis (Harrell 2001). Survival models for pink and sockeye salmon assuming no effects, temperature effects (temperature), or sex effects (sex), as well as models including a combination of these effects (temperature + sex) and their interaction (temperature × sex), were fitted to the data. Model selection was carried out using Akaike Information Criterion corrected for small sample sizes (AIC_c; Burnham and Anderson 2002). According to this criterion, the model with the lowest AIC_c value is the most parsimonious one describing the data and other models differing from this one in <2 units (Δ_i) are regarded as also having substantial support from the data. To account for model selection uncertainty, model-averaged cumulative mortality and associated 95% confidence intervals were computed using the weight AIC_c (w_i) of the models included in a 95% confidence set for the best model (Burnham and Anderson 2002). Ratios between the model-averaged estimates were used as a measure of the effect size of the temperature treatments on cumulative mortality (presented as percentages). The parametric distribution used for the survival data analysis was log logistic for pink salmon and Weibull for sockeye salmon. The adequacy of these distributions and the fit of the models were assessed graphically as described by Harrell (2001). Model fitting and selection were performed using R-2.13.1 (R Development Core Team 2008).

Statistical Analysis

It should be noted that logistical constraints and access to salmon prevented further tank/treatment replication. However, we conducted some preliminary analyses and found no strong tank effects; therefore, we pooled results across tanks and used individual fish as the replicates in the statistical analyses. Nevertheless, the samples sizes and inclusion of some replicate tanks in this study were considered exceptional in light of previous studies of large wild adult salmon. Statistical differences between groups for blood plasma variables were determined using a two-factor ANOVA with temperature and sex as factors (SAS, ver. 9.1; SAS Institute) unless specified otherwise. Species were analyzed separately. Tukey-Kramer pairwise comparisons were made a posteriori (Zar 1999). In all cases, homogeneity of variances was assessed by F_{max} tests, and normality was tested using Kolmogorov-Smirnov tests (Sokal and Rohlf 1995). Data were $\log_{10}$-transformed if the assumption of homogeneity of variances could not be met. Where the assumption of normality could not be met, a Freidman nonparametric two-factor ANOVA was used with temperature and sex as factors (Zar 1999). Sexes were analyzed separately for plasma sex steroid levels using either *t*-tests or Wilcoxon two-sample tests if the assumption of normality could not be met. Differences in the number of pink salmon females that became ripe were compared between temperature treatments using a χ^2 contingency table. Length-adjusted gonad masses were compared between temperature treatments using ANCOVA with postorbital fork length as the covariate.

Results

Mortality Patterns and Maturation

Observed mortality was greater at 19° than at 13°C at experimental days 5 and 10 for both species and sexes (fig. 2). Accordingly, the most parsimonious model describing the cumulative mortality data showed that temperature alone best described the mortality patterns for pink salmon (table 1). Model-averaged mortality estimates indicate that the 19°C treatment mortality patterns for male and female pink salmon are on average 30% and 32%, respectively, higher compared with those of the 13°C treatment (fig. 2). Sex-specific differences in mortality patterns existed for sockeye salmon because females had greater observed mortality than males, a pattern not observed in pink salmon. Model selection supported this because the most parsimonious model describing the cumulative mortality data for sockeye salmon had an interaction between temperature and sex (table 1). Indeed, the 10-d model-averaged mortality estimates indicate that female sockeye salmon suffer on average 92% (at 13°C) and 62% (at 19°C) higher mortality than male sockeye salmon (fig. 2). Observed mortality of female sockeye salmon did not vary substantially by temperature, and model-averaged estimates indicate that their mortality is on average only 10% higher at 19° than at 13°C (fig. 2). Conversely, model-averaged estimates indicate that the 19°C treatment mortality in male sockeye salmon is on average 34% higher than at 13°C (fig. 2). Male sockeye salmon had the lowest mortality of all the groups, suggesting that male sockeye salmon were the most thermally tolerant.

There was evidence that exposure to the high-temperature treatment delayed or inhibited final maturation (table 2). While there were no differences in length-adjusted gonad mass between female pink salmon held at 13° and 19°C (ANCOVA, $P > 0.05$), more female pink salmon held at 13°C (19 out of 30 [63.3%]) became ripe compared with females held at 19°C (11 out of 48 [22.9%]; $\chi^2 = 11.09$, $P < 0.001$). Within the 19°C treatment, female pink salmon that became ripe had greater length-adjusted gonad mass than females that did not become ripe (ANCOVA, $F_{1,45} = 17.73$, $P < 0.0001$), although this difference was not detected within the females held at 13°C ($P = 0.09$). Only one female sockeye salmon (held at 13°C) that died during the 10-d holding period was ripe.

Plasma Variables

The effect of the holding temperature on blood plasma physiology was limited to effects on osmoregulatory and reproductive indices. Sockeye salmon held at 19°C had higher plasma osmolality ($F_{1,34} = 19.37$, $P < 0.0005$) and chloride ($F_{1,34} = 19.32$, $P < 0.0005$) than those held at 13°C on experimental day 5 (fig. 3). Females held at 19°C had lower testosterone than fish held at 13°C in sockeye (Wilcoxon two-sample test, $T = 10.00$, $P < 0.05$) and pink (Wilcoxon two-sample test, $T =$

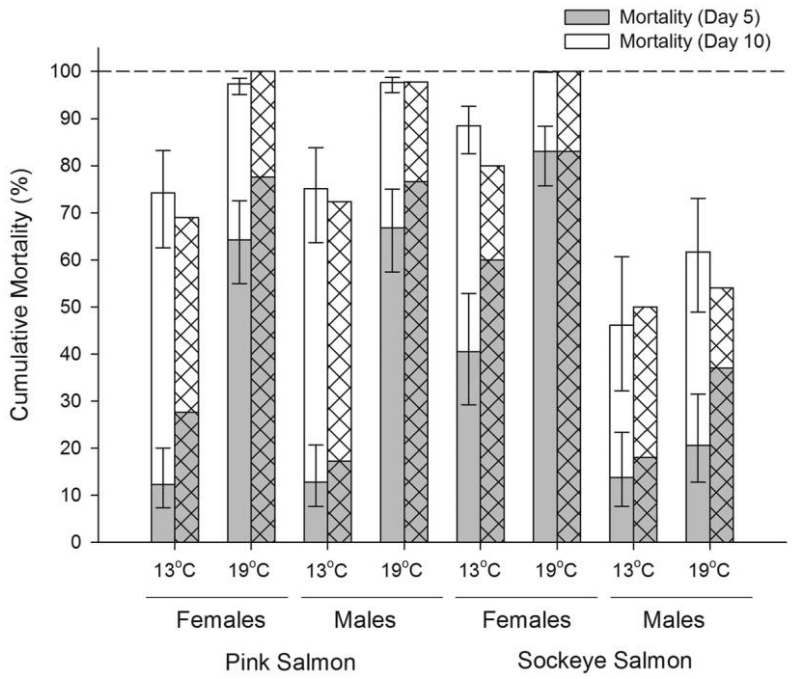

Figure 2. Model-averaged estimated (open bars) and observed (hatched bars) cumulative mortality at experimental days 5 and 10 for female and male pink and sockeye salmon held for 10 d at the treatment temperatures of 13° and 19°C. Error bars denote 95% confidence intervals for the model-averaged estimates.

217.00, $P < 0.001$) salmon, and female sockeye salmon held at 19°C had lower 17β-estradiol than those held at 13°C (t-test, $t = 2.53$, $P < 0.05$; fig. 4).

Female pink salmon had higher pretreatment chloride than males (Freidman ANOVA, $F_{1,93} = 5.63$, $P < 0.02$), and this difference was maintained throughout the temperature treatments (Freidman ANOVA, $F_{1,80} = 15.47$, $P < 0.0005$). Similarly, female pink ($F_{1,92} = 35.16$, $P < 0.0001$) and sockeye ($F_{1,124} = 23.60$, $P < 0.0001$) salmon had higher pretreatment cortisol than males, yet this was maintained throughout the temperature treatments for sockeye salmon only ($F_{1,34} = 17.58$, $P < 0.0005$; fig. 5). Female sockeye salmon had higher pretreatment ($F_{1,124} = 14.13$, $P < 0.0005$) and treatment ($F_{1,34} = 5.38$, $P < 0.05$) glucose and pretreatment (Freidman ANOVA, $F_{1,125} = 10.43$, $P < 0.005$) and treatment ($F_{1,34} = 8.66$, $P < 0.01$) lactate compared with males. Conversely, male pink salmon had higher treatment lactate levels compared with females (Freidman ANOVA, $F_{1,80} = 21.06$, $P < 0.0001$).

Discussion

Strong temperature- and sex-specific differences in mortality were detected in this study. After 10 d at 19°C, nearly all female sockeye salmon and both sexes of pink salmon had died, while mortality was lower in pink salmon held at 13°C and male sockeye salmon held at both temperatures. Male sockeye salmon were the most thermally tolerant group in this experiment, contrary to our prediction that pink salmon would be less affected by the temperature treatments. The pink salmon mortality patterns may have been influenced by the fact that these fish were closer to final maturation than the sockeye salmon at time of capture and the start of the experiment. It is important to note that there may be considerable interannual variation in mortality patterns in Pacific salmon populations that are often related to in-river migration conditions (Gil-

Table 1: Model selection statistics summary for models describing survival for pink and sockeye salmon held at 13° and 19°C

Model	AIC_c	Δ_i	w_i	No. parameters
Pink salmon:				
Temperature	**609.53**	**.00**	**.59**	**3**
Temperature + sex	**611.01**	**1.48**	**.28**	**4**
Temperature × sex	**612.45**	**2.92**	**.14**	**5**
No effects	670.81	61.28	.00	2
Sex	672.75	63.22	.00	3
Sockeye salmon:				
Temperature × sex	**339.19**	**.00**	**.71**	**5**
Temperature + sex	**340.98**	**1.79**	**.29**	**4**
Sex	349.39	10.19	.00	3
Temperature	372.17	32.97	.00	3
No effects	373.70	34.51	.00	2

Note. Models are ranked by increasing order of their AIC_c value, and statistics for models included in the 95% confidence set for the best models are shown in bold. See text for description of AIC_c, Δ_i, and w_i.

Table 2: Maturation status and gonadosomatic indices of female sockeye and pink salmon that died during the 10-d treatment period

Species and holding temperature	Total no. females	Ripe		Not ripe	
		n	GSI	*n*	GSI
Sockeye salmon:					
13°C	16	1	17.4	15	13.8 (2.3)
19°C	19	0	NA	19	15.3 (5.4)
Pink salmon:					
13°C	30	19	21.1 (3.1)^{A}	11	18.2 (4.0)^{A}
19°C	48	11	22.2 (2.0)^{A}	37	18.1 (2.4)^{B}

Note. For the gonadosomatic index (GSI), data are means, with SDs in parentheses. All pink salmon were killed immediately after the experiment and were included in the analysis; any surviving female sockeye salmon were kept alive after the temperature holding period. Superscript letters that are different within a row indicate statistical significance at $P < 0.05$.

housen 1990; Macdonald et al. 2010). There were also additional stressors in this study, such as capture, transport, handling, and laboratory holding, that likely enhanced rates of mortality from those experienced naturally. Therefore, it is difficult to generalize the details of these results (e.g., actual levels of mortality) to other migration years and systems, especially because of the relatively high mortality in the 13°C treatment. However, the effect of the elevated water temperature treatment was clear in that both sexes of both species suffered greater mortality at 19° than at 13°C.

The measured blood plasma properties did not provide direct evidence for a physiological mechanism for the higher mortality observed at 19°C. Because fish were held at 19°C for a relatively short period of time (≤10 d), mortality was unlikely to be due to energy exhaustion, especially for the female sockeye salmon that were approximately 1 mo from spawning. Therefore, the most likely cause of death may be an increase in disease and parasite progression (e.g., Servizi and Jensen 1977; Crossin et al. 2008) and/or impaired cardiorespiratory function leading to insufficient oxygen transport capacity (e.g., Clark et al. 2008; Farrell et al. 2008). Because some stocks of Pacific salmon can tolerate temperatures much greater than 19°C for short periods without lethally impacting cardiorespiratory performance (Steinhausen et al. 2008; Clark et al. 2011) and cardiovascular impairments would likely be accompanied by changes in blood properties (e.g., lactate linked with hypoxemia), we believe that temperature-dependent disease and parasite progression (e.g., *Parvicapsula minibicornis*, *Saprolegnia* spp., and *Flexibactor columnaris*) were likely large contributors to the higher mortality observed at 19°C in this study. The relatively high mortality in fish held at 13°C (50%–80% after 10 d) potentially suggests that disease and parasite progressions may have been under way before collection and exposure to the temperature treatments. Indeed, there were en route losses of approximately 80% for Harrison sockeye salmon in the wild in 2008, and these fish would have experienced extended freshwater residence time as a result of their early entry into the Fraser River (http://www.psc.org), increasing potential exposure to diseases and pathogens and allowing more time for infections to become virulent.

The experiments revealed the higher and more rapid mortality of female sockeye salmon compared with males, independent of temperature treatment. A similar pattern has been reported previously for experimentally held and freely migrating Fraser River sockeye salmon (Patterson et al. 2004; Crossin et al. 2008). A key regulator of Pacific salmon population productivity is considered to be the total number of eggs deposited during the spawning period (Quinn 2005) because the number of eggs from females is limited while males are capable of spawning with several females (Mehranvar et al. 2004). Consequently, a higher level of premature mortality in females could have a proportionally greater negative impact on the abundance of Pacific salmon populations.

Insight into the mechanisms causing higher mortality in female sockeye salmon, and their apparent increased sensitivity to stress, comes from an examination of their physiology. Female salmonids respond to stress differently at the transcriptional level compared with males, emphasizing the importance of accounting for sex when studying physiological responses to stress (Momoda et al. 2007). Previous work has demonstrated that female sockeye salmon have higher routine heart rates than males when confined, which may reduce their scope in heart rate and their ability to tolerate additional stressors (Sandblom et al. 2009). It is well established that cortisol levels are naturally elevated in maturing female sockeye salmon compared with males (Macdonald et al. 2000; Patterson et al. 2004; Crossin et al. 2008; Sandblom et al. 2009; Clark et al. 2010). Consistent with this, female sockeye salmon in our study had higher pretreatment plasma cortisol, along with higher glucose and lactate levels, than males. The elevated cortisol in female sockeye salmon, which was maintained through the temperature treatments, is perhaps to mobilize energy stores to fuel the migration and the greater relative reproductive investment of females (Mommsen et al. 1999). Nevertheless, cortisol is an important biomarker for stress. Macdonald et al. (2000) previously observed higher cortisol levels in compromised (external fungus,

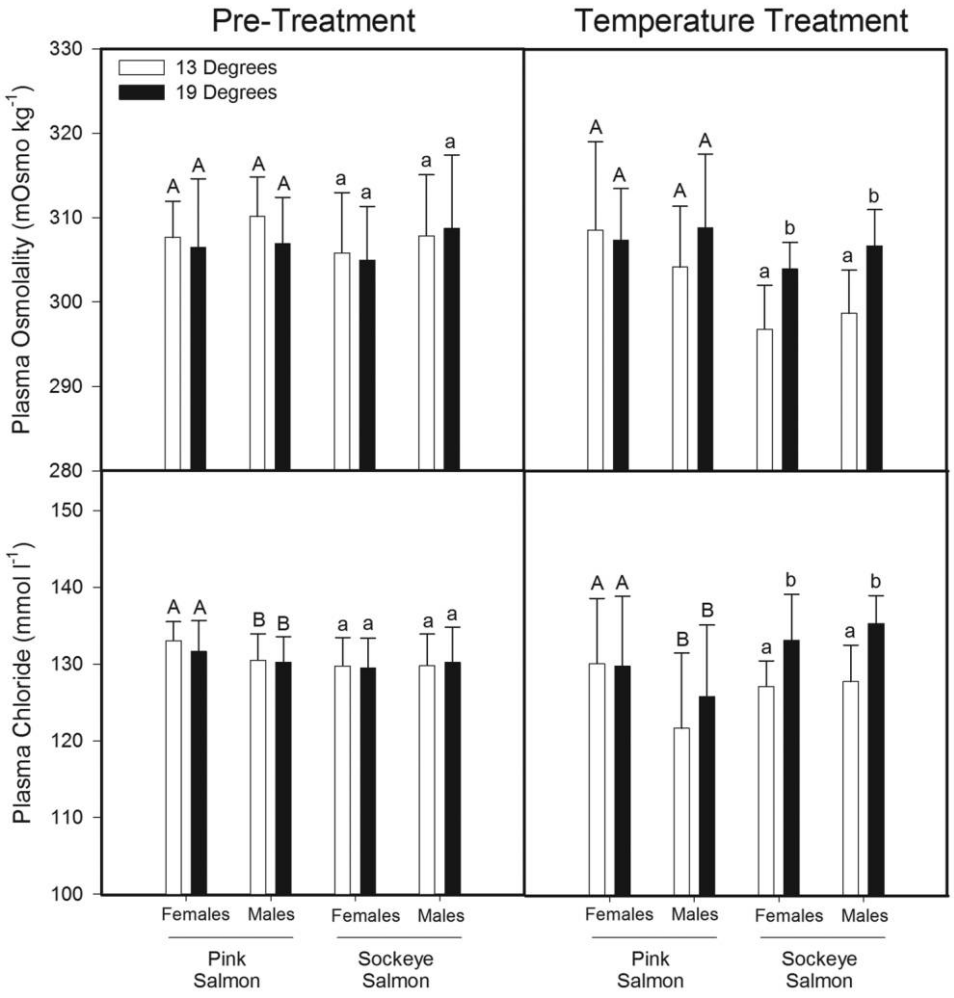

Figure 3. Pretreatment and treatment (experimental day 5) plasma osmolality and chloride for female and male pink and sockeye salmon held at 13° and 19°C (mean ± SD). Uppercase letters indicate statistical differences among pink salmon groups, and lowercase letters indicate statistical differences among sockeye salmon groups.

skin lesions, and lethargic behavior) female versus "healthy" male and female sockeye salmon. Thus, the higher pretreatment cortisol (and glucose and lactate) levels of female sockeye salmon in our study may also suggest that they were less healthy or more stressed before the temperature exposure period, consistent with the rapid mortality that occurred. Indeed, prespawn mortality of female sockeye salmon in 2008 was high throughout the Fraser River watershed (~34% averaged across all stocks), which led to lower numbers of successful spawners (K. Benner, Fisheries and Oceans Canada, personal communication) and suggests that 2008 was an anomalous year. While the exact physiological mechanisms responsible for the higher mortality in female sockeye salmon remain unknown, higher basal stress levels and susceptibility to stress and disease may have played a role.

Temperature and handling/confinement stress, along with proximity to reproductive maturation, likely contributed to the higher mortality of pink salmon held at 19°C compared with those held at 13°C. These are ecologically important results because Lower Fraser River pink salmon spawn in the main stem of the Fraser River and its major tributaries (e.g., Harrison River) and therefore may experience high water temperatures during final maturation, which may lead to high rates of prespawn mortality. However, because some female pink salmon became ripe in the 19°C treatment, it is likely that some successful spawning still occurs at elevated water temperatures in natural conditions. Captive pink salmon in this study were observed attempting to dig redds, eliciting courtship behaviors, and interacting aggressively with conspecifics, similar to the behavior observed in another study that held pink salmon in captivity (Williams and Brett 1987). In contrast, sockeye salmon were observed to school and swim into the current. Additionally, pink salmon sex steroid levels in our study were comparable to levels detected in migrating pink salmon on arrival at spawning grounds (Williams et al. 1986). These behavioral observations and sex steroid patterns suggest that the pink salmon

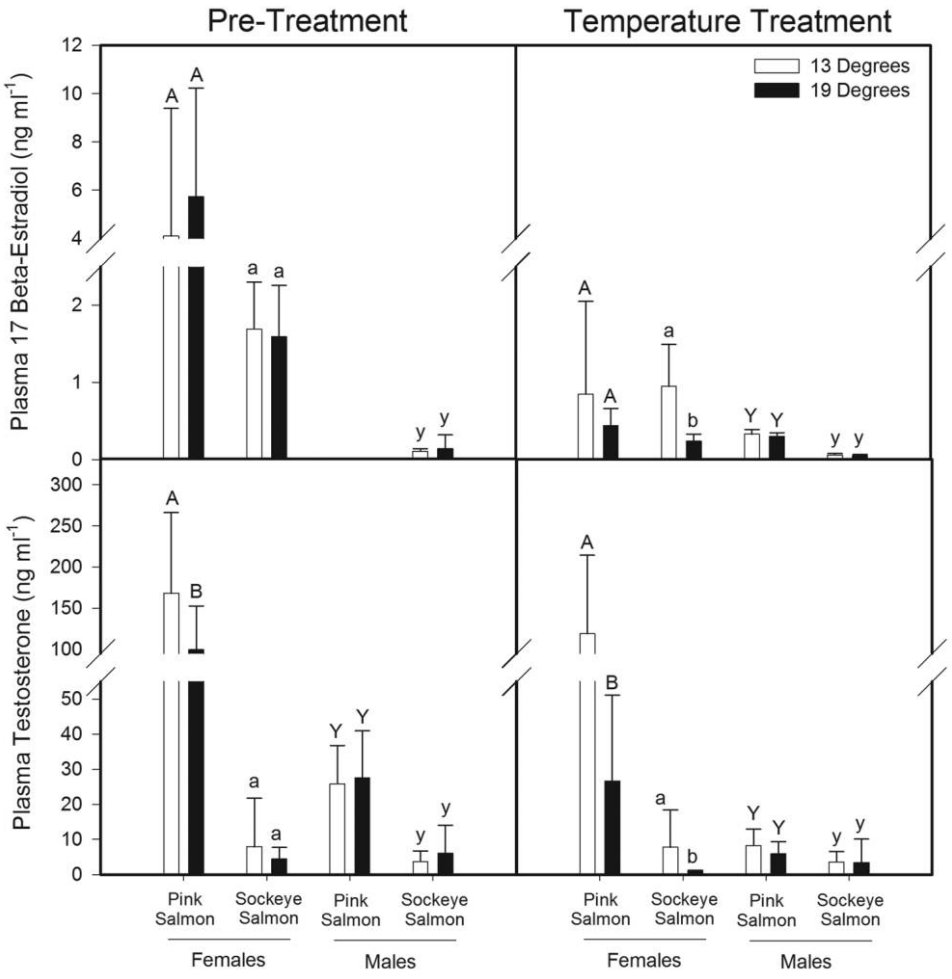

Figure 4. Pretreatment and treatment (experimental day 5) plasma 17β-estradiol and testosterone for female and male pink and sockeye salmon held at 13° and 19°C (mean ± SD). Uppercase letters indicate statistical differences among pink salmon groups, and lowercase letters indicate statistical differences among sockeye salmon groups. There were no direct sex comparisons for plasma 17β-estradiol and testosterone.

used in our study were close to final maturation. The relative increases in plasma cortisol, glucose, and lactate in pink salmon from pretreatment levels to temperature treatment levels likely reflect an elevated stress response associated with proximity to final maturation. Enhanced senescence may have also been a factor, as plasma cortisol and lactate have been reported to increase in moribund Pacific salmon (Hruska et al. 2010; Jeffries et al. 2011). Mortality of pink salmon associated with natural senescence may help to explain the mortality patterns observed at both temperatures in our study.

In addition to the implications for survival, exposure to elevated water temperature may also delay or inhibit final maturation in salmonids through its effects on steroid biosynthesis and the inhibition of the preovulatory shift to maturational hormone production (Pankhurst and King 2010). Chronic exposure to 19°C water resulted in a reduction in plasma testosterone and 17β-estradiol levels in female pink and sockeye salmon after 5 d. These reduced sex steroid levels are consistent with those observed by Macdonald et al. (2000), who suggested that the temperature threshold for sex steroid production is between 15° and 19°C for Fraser River sockeye salmon. Decreased sex steroid biosynthesis may lead to reduced 17α,20β-dihydroxy-4-pregen-3-one (hormone required for final maturation) production, which decreases the likelihood of females reaching maturity and having viable eggs (Macdonald et al. 2000). Indeed, in comparison with fish at 13°C, female pink salmon held at 19°C were less likely to become ripe, which may be linked with the reduced plasma sex steroid levels and demonstrates a potential reproductive consequence to exposure to elevated water temperatures during final maturation. In contrast, temperature effects on final maturation were not measurable in female sockeye salmon because the temperature treatments occurred over a month before these fish historically reach maturity. The mechanisms for decreased steroid biosynthesis and the possible link to delayed or inhibited maturation in temperature-challenged migrating Pacific salmon warrant further investigation.

Water temperature appeared to affect ion balance, with

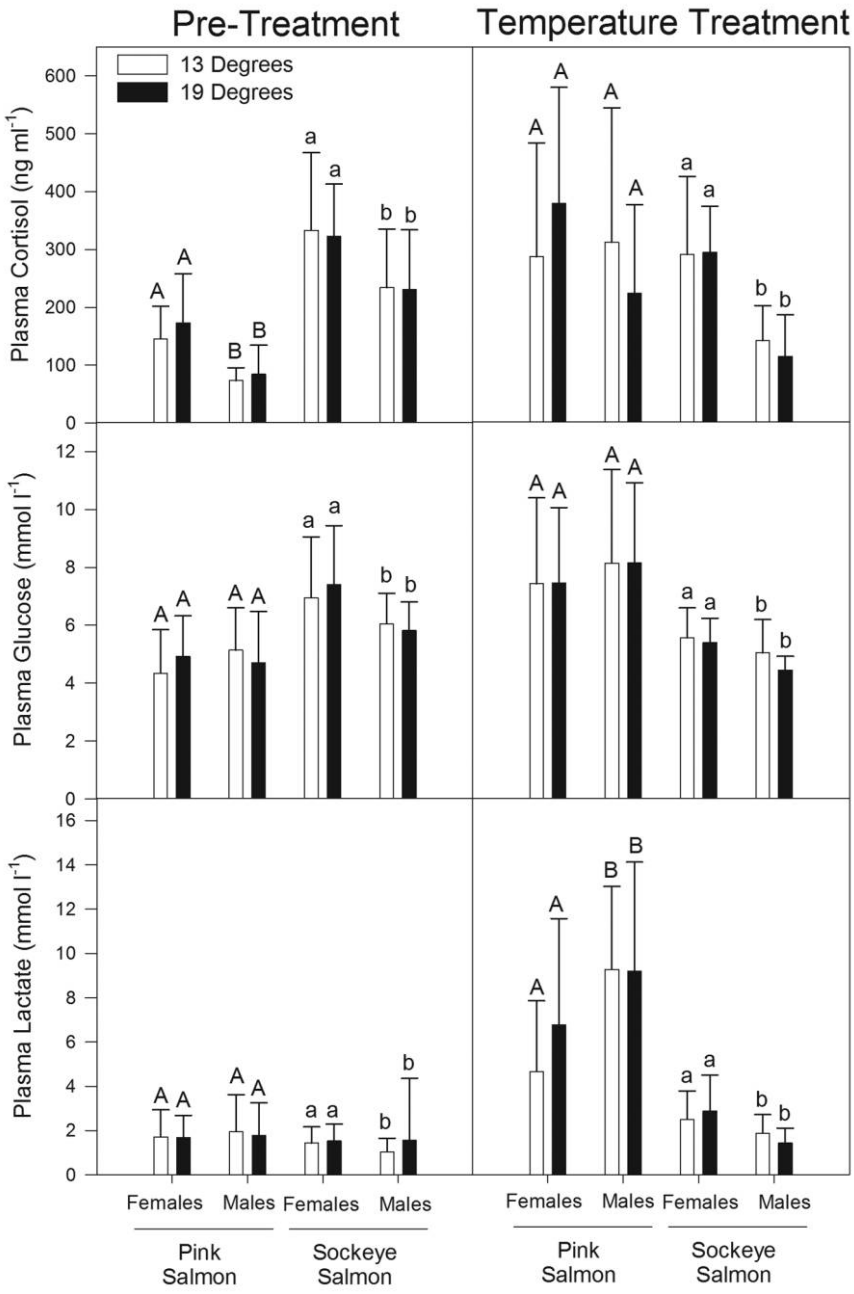

Figure 5. Pretreatment and treatment (experimental day 5) plasma cortisol, glucose, and lactate for female and male pink and sockeye salmon held at 13° and 19°C (mean ± SD). Uppercase letters indicate statistical differences among pink salmon groups, and lowercase letters indicate statistical differences among sockeye salmon groups.

plasma chloride and osmolality being higher in adult sockeye salmon held at 19°C compared with fish held at 13°C (the higher plasma chloride likely contributed to the higher osmolality), indicative of an osmoregulatory disturbance. Plasma chloride has been shown to increase with temperature in rainbow trout (*Oncorhynchus mykiss* Walbaum; Smit et al. 1981; Wagner et al. 1997), in an African cichlid (*Tilapia mossambica* Peters; Allanson et al. 1971), and even in the nonteleost Adriatic sturgeon (*Acipenser naccarii* Bonaparte; Cataldi et al. 1998). Branchial chloride uptake increases at higher temperatures in Arctic grayling (*Thymallus arcticus* Pallas), and this increase may be required for a blood buffer readjustment at the higher temperature to maintain acid-base equilibrium (Cameron 1976). Fish typically show a decrease in plasma pH with an increase in temperature (Reeves 1977), and chloride ions can be used to counter the increase in plasma H^+ to maintain ionic balance. Therefore, the higher plasma chloride detected in 19°C-held sockeye salmon may be associated with maintaining

blood acid-base equilibrium at warm water temperatures and is indicative of a thermal stress response.

In summary, a 10-d exposure to an ecologically relevant high temperature (19°C) resulted in higher mortality in both pink and sockeye salmon, with evidence for sex-specific mortality patterns in sockeye salmon. This suggests that during years with extreme water temperatures, the magnitude of temperature-associated en route and prespawn mortality will vary by species and sex in Pacific salmon. It must be noted that the qualitative differences in mortality patterns detected in our study may have been influenced by the fact that the pink salmon were closer to final maturation than the sockeye salmon. While fisheries management models have evolved to include information on river temperature, our data suggest that the models would be improved by including sex and proximity to final maturation as additional factors. Additionally, our study demonstrated that there are reproductive consequences to exposure to high water temperature during final maturation, a response to temperature with profound ecological implications. If Pacific salmon populations are unable to adapt to the changing thermal regime in natal rivers, populations at the southern periphery of their species' distribution may continue to decline or possibly become extirpated.

Acknowledgments

We thank Jenn Burt, Alison Collins, Mike Donaldson, Matt Drenner, Erika Eliason, Marika Gale, Merran Hague, Kim Hruska, Lucas Pon, David Roscoe, Lisa Thompson, Charlotte Whitney, Jeremy Lotto, Chris Brown, Sarah McConnachie, Graham Raby, and Quinten Renault for field assistance. Many thanks to Jayme Hills, Vanessa Ives, Miki Nomura, Jessica Carter, and D'Arcy McKay, with the Fraser River Environmental Watch Program, and Colin Wallace, John Candy, and the Molecular Genetics Laboratory at the Pacific Biological Station. We also thank Mike Lapointe and Steve Latham from the Pacific Salmon Commission, Bryan Smith and the Fisheries and Oceans Canada Cultus Lake Laboratory staff, and Bill Milsom and Greg Goss for helpful discussions. This research was approved by the University of British Columbia Animal Ethics Committee (animal care permit A08-0388-010) in accordance with the Canadian Council on Animal Care. This project was funded by a Natural Sciences and Engineering Research Council (NSERC) strategic grant to S.G.H., K.M.M., and others and by the Fisheries and Oceans Canada's Environmental Watch program. T.D.C. was supported in part by a Killam Postdoctoral Fellowship at the University of British Columbia. K.M.J. was supported by a NSERC postgraduate scholarship and a Pacific Century Graduate Scholarship at the University of British Columbia.

Literature Cited

Allanson B.R., A. Bok, and N.I. van Wyk. 1971. The influence of exposure to low temperature on *Tilapia mossambica* Peters (Cichlidae). II. Changes in serum osmolality, sodium and chloride ion concentrations. J Fish Biol 3:181–185.

Beacham T.D., J.R. Candy, B. McIntosh, C. MacConnachie, A. Tabata, K. Kaukinen, L.T. Deng, K.M. Miller, R.E. Withler, and N. Varnavskaya. 2005. Estimation of stock composition and individual identification of sockeye salmon on a Pacific rim basis using microsatellite and major histocompatibility complex variation. Trans Am Fish Soc 134:1124–1146.

Beechie T., E. Buhle, M. Ruckelshaus, A. Fullerton, and L. Holsinger. 2006. Hydrologic regime and the conservation of salmon life history diversity. Biol Conserv 130:560–572.

Bradford M.J., J. Lovy, D.A. Patterson, D.J. Speare, W.R. Bennett, A.R. Stobbart, and C.P. Tovey. 2010. *Parvicapsula minibicornis* infections in gill and kidney and the premature mortality of adult sockeye salmon (*Oncorhynchus nerka*) from Cultus Lake, British Columbia. Can J Fish Aquat Sci 67:673–683.

Burnham K.P. and D.R. Anderson. 2002. Model selection and multi-model inference: a practical information-theoretic approach. Springer, New York.

Cameron J.N. 1976. Branchial ion uptake in Arctic grayling: resting values and effects of acid-base disturbance. J Exp Biol 64:711–725.

Cataldi E., P. Di Marco, A. Mandich, and S. Cataudella. 1998. Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): effects of temperature and stress. Comp Biochem Physiol A 121:351–354.

Clark T.D., K.M. Jeffries, S.G. Hinch, and A.P. Farrell. 2011. Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. J Exp Biol 214:3074–3081.

Clark T.D., E. Sandblom, G.K. Cox, S.G. Hinch, and A.P. Farrell. 2008. Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). Am J Physiol 295:R1631–R1639.

Clark T.D., E. Sandblom, S.G. Hinch, D.A. Patterson, P.B. Frappell, and A.P. Farrell. 2010. Simultaneous biologging of heart rate and acceleration, and their relationships with energy expenditure in free-swimming sockeye salmon (*Oncorhynchus nerka*). J Comp Physiol B 180:673–684.

Cooke S.J., S.G. Hinch, A.P. Farrell, M.F. Lapointe, S.R.M. Jones, J.S. Macdonald, D.A. Patterson, M.C. Healey, and G. Van Der Kraak. 2004. Abnormal migration timing and high *en route* mortality of sockeye salmon in the Fraser River, British Columbia. Fisheries 29:22–33.

Crossin G.T., S.G. Hinch, S.J. Cooke, D.W. Welch, D.A. Patterson, S.R.M. Jones, A.G. Lotto, et al. 2008. Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migration. Can J Zool 86:127–140.

Crossin G.T., S.G. Hinch, A.P. Farrell, M.P. Whelly, and M.C. Healey. 2003. Pink salmon (*Oncorhynchus gorbuscha*) migratory energetics: response to migratory difficulty and comparisons with sockeye salmon (*Oncorhynchus nerka*). Can J Zool 81:1986–1995.

Eaton J.G. and R.M. Scheller. 1996. Effects of climate warming

on fish thermal habitat in streams of the United States. Limnol Oceanogr 41:1109–1115.

Eliason E.J., T.D. Clark, M.J. Hague, L.M. Hanson, Z.S. Gallagher, K.M. Jeffries, M.K. Gale, D.A. Patterson, S.G. Hinch, and A.P. Farrell. 2011. Differences in thermal tolerance among sockeye salmon populations. Science 332:109–112.

English K.K., W.R. Koski, C. Sliwinski, A. Blakley, A. Cass, and J.C. Woodey. 2005. Migration timing and river survival of late-run Fraser River sockeye salmon estimated using radiotelemetry techniques. Trans Am Fish Soc 134:1342–1365.

eWatch. 2010. Fisheries and Oceans Canada Fraser River environmental watch program. http://www.pac.dfo-mpo.gc.ca/science/habitat/frw-rfo/index-eng.htm.

Farrell A.P., P.E. Gallaugher, J. Fraser, D. Pike, P. Bowering, A.K.M. Hadwin, W. Parkhouse, and R. Routledge. 2001. Successful recovery of the physiological status of coho salmon on board a commercial gillnet vessel by means of a newly designed revival box. Can J Fish Aquat Sci 58:1932–1946.

Farrell A.P., S.G. Hinch, S.J. Cooke, D.A. Patterson, G.T. Crossin, M. Lapointe, and M.T. Mathes. 2008. Pacific salmon in hot water: applying aerobic scope models and biotelemetry to predict the success of spawning migrations. Physiol Biochem Zool 81:697–708.

Ferrari M.R., J.R. Miller, and G.L. Russell. 2007. Modeling changes in summer temperature of the Fraser River during the next century. J Hydrol 342:336–346.

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

Groot C. and L. Margolis, eds. 1991. Pacific salmon life histories. University of British Columbia Press, Vancouver.

Hague M.J., M.R. Ferrari, J.R. Miller, D.A. Patterson, G.L. Russell, A.P. Farrell, and S.G. Hinch. 2011. Modelling the future hydroclimatology of the lower Fraser River and its impacts on the spawning migration survival of sockeye salmon. Glob Change Biol 17:87–98.

Harrell F.E. 2001. Regression modelling strategies. Springer, New York.

Hruska K.A., S.G. Hinch, M.C. Healey, D.A. Patterson, S. Larsson, and A.P. Farrell. 2010. Influences of sex and activity level on physiological changes in individual adult sockeye salmon during rapid senescence. Physiol Biochem Zool 83:663–676.

Jeffries K.M., S.G. Hinch, M.R. Donaldson, M.K. Gale, J.M. Burt, L.A. Thompson, A.P. Farrell, D.A. Patterson, and K.M. Miller. 2011. Temporal changes in blood variables during final maturation and senescence in male sockeye salmon *Oncorhynchus nerka*: reduced osmoregulatory ability can predict mortality. J Fish Biol 79:449–465.

Jensen J.O.T., W.E. McLean, W. Damon, and T. Sweeten. 2004. Puntledge River high temperature study: influence of high water temperatures on adult pink salmon mortality, maturation, and gamete viability. Can Tech Rep Fish Aquat Sci 2523:1–50.

Keefer M.L., C.A. Peery, and M.J. Heinrich. 2008. Temperature-mediated *en route* migration mortality and travel rates of endangered Snake River sockeye salmon. Ecol Freshw Fish 17:136–145.

Keefer M.L., G.A. Taylor, D.F. Garletts, G.A. Gauthier, T.M. Pierce, and C.C. Caudill. 2010. Prespawn mortality in adult spring Chinook salmon outplanted above barrier dams. Ecol Freshw Fish 19:361–372.

Macdonald J.S., M.G.G. Foreman, T. Farrell, I.V. Williams, J. Grout, A. Cass, J.C. Woodey, et al. 2000. The influence of extreme water temperatures on migrating Fraser River sockeye salmon (*Oncorhynchus nerka*) during the 1998 spawning season. Can Tech Rep Fish Aquat Sci 2326:1–117.

Macdonald J.S., D.A. Patterson, M.J. Hague, and I.C. Guthrie. 2010. Modeling the influence of environmental factors on spawning migration mortality for sockeye salmon fisheries management in the Fraser River, British Columbia. Trans Am Fish Soc 139:768–782.

Mantua N., I. Tohver, and A. Hamlet. 2010. Climate change impacts on streamflow extremes and summertime stream temperature and their possible consequences for freshwater salmon habitat in Washington State. Clim Change 102:187–223.

Martins E.G., S.G. Hinch, D.A. Patterson, M.J. Hague, S.J. Cooke, K.M. Miller, M.F. Lapointe, K.K. English, and A.P. Farrell. 2011. Effects of river temperature and climate warming on stock-specific survival of adult migrating Fraser River sockeye salmon (*Oncorhynchus nerka*). Glob Change Biol 17: 99–114.

Mehranvar L., M. Healey, A. Farrell, and S. Hinch. 2004. Social versus genetic measures of reproductive success in sockeye salmon, *Oncorhynchus nerka.* Evol Ecol Res 6:1167–1181.

Mommsen T.P., M.M. Vijayan, and T.W. Moon. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fish 9:211–268.

Momoda T.S., A.R. Schwindt, G.W. Feist, L. Gerwick, C.J. Bayne, and C.B. Schreck. 2007. Gene expression in the liver of rainbow trout, *Oncorhynchus mykiss*, during the stress response. Comp Biochem Physiol D 2:303–315.

Morrison J., M.C. Quick, and M.G.G. Foreman. 2002. Climate change in the Fraser River watershed: flow and temperature projections. J Hydrol 263:230–244.

Pankhurst N.W. and H.R. King. 2010. Temperature and salmonid reproduction: implications for aquaculture. J Fish Biol 76:69–85.

Patterson D.A., J.S. Macdonald, S.G. Hinch, M.C. Healey, and A.P. Farrell. 2004. The effect of exercise and captivity on energy partitioning, reproductive maturation and fertilization success in adult sockeye salmon. J Fish Biol 64:1039–1059.

Patterson D.A., J.S. MacDonald, K.M. Skibo, D. Barnes, I. Guthrie, and J. Hills. 2007. Reconstructing the summer thermal history for the lower Fraser River, 1941 to 2006, and implications for adult sockeye salmon (*Oncorhynchus nerka*) spawning migration. Can Tech Rep Fish Aquat Sci 2724:1–43.

Quinn T.P. 2005. The behavior and ecology of Pacific salmon and trout. American Fisheries Society, Bethesda, MD.

Quinn T.P. and D.J. Adams. 1996. Environmental changes affecting the migratory timing of American shad and sockeye salmon. Ecology 77:1151–1162.

Rand P.S., S.G. Hinch, J. Morrison, M.G.G. Foreman, M.J. MacNutt, J.S. Macdonald, M.C. Healey, A.P. Farrell, and D.A. Higgs. 2006. Effects of river discharge, temperature, and future climates on energetics and mortality of adult migrating Fraser River sockeye salmon. Trans Am Fish Soc 135:655–667.

R Development Core Team. 2008. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.r-project.org.

Reeves R.B. 1977. Interaction of body-temperature and acid-base-balance in ectothermic vertebrates. Annu Rev Physiol 39:559–586.

Sandblom E., T.D. Clark, S.G. Hinch, and A.P. Farrell. 2009. Sex-specific differences in cardiac control and hematology of sockeye salmon (*Oncorhynchus nerka*) approaching their spawning grounds. Am J Physiol 297:R1136–R1143.

Servizi J.A. and J.O.T. Jensen. 1977. Resistance of adult sockeye salmon to acute thermal shock. Report 34. International Pacific Salmon Fisheries Commission, New Westminster, British Columbia.

Smit G.L., J. Hattingh, and J.T. Ferreira. 1981. The physiological responses of blood during thermal adaptation in three freshwater fish species. J Fish Biol 19:147–160.

Sokal R.R. and F.J. Rohlf. 1995. Biometry: the principals and practice of statistics in biological research. 3rd ed. W. H. Freeman, New York.

Steinhausen M.F., E. Sandblom, E.J. Eliason, C. Verhille, and A.P. Farrell. 2008. The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). J Exp Biol 211: 3915–3926.

Taylor S.G. 2008. Climate warming causes phenological shift in pink salmon, *Oncorhynchus gorbuscha*, behavior at Auke Creek, Alaska. Glob Change Biol 14:229–235.

Wagner E.J., T. Bosakowski, and S. Intelmann. 1997. Combined effects of temperature and high pH on mortality and the stress response of rainbow trout after stocking. Trans Am Fish Soc 126:985–998.

Williams I.V. and J.R. Brett. 1987. Critical swimming speed of Fraser and Thompson River pink salmon (*Oncorhynchus gorbuscha*). Can J Fish Aquat Sci 44:348–356.

Williams I.V., J.R. Brett, G.R. Bell, G.S. Traxler, J. Bagshaw, J.R. McBride, U.H.M. Fagerlund, et al. 1986. The 1983 early run Fraser and Thompson River pink salmon: morphology, energetics and fish health. Int Pac Salmon Fish Comm Bull 23: 1–55.

Zar J.H. 1999. Biostatistical analysis. 4th ed. Prentice Hall, Upper Saddle River, NJ.