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

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Abstract: Since 1996, some populations of Fraser River sockeye salmon (*Oncorhynchus nerka* Walbaum in Artedi, 1792) have begun spawning migrations weeks earlier than normal, and most perish en route as a result. We suspect that a high midsummer river temperature is the principal cause of mortality. We intercepted 100 sockeye during normal migration near a spawning stream and measured somatic energy and aspects of plasma biochemistry. Fish were then held at either 10 or 18 °C for 24 days. Before release, fish were biopsied again and implanted with acoustic transmitters. A group of biopsied but untreated control salmon were released at the same time. Sixty-two percent (8 of 13) of control salmon and 68% (21 of 31) of 10 °C salmon reached spawning areas. The 18 °C-treated fish were half as successful (35%; 6 of 17). During the holding period, mortality was 2 times higher and levels of *Parvicapsula minibicornis* (Kent, Whitaker and Dawe, 1997) infection were higher in the 18 °C-treated group than in the 10 °C-treated group. The only physiological difference between treatments was a change in gill Na^+,K^+-ATPase activity. This drop correlated negatively with travel times for the 18 °C-treated males. Reproductive-hormone levels and stress measures did not differ between treatments but showed significant correlations with individual travel times.

Résumé : Depuis 1996, quelques populations de saumons rouges (*Oncorhynchus nerka* Walbaum in Artedi, 1792) du Fraser commencent leur migration de fraie quelques semaines avant la période normale et, en conséquence, la plupart des poissons périssent en route. Nous soupçonnons que c'est principalement la forte température de la rivière en mi-été qui cause cette mortalité. Nous avons intercepté 100 saumons rouges durant leur période normale de migration près d'un cours d'eau de fraie et nous avons mesuré leur énergie somatique et quelques aspects de la biochimie de leur plasma. Les poissons ont ensuite été retenus pendant 24 jours à 10 ou à 18 °C. Avant leur libération, nous avons fait une nouvelle biopsie et leur avons implanté un transmetteur acoustique. Un groupe de poissons ayant subi une biopsie, mais non le traitement, ont été libérés en même temps. Soixante-deux pour cent (8 sur 13) des saumons témoins et 68 % (21 sur 31) des poissons gardés à 10 °C ont atteint les zones de fraie. Les poissons traités à 18 °C n'ont eu que la moitié de ce succès (35 %; 6 sur 17). Durant la période de rétention, les poissons gardés à 18 °C ont connu une mortalité deux fois plus élevée et leur niveau d'infection à *Parvicapsula minibicornis* (Kent, Whitaker et Dawe, 1997) était plus important que celui du groupe traité à 10 °C. La seule différence physiologique chez les deux groupes de poissons traités concerne un changement de l'activité de la Na^+,K^+-ATPase des branchies. Cette chute est en corrélation négative avec les durées des déplacements chez les mâles gardés à 18 °C. Il n'y a pas de différence dans les hormones reproductives et les mesures du stress entre les groupes de poissons traités, mais ces variables sont en corrélation significative avec les durées des déplacements individuels.

[Traduit par la Rédaction]

Received 15 May 2007. Accepted 18 October 2007. Published on the NRC Research Press Web site at cjz.nrc.ca on 18 February 2008.

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 doi:10.1139/Z07-122

Introduction

Each summer, homing sockeye salmon (*Oncorhynchus nerka* Walbaum in Artedi, 1792) begin their spawning migrations through the Fraser River of British Columbia, Canada, and for a given population, the timing of river entry rarely deviates by more than a week interannually (Woodey 1987), though timing can vary appreciably among populations (Hodgson and Quinn 2002). Upon return from the high seas, the late-summer run of homing Fraser River sockeye salmon (hereinafter "late-run" sockeye) represents a group of populations that are characterized not only by their river-entry date but also by a holding behaviour in the Strait of Georgia near the Fraser River estuary that generally lasts 2–6 weeks before fish finally enter the river and migrate to natal streams. Since 1995, however, variable but large segments of late-run sockeye populations have abandoned this holding behaviour (Cooke et al. 2004) and have advanced freshwater entry by 2–8 weeks. Because spawning dates for these fish have not changed, late-run sockeye entering prematurely spend several weeks longer in fresh water relative to historic norms (Lapointe et al. 2003). The reason for this change in behaviour is not known, but here we examine its consequence.

Given the highly adaptive nature of migration timing, not only in salmon but in many other organisms (Brown and Brown 2000; Drent et al. 2003; Prop et al. 2003; Bêty et al. 2004), serious fitness consequences are expected when migration dates deviate significantly from historic averages. Early river entry by late-run sockeye has been associated with high levels of mortality (60%–90%), exceeding 4 million fish since this was first noted in 1996 (M.F. Lapointe, Pacific Salmon Commission, Vancouver, British Columbia, unpublished data). Recent studies have shown that these early-entering fish are reproductively more advanced than those entering at normal times (Cooke et al. 2006; Crossin et al. 2007). In addition, premature entry means that late-run sockeye leave cool coastal waters (10–13 °C) and enter the Fraser River in midsummer, when river temperatures are ≥18 °C, rather than at 10–14 °C in late summer and autumn, which is what they would normally experience after estuarine holding (Patterson et al. 2007). This seems anomalous, as no sockeye populations anywhere have been known to initiate spawning migrations into rivers at times when average seasonal temperatures are higher than 19 °C (Hodgson and Quinn 2002). Compounding matters is an ~1.5 °C rise in peak summer temperatures in the main stem of the Fraser River over the past 60 years due to climate change (Patterson et al. 2007). The year that the present study was conducted (2004), the earliest migrants of the Weaver Creek population of late-run sockeye experienced river temperatures in excess of 21 °C, which greatly exceeds the optimum temperature for metabolic aerobic scope for this population (Lee et al. 2003) and approaches the 5-day lethal temperature of 22 °C for adult sockeye salmon (Servizi and Jensen 1977).

The high routine metabolic cost associated with exposure to high water temperature likely results in the rapid depletion of somatic energy stores in salmon that have ceased feeding. This may put them at risk of exhaustion prior to spawning because somatic energy reserves are vital for the successful completion of migration and spawning. Most sockeye salmon have little reserve energy after spawning (Brett 1995; Hendry and Berg 1999; Crossin et al. 2004; Hinch et al. 2006) and die, in part, from exhaustion. Exposure to high temperature may also promote disease in salmon (Fagerlund et al. 1995), like that caused by the myxosporean kidney parasite *Parvicapsula minibicornis* (Kent, Whitaker and Dawe, 1997), which has been implicated in the mortality of early-migrating Fraser River sockeye. Despite the potential consequences of exposure to high river temperatures, no study has directly investigated whether the temperature experienced by homing sockeye affects their migration success and, because they have only one lifetime reproductive opportunity, their fitness.

We tested the hypothesis that exposure of sockeye to higher temperatures, 18 °C and above, reduces migration success compared with that of sockeye exposed to a lower temperature (10 °C). Late-run sockeye from the Weaver Creek population were caught en route to spawning grounds and were experimentally exposed to temperatures that have commonly been encountered by early migrants (18 °C) and to temperatures historically encountered by normal-timed fish (10 °C; Patterson et al. 2007). These temperatures also bracket the optimal temperature for swimming performance (14–15 °C) for this population (Lee et al. 2003). The holding temperatures also spanned a threshold for *P. minibicornis* infection (Wagner et al. 2005). Fish were biopsied to assess aspects of their physiology, and were then implanted with acoustic transmitters and returned to the river, where migration behaviour was observed by means of underwater acoustic receivers as fish swam to the spawning stream. Biopsy was used to examine concentrations of plasma ions, metabolites, reproductive hormones, heat-shock proteins, and levels of somatic energy, and to assess whether handling and holding affected behaviour and survival of fish. Moribund fish were examined during the holding component of the study to assess *P. minibicornis* disease development. We predicted that warm-treated sockeye would have less somatic energy, elevated stress measures (i.e., high plasma cortisol, lactate, glucose, and heat shock protein levels), and higher levels of parasitic infection relative to cool-treated sockeye. We predicted that after release, warm-treated sockeye would exhibit higher rates of migration mortality.

Materials and methods

Study animals and capture technique

Sockeye salmon were captured by beach seine 10 km downstream from the spawning grounds at Weaver Creek (Fig. 1) on 13 and 14 September 2004. Weaver Creek sockeye historically migrate into the Fraser River from the Pacific Ocean between mid-September and mid-October, but premature entry begins as early as mid-August (Lapointe et al. 2003; Cooke et al. 2004). We sampled fish at the start of the historic migration window, when the river temperature was ~15 °C. Fish were then transferred by dip net into a holding pen anchored on the river bank, then Floy-tagged and biopsied. Fish were subsequently held at the Fisheries and Oceans Canada Chehalis Hatchery (located ~5 km from the capture site) and scale analyses were conducted to determine population of origin (Gable and Cox-Rogers 1993).

Weaver Creek sockeye were transported to two experimental holding tanks at the Cultus Lake Research Laboratory the next day, while salmon identified as belonging to comigrating populations were returned to the capture site and released. No mortality occurred during holding at the hatchery or prior to release. Population identity of Weaver Creek sockeye was later verified by DNA analyses (Beacham et al. 1995, 2004). All procedures were approved by the Animal Care Committees of The University of British Columbia and Fisheries and Oceans Canada. Capture, transplant, and release permits were issued by Fisheries and Oceans Canada.

Experimental design

Weaver Creek sockeye (N = 100) were transported in large aerated transport tanks from the capture site 50 km to the Fisheries and Oceans Canada Cultus Lake Research Laboratory (Fig. 1). They were randomly assigned to the two experimental tanks (3.7 m in diameter, 1.5 m deep) and maintained at 15 °C for the first 2 days. Over the subsequent 2 days, the water temperature was raised to 18 °C in one tank (N = 50 fish) and lowered to 10 °C in the other (N = 50 fish). Fish were held for 24 days at these temperatures. A unidirectional flow was maintained in both tanks around the periphery at 0.35–0.50 $m \cdot s^{-1}$ (measured at the midwater level; see Patterson et al. 2004). By assuming a 6.5-day migration at 15 °C in the Fraser River prior to reaching the capture site (English et al. 2005), the cumulative freshwater temperature experienced by the fish (degree-days; DD) prior to release from the holding tanks was 510 DD for the warm-treated fish and 325 DD for the cool-treated fish. This range brackets the cumulative temperature threshold (~400 DD) for the full expression of the kidney parasite (*P. minibicornis*) (Cooke et al. 2004; Wagner et al. 2005). During holding, moribund salmon (<2 h dead) were removed and histological examinations of the kidneys were conducted to determine the severity of *P. minibicornis* infection. Biopsy of additional tissues or plasma was not performed on dead or moribund fish, as these samples are time-sensitive. Assay and biopsy details are provided below.

On 5 October, temperatures in the experimental tanks were returned to ambient (15 °C) over a 12 h period in preparation for their return to the river. On 7 October, fish were biopsied again, gastrically implanted with acoustic transmitters (details below), and transported to the release location at Derby Reach on the Fraser River, ~85 km downstream of the initial capture site on the Harrison River (Fig. 1). Temperatures in the Fraser River at release and in the Harrison River when migrants re-ascended were similar (~12–14 °C). Acoustic receivers positioned at sites along the migration route (see details below; Fig. 1) allowed calculation of migration and survival rates for individual fish. Radio-tagging studies show that Weaver Creek sockeye can swim from Derby Reach to spawning areas in 3–7 days. Peak spawning for this population occurred in mid-October (10–15).

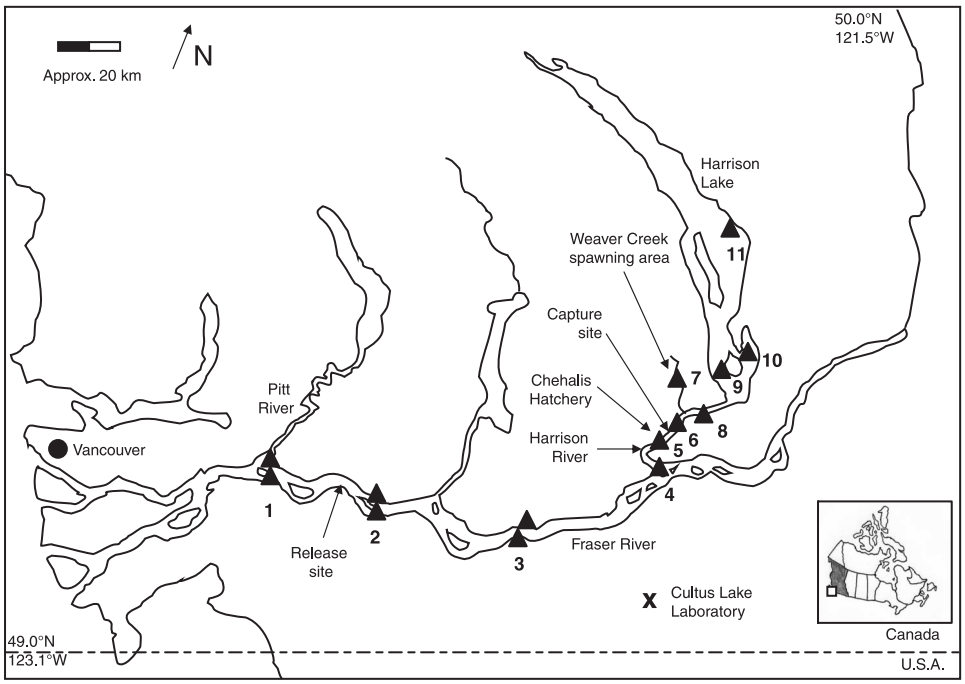
Experimental controls

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

Biopsy, tagging, and biotelemetry

The biopsy procedures followed those described by Cooke et al. (2005). Fish were individually dip-netted and placed in a V-shaped foam-lined trough with a continuous flow of fresh ambient water directed into the mouth and across the gills. A Floy-tag printed with a unique identifying number was inserted through the dorsal musculature of each fish anterior to the dorsal fin. Biopsies collected (*i*) a small section of adipose fin (0.5 g) for DNA confirmation of population identity (collected at capture only), (*ii*) a single scale for determining fish age and population identity (collected at capture only), (*iii*) 3 mL of blood from the caudal vein with an heparinized syringe (1.5 inches (1 inch = 25.4 mm), 21 gauge; Houston 1990) for assessing plasma chemistry, and (*iv*) <4 mm of gill tissue from the tips of six to eight filaments (<0.3 g) for assessing gill Na^+,K^+-ATPase activity (McCormick 1993). From the 3 mL sample of blood, a small volume (<0.01 mL) was removed by capillary tube and centrifuged for 3 min to measure hematocrit (percentage packed red blood cells). Blood samples were then centrifuged for 7 min to separate plasma from red blood cells, both of which were pipetted into separate cryo-vials and placed immediately on dry ice until they could be transferred to a –86 °C freezer. Gill tissues were stored similarly. Fish fork length was measured. Gross somatic energy (GSE, $MJ \cdot kg^{-1}$) was assessed using a microwave energy meter (Distell Fish Fatmeter model 692, Distell Inc., Fauldhouse, West Lothian, Scotland, UK), with readings taken from two locations on the left side of the fish (see Crossin and Hinch 2005). Equipment malfunctions meant that GSE was not measured in any of the salmon at initial capture, though it was measured in all fish prior to release. As a result, this variable was not included in the multivariate analysis of variance (MANOVA) model that examined for physiological differences prior to holding. However, GSE was measured at capture in a separate group of Weaver Creek fish that were collected at the same time and sacrificed for more extensive physiological analyses not reported here, giving us some indication of mean GSE at capture. Sex was determined visually whenever possible (sexual dimorphism is expressed in sockeye approaching the spawning grounds), or otherwise from the ratio of plasma 17β-estradiol to testoster-

Fig. 1. Map of the lower Fraser and Harrison rivers and the Weaver Creek spawning area in southwestern British Columbia, with an inset map of Canada indicating the locations of British Columbia (shaded area) and the study site (box). Points of sockeye salmon (*Oncorhynchus nerka*) capture and release, sites of laboratory holding facilities, and sites of interest are indicated with arrows or symbols. Numbers refer to specific individual and paired VR-2 acoustic receiver sites (▲) that are listed in the Materials and methods section.



one, which is higher in maturing female salmon (Fostier et al. 1983).

Acoustic transmitters (16 mm diameter, 56 mm length, ~25.0 g in air; V16–3H-R04K coded pingers, Vemco Inc., Shad Bay, Nova Scotia) were inserted into the stomach with a smooth plastic applicator (see Cooke et al. 2005). After tagging, fish were placed in a transport tank containing aerated 15 °C water and moved to the release site, where they were released individually. The total time required to process a fish, including biopsy, transmitter insertion, and length assessment, was 2–3 min. The biopsy and transmitter-insertion procedures were done on fish that were not anesthetized, and this technique had been carefully evaluated in previous studies to ensure that fish handled in this manner do not suffer any deleterious effects on migratory behaviour and survival (Cooke et al. 2005, 2006).

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

Laboratory assays

Plasma testosterone and 17β-estradiol levels were measured by radioimmunoassay (McMaster et al. 1992). Plasma osmolality and concentrations of lactate, glucose, and cortisol were determined with protocols described by Farrell et al. (2001), and gill-tissue Na^+,K^+-ATPase activity was determined with a kinetic assay (McCormick 1993). Pre- and post-treatment red blood cell samples were analyzed for heat shock 70 protein (Hsp70) levels. Hsp70 levels were determined by SDS–PAGE (Forsyth et al. 1997) using rabbit polyclonal anti-chinook salmon Hsp70 primary antibody (Stressgen, Victoria, British Columbia). Two 5 μm sections of kidney from the moribund or recently dead salmon were fixed in Davidson's solution, mounted on glass slides, and stained with hematoxylin and eosin. The number of spores per 25 glomeruli was used as a measure of infection, with 25 spores per 25 glomeruli representing maximal (100%) infection (Jones et al. 2003; Wagner et al. 2005).

Statistical analyses

All analyses were conduced with JMP® version 4.0 (SAS Institute Inc., Raleigh, North Carolina). All data were $\log_{10}$-transformed to reduce heteroscedasticity, but non-transformed values are used in the tables and figures. All physiological, reproductive, and energetic variables were then analyzed for normality. MANOVA on $\log_{10}$-transformed data (McGarigal et al. 2000) was used to

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

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

Results

At capture there were no significant differences in any of the physiological variables (pretreatment plasma osmolality, plasma Na^+, plasma K^+, plasma glucose, plasma lactate, hematocrit, and gill Na^+,K^+-ATPase activity) between warm- and cool-treated fish (MANOVA, $F_{[7,33]} = 1.933$, $P = 0.101$, $N = 41$). GSE could not be measured at capture, but in a separate group of Weaver Creek fish captured at the same time for a different study, GSE was 6.12 ± 0.2 MJ·kg^{-1}.

The temperature experienced during treatment had a significant effect on survival: 31 fish survived at 10 °C (15 females and 16 males) and 17 fish at 18 °C (8 females and 9 males). Thus, the survival rate was much lower for warm-treated (34%) than for cool-treated sockeye (62%; ANOVA, $P = 0.011$). Despite these differences in survival, when survival was expressed as a function of cumulative thermal experience (Fig. 2*a*), the two curves followed a similar decreasing trajectory.

The expression of *P. minibicornis* in moribund fish was temperature-dependent. The kidneys of 18 °C-treated fish (hereinafter 18 °C fish, $N = 14$) had significantly higher levels of *P. minibicornis* spores than 10 °C-treated fish (hereinafter 10 °C fish, $N = 13$, $P = 0.002$; Fig. 2*b*). The infection scores were maximal (25) in 7 of the 14 fish that were exposed to 18 °C and that had accrued >350 DD, whereas none of the 10 °C fish showed histological evidence of infection. Qualitatively, it is easy to see that warm-treated sockeye also had more external fungal infections (e.g., *Saprolegnia* spp.).

After Bonferroni correction, the only significant differences in physiology between warm- and cool-treatment groups were an increase in gill Na^+,K^+-ATPase activity in cool-treated fish and a decrease in activity in warm-treated fish (Table 1; females: $P = 0.003$, $N = 21$; males: $P = 0.002$, $N = 20$). There was a trend toward a post-treatment decline in plasma glucose concentration in warm-treated females, though this difference was not significant after Bonferroni correction (Table 1). Unlike other physiological variables, GSE and cortisol concentration were only measured in fish at the end of the treatment period, so we could not assess how these changed during experimental holding. There was, however, no significant difference in either GSE or cortisol concentration between warm- and cold-treated sockeye at the end of thermal treatments prior to being returned to the river (Table 1).

Regarding the control sockeye, sex-steroid assays were not run, owing to budget constraints, but we were confident in assigning sex on the basis of secondary sexual characteristics. Physiological comparisons with the thermally treated fish (excluding the sex steroids) were therefore done with one-way ANOVA using treatment (10 °C, 18 °C, and control) as the only model effect. Prior to these analyses, we compared all the post-treatment physiological variables in males and females from both thermal-treatment groups with two-way ANOVA, using treatment and sex as model effects. After Bonferroni correction, the analysis did not reveal any significant differences between the sexes, but at the $\alpha = 0.05$ level, differences in plasma Na^+ concentration ($P = 0.019$), plasma Cl^- concentration ($P = 0.008$), gill ATPase activity ($P = 0.009$), and GSE ($P = 0.009$) were observed. Between treatments, there was a Bonferroni-corrected significant difference in gill ATPase activity ($P < 0.0001$) and a $\alpha = 0.05$ difference in glucose concentration ($P = 0.007$). These differences are consistent with the results of paired *t* tests presented for each sex in Table 1. When one-way ANOVA was used to compare the physiology of the control fish (which were captured, biopsied, and handled the same as the treated fish but were not held or exposed to thermal treatment) with that of the thermally treated fish, the control fish did not differ from the 10 °C fish (GSE, hematocrit, Na^+, Cl^-, glucose, and lactate concentrations, osmolality, and gill Na^+,K^+-ATPase activity, all $P > 0.05$), but both the 10 °C and the control fish differed significantly from the 18 °C fish in plasma Na^+ and glucose concentrations and gill ATPase activity (see Table 2).

After the treatment period, 48 thermally treated salmon were transported ~85 km downstream from the initial collection site and released back into the Fraser River. In total, the 18 °C fish ($N = 17$) had accumulated 506–510 DD and the 10 °C fish ($N = 31$) 323–327 DD. Thirteen control fish were also transported to and released at the same site. As during the holding period, fish held at 18 °C showed elevated mortality rates after release into the river (Fig. 3), and only 35% of these (1 female, 5 males) succeeded in reaching the spawning grounds. In contrast, fish held at 10 °C ($N = 31$) were 68% (10 females, 11 males) successful and control fish were 62% (3 females, 5 males) successful. Fish held at 18 °C migrated to site 4 (Fraser River – Harrison River confluence) significantly more slowly than either the control or the 10 °C sockeye ($P = 0.045$; Fig. 4). This was the only site where travel times were significantly different. Interestingly, travel times to spawning areas at site 7 by successful 18 °C males and the one successful 18 °C female did not differ from the travel times of successful 10 °C fish (Fig. 4). When survival to spawning areas was examined by sex, rates were significantly lower ($\chi^2 = 6.727$, $P = 0.035$) for

Table 1. Comparison of the physiological attributes of Weaver Creek sockeye salmon (*Oncorhynchus nerka*) prior to and after experimental thermal treatment.

Physiological variable	Treatment temp. (°C)	Capture value[a]	Post-treatment release value	*N*	*P* Within treatment	*P* Mean between treatments
Females						
Gross somatic energy (GSE; MJ·kg^{-1})	10	na	5.42 ± 0.1	15	na	0.479
	18	na	5.29 ± 0.2	8	na	
Hematocrit (%)	10	36.7 ± 0.9	36.0 ± 1.0	15	0.434	0.879
	18	37.6 ± 1.4	35.1 ± 1.4	7	0.095	
Plasma Na^+ (mmol·L^{-1})	10	150.6 ± 2.6	147.6 ± 1.3	15	0.322	0.670
	18	156.6 ± 3.5	144.1 ± 1.9	7	0.0005*	
Plasma K^+ (mmol·L^{-1})	10	1.6 ± 0.3	2.4 ± 0.1	15	0.026*	0.996
	18	2.2 ± 0.3	1.8 ± 0.2	7	0.292	
Plasma Cl^- (mmol·L^{-1})	10	131.1 ± 1.6	132.4 ± 0.8	15	0.456	0.053
	18	138.9 ± 2.1	132.1 ± 1.2	7	0.030*	
Plasma osmolality (mosmol·kg^{-1})	10	304.7 ± 4.9	306.3 ± 1.9	15	0.338	0.491
	18	314.5 ± 6.8	298.0 ± 2.8	7	0.062	
Plasma glucose (mmol·L^{-1})	10	5.5 ± 0.2	5.8 ± 0.2	15	0.226	0.047*
	18	5.3 ± 0.3	4.9 ± 0.3	7	0.365	
Plasma lactate (mmol·L^{-1})	10	5.6 ± 1.3	2.1 ± 0.2	15	0.029*	0.290
	18	7.4 ± 1.8	2.4 ± 0.3	7	0.0053*	
Plasma cortisol (ng·mL^{-1})	10	na	325.4 ± 35.9	15	na	0.743
	18	na	362.4 ± 52.6	7	na	
Plasma testosterone (pg·mL^{-1})	10	17 563 ± 1 567	18 844 ± 2 174	15	0.437	0.939
	18	18 854 ± 2 145	19 021 ± 3 182	7	0.817	
Plasma 11-ketotestosterone (pg·mL^{-1})	10	na	2 693 ± 793		na	0.426
	18	na	1 150 ± 1 160		na	
Plasma 17β-estradiol (pg·mL^{-1})	10	13 229 ± 4 222	10 886 ± 5 206	12	0.261	0.937
	18	5 614 ± 4 951	5 187 ± 10 412	3	0.951	
Gill Na^+,K^+-ATPase (μmol ADP·mg protein^{-1}·h^{-1})	10	1.8 ± 0.1	2.2 ± 0.1	14	0.008*	0.0035**
	18	1.8 ± 0.2	1.5 ± 0.1	8	0.383	
Erythrocyte Hsp70 (relative to reference)	10	1.72 ± 0.26	1.60 ± 0.23	6	0.120	0.321
	18	1.34 ± 0.26	1.25 ± 0.23	6	0.419	
Males						
Gross somatic energy (GSE; MJ·kg^{-1})	10	na	5.13 ± 0.1	16	na	0.566
	18	na	5.06 ± 0.1	8	na	
Hematocrit (%)	10	36.3 ± 1.2	34.6 ± 1.0	11	0.013*	0.432
	18	37.5 ± 1.4	31.0 ± 1.4	8	0.040*	
Plasma Na^+ (mmol·L^{-1})	10	156.6 ± 2.2	150.7 ± 1.7	15	0.078	0.557
	18	159.4 ± 2.9	150.2 ± 2.2	9	0.048*	
Plasma K^+ (mmol·L^{-1})	10	1.4 ± 0.3	2.7 ± 0.3	15	0.0004*	0.601
	18	1.9 ± 0.4	1.9 ± 0.3	9	0.866	
Plasma Cl^- (mmol·L^{-1})	10	132.3 ± 1.7	127.2 ± 0.9	15	0.025*	0.174
	18	136.2 ± 2.3	129.1 ± 1.2	8	0.004*	
Plasma osmolality (mosmol·kg^{-1})	10	310.9 ± 4.26	300.0 ± 2.6	15	0.095	0.503
	18	315.8 ± 5.68	300.9 ± 3.5	8	0.029*	
Plasma glucose (mmol·L^{-1})	10	6.4 ± 0.3	5.2 ± 0.2	15	0.0054*	0.562
	18	5.9 ± 0.4	5.3 ± 0.3	9	0.032*	
Plasma lactate (mmol·L^{-1})	10	6.6 ± 1.2	2.5 ± 0.4	15	0.011*	0.422
	18	7.0 ± 1.5	2.5 ± 0.5	7	0.002**	
Plasma cortisol (ng·mL^{-1})	10	na	108.6 ± 15.4	11	na	0.365
	18	na	119.8 ± 18.1	9	na	
Plasma testosterone (pg·mL^{-1})	10	12 596 ± 1 411	13 208 ± 824	7	0.860	0.162
	18	11 268 ± 1 411	11 529 ± 868	8	0.934	
Plasma 11-ketotestosterone (pg·mL^{-1})	10	na	10 464 ± 980		na	0.456
	18	na	11 586 ± 1 096		na	

Table 1 (*concluded*).

Physiological variable	Treatment temp. (°C)	Capture value[a]	Post-treatment release value	*N*	*P* Within treatment	*P* Mean between treatments
Gill Na^+,K^+-ATPase (μmol ADP·mg protein^{-1}·h^{-1})	10	1.8 ± 0.1	2.6 ± 0.1	16	0.0001**	0.0016**
	18	1.8 ± 0.1	1.6 ± 0.1	9	0.028*	
Erythrocyte Hsp70 (relative to reference)	10	1.38 ± 0.22	1.26 ± 0.09	6	0.115	0.343
	18	1.30 ± 0.22	1.13 ± 0.09	6	0.619	

Note: Analyses were conducted using paired *t* tests. All variables were $\log_{10}$-transformed prior to analysis. Asterisks indicate significant treatment-related changes within treatments and significant mean differences between treatments: *, $P < 0.05$; **, Bonferroni-corrected $P < 0.0036$ (females, 14 variables) and $P < 0.0039$ (males, 13 variables).

[a]GSE values at capture were not measured, but mean GSE for a group of 20 comigrating Weaver Creek sockeye not analyzed in this study was measured at 6.12 ± 0.2 MJ·kg (mean ± SE).

Table 2. Results (mean ± SE) of a two-way ANOVA with treatment and sex as model effects, comparing physiological variables between thermally treated (18 and 10 °C) and control sockeye salmon (*Oncorhynchus nerka*) from the Weaver Creek population at the time of release to the Fraser River.

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

Note: Values followed by a different letter are significantly different. *, statistical significance at $\alpha = 0.05$;**, significance after Bonferroni correction (nine variables, $P = 0.0056$).

females held at 18 °C (1 of 8, or 13%) compared with 10 °C females (10 of 15, or 67%) and control fish (3 of 6, or 50%), and lower than those of all the male groupings, which did not differ from one another ($\chi^2 = 0.568$, $P = 0.753$; 10 °C males: 11 of 16, or 69%; control males: 5 of 7, or 71%; 18 °C males: 5 of 9, or 56%) (Fig. 3).

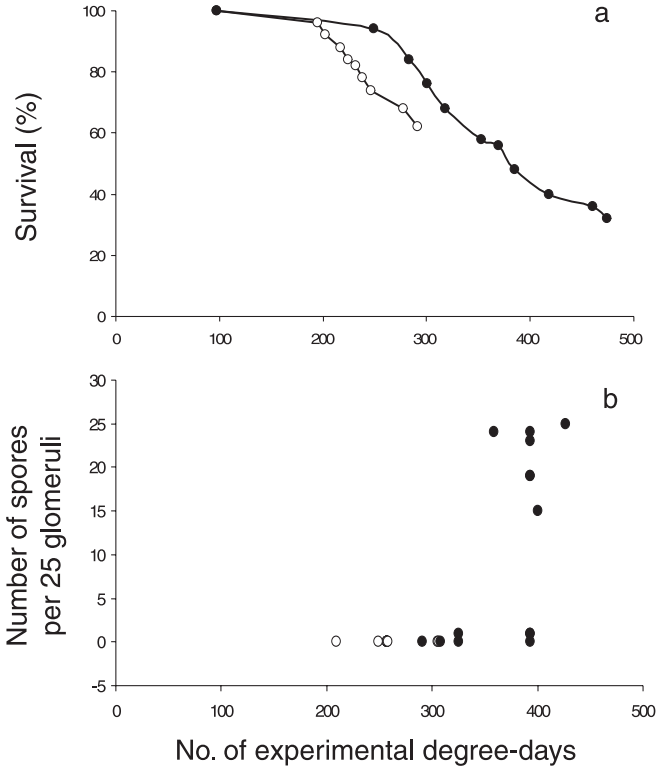
An individual's physiological state prior to release was correlated with its subsequent migration behaviour. For convenience we only present correlations with sites where they were significant: sites 3 (Mission), 4 (Harrison River – Fraser River confluence), and 7 (spawning grounds) (Table 3). Independently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

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

Discussion

Exposure to high but sublethal temperatures had a profound negative effect on survival of sockeye, especially females, during the exposure period. It also had a negative effect on migratory performance and survival in both sexes after their release back into the Fraser River. Greater than

Fig. 2. Survival of sockeye salmon (*Oncorhynchus nerka*) during experimental temperature treatments (*a*) and the severity of *Parvicapsula minibicornis* infection in the kidneys of fish that died during temperature treatment (*b*) relative to the number of degree-days accumulated over the 24-day holding period at the Cultus Lake Laboratory (●, 10 °C-treated fish (N = 13); ○, 18 °C-treated fish (N = 14)). The fish had already accumulated ~97.5 degree-days prior to the experiment. The severity of infection (*b*) was determined from histological counts of infected spores per 25 glomeruli (*y* axis). Many of the symbols lie on top of one another.



two-thirds of the salmon exposed to 10 °C successfully completed the upriver migration after release, whereas less than one-third of those exposed to 18 °C survived. The control fish, which were handled and biopsied but not thermally treated, migrated at similar speeds and survived at similar levels to the 10 °C fish. Thus, exposure to high temperature may be an important factor contributing to the ~60%–95% mortality experienced by early migrants from the Weaver Creek population, which have tended to migrate through water at higher than normal temperatures in recent years (Cooke et al. 2004).

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

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

The 28 days of holding did not appear to impose an energetic constraint on the experimental sockeye that could account for the loss of fish en route to spawning areas. Energy-modeling exercises using swim-speed information obtained from migrating sockeye show that fish can become energy-exhausted if excessive swim speeds are maintained (Hinch and Bratty 2000) and (or) if normal swim speeds are maintained for many more days or weeks than usual (Rand and Hinch 1998), both of which have fitness consequences to these semelparous animals. Previous energetic analysis of dying, post-spawning Fraser River sockeye indicates that death occurs when somatic energy levels fall below 4 $MJ \cdot kg^{-1}$ (Crossin et al. 2004). Despite the fish being held for 28 days, energy levels in the thermally treated fish were well above this terminal level at release (5.29–5.42 $MJ \cdot kg^{-1}$ for females and 5.06–5.13 $MJ \cdot kg^{-1}$ for males), thus any mortality during holding was likely not caused by energy exhaustion. Furthermore, current speeds in the holding tanks were set so that fish were maintaining their station and swimming in place (swim speeds 10–15 $cm \cdot s^{-1}$). Underwater video studies of river-migrating sockeye show that swim speeds (i.e., the speeds at which their swimming is propelling them if they were in still water) are typically 30–40 $cm \cdot s^{-1}$ (Hinch and Rand 2000). Thus, the swim speeds of the experimental fish were between 50% and 75% of those of freely migrating fish, which would have conferred a significant energy saving on the experimental fish. Energetic analyses reveal that Weaver Creek sockeye use ~0.12 $MJ \cdot kg^{-1} \cdot d^{-1}$ to swim and ripen the gonads during mi-

Fig. 3. Percent en-route survival from point of release to various upriver receiver stations for 18 °C-treated, 10 °C-treated, and control sockeye salmon (*Oncorhynchus nerka*). Site 3 is at Mission, British Columbia, site 4 is at the confluence of the Fraser and Harrison rivers, and site 7 is at spawning areas in Weaver Creek. A different letter over the bar indicates a significant difference in survival between treatments (P = 0.032).

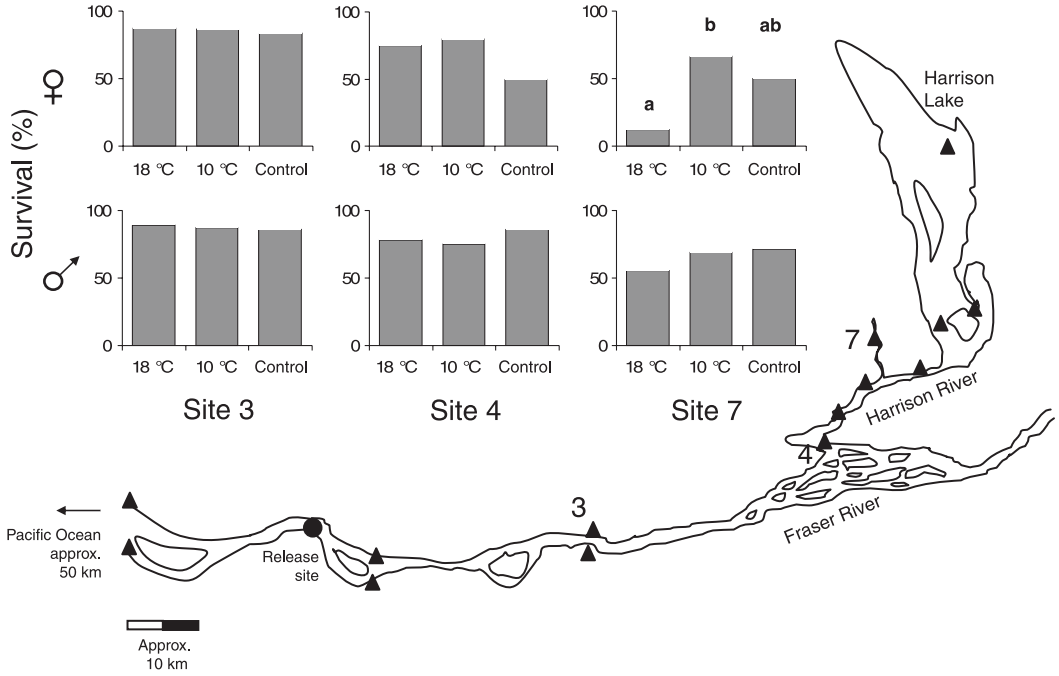


Fig. 4. Travel times from point of release to various upriver locales for 18 °C-treated, 10 °C-treated, and control sockeye salmon (*Oncorhynchus nerka*). Site 3 is at Mission, British Columbia, site 4 is at the confluence of the Fraser and Harrison rivers, and site 7 is at spawning areas at Weaver Creek. The distance from the Fraser River release site to spawning grounds is ~90 km. Shaded bars represent females and open bars males. A different letter over the bar indicates a significant difference at $P < 0.05$. Error bars are ±1 SE.

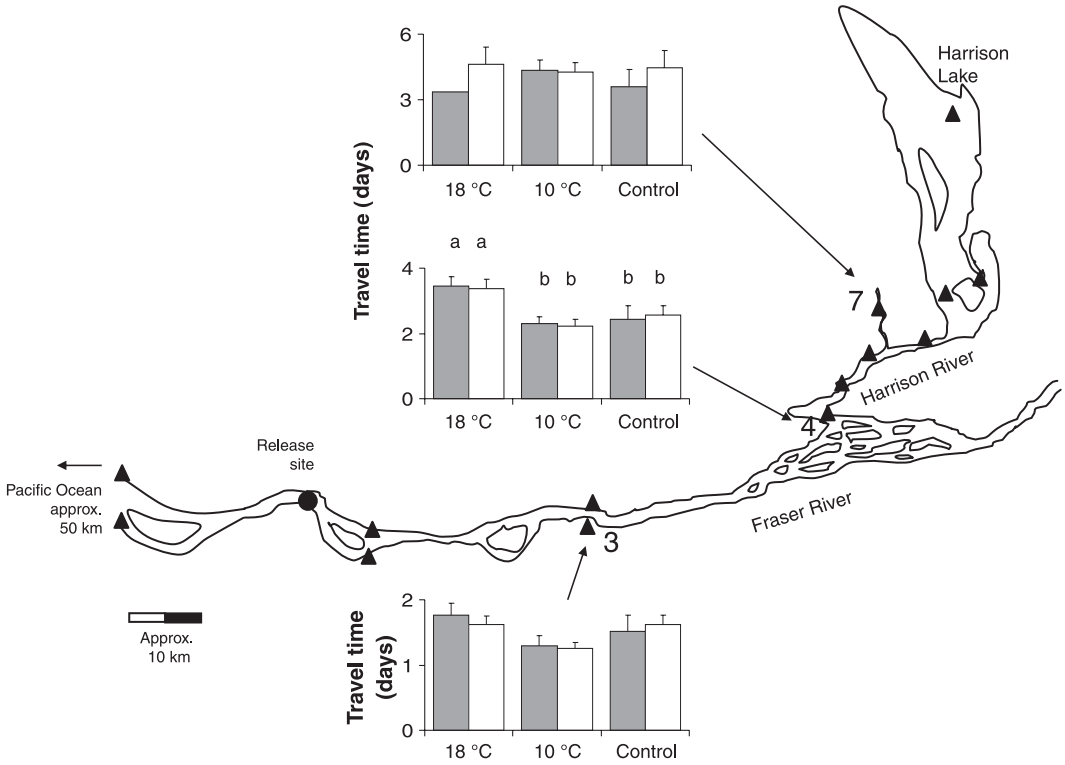


Table 3. Pearson's correlation coefficients relating gross somatic energy and blood plasma biochemistry of surviving sockeye salmon (*Oncorhynchus nerka*) to upriver travel times as determined by acoustic telemetry.

Treatment × sex	Physiological variable	Travel time to site 3 (~35 km upriver)	*N*	Travel time to site 4 (~70 km upriver)	*N*	Travel time to site 7 (~85 km upriver)	*N*
18 °C-treated females	Gross somatic energy ($MJ \cdot kg^{-1}$)	0.791 (0.034)	7	0.849 (0.069)	5	na	1
	Hematocrit (%)	–0.679 (0.138)	6	–0.566 (0.320)	5	na	1
	Plasma Na^+ ($mmol \cdot L^{-1}$)	0.159 (0.764)	6	–0.001 (0.998)	5	na	1
	Plasma K^+ ($mmol \cdot L^{-1}$)	–0.123 (0.817)	6	0.476 (0.418)	5	na	1
	Plasma Cl^- ($mmol \cdot L^{-1}$)	–0.550 (0.258)	6	–0.550 (0.337)	5	na	1
	Plasma osmolality ($mosmol \cdot kg^{-1}$)	–0.497 (0.316)	6	–0.405 (0.499)	5	na	1
	Plasma glucose ($mmol \cdot L^{-1}$)	–0.787 (0.063)	6	–0.934 (0.020*)	5	na	1
	Plasma lactate ($mmol \cdot L^{-1}$)	0.872 (0.024*)	6	0.461 (0.435)	5	na	1
	Plasma cortisol ($ng \cdot mL^{-1}$)	0.370 (0.471)	6	0.765 (0.132)	5	na	1
	Plasma testosterone ($pg \cdot mL^{-1}$)	–0.388 (0.447)	6	–0.939 (0.018*)	5	na	1
	Plasma 17β-estradiol ($pg \cdot mL^{-1}$)	–0.615 (0.578)	3	–0.331 (0.785)	3	na	1
	Plasma 11-ketotestosterone ($pg \cdot mL^{-1}$)	–0.620 (0.189)	6	–0.884 (0.046*)	5	na	1
	Gill Na^+,K^+-ATPase (μmol ADP·mg $protein^{-1} \cdot h^{-1}$)	–0.532 (0.219)	7	–0.507 (0.384)	5	na	1
10 °C-treated females	Gross somatic energy ($MJ \cdot kg^{-1}$)	0.094 (0.759)	13	–0.014 (0.965)	12	0.179 (0.621)	10
	Hematocrit (%)	0.011 (0.972)	13	–0.008 (0.979)	12	0.271 (0.448)	10
	Plasma Na^+ ($mmol \cdot L^{-1}$)	–0.213 (0.485)	13	–0.378 (0.226)	12	–0.017 (0.964)	10
	Plasma K^+ ($mmol \cdot L^{-1}$)	0.286 (0.343)	13	0.218 (0.497)	12	0.605 (0.064)	10
	Plasma Cl^- ($mmol \cdot L^{-1}$)	0.479 (0.098)	13	0.249 (0.435)	12	0.277 (0.438)	10
	Plasma osmolality ($mosmol \cdot kg^{-1}$)	–0.044 (0.886)	13	–0.002 (0.995)	12	0.102 (0.780)	10
	Plasma glucose ($mmol \cdot L^{-1}$)	–0.023 (0.942)	13	0.253 (0.427)	12	–0.253 (0.480)	10
	Plasma lactate ($mmol \cdot L^{-1}$)	0.757 (0.0027**)	13	0.552 (0.063)	12	0.455 (0.186)	10
	Plasma cortisol ($ng \cdot mL^{-1}$)	–0.360 (0.228)	13	–0.553 (0.062)	12	0.435 (0.209)	10
	Plasma testosterone ($pg \cdot mL^{-1}$)	–0.251 (0.409)	13	–0.313 (0.322)	12	0.477 (0.163)	10
	Plasma 17β-estradiol ($pg \cdot mL^{-1}$)	–0.760 (0.0036**)	11	–0.620 (0.056)	10	–0.713 (0.047*)	8
	Plasma 11-ketotestosterone ($pg \cdot mL^{-1}$)	–0.266 (0.379)	13	–0.338 (0.283)	12	–0.446 (0.197)	10
	Gill Na^+,K^+-ATPase (μmol ADP·mg $protein^{-1} \cdot h^{-1}$)	–0.379 (0.225)	12	–0.271 (0.395)	12	–0.088 (0.808)	10
18 °C-treated males	Gross somatic energy ($MJ \cdot kg^{-1}$)	0.361 (0.340)	9	0.684 (0.062)	8	–0.086 (0.891)	5
	Hematocrit (%)	0.084 (0.842)	8	0.599 (0.155)	7	–0.253 (0.747)	4
	Plasma Na^+ ($mmol \cdot L^{-1}$)	–0.590 (0.094)	9	–0.022 (0.958)	8	–0.006 (0.993)	5
	Plasma K^+ ($mmol \cdot L^{-1}$)	–0.161 (0.679)	9	0.352 (0.393)	8	0.065 (0.918)	5
	Plasma Cl^- ($mmol \cdot L^{-1}$)	0.019 (0.965)	8	0.587 (0.166)	7	0.231 (0.709)	5
	Plasma osmolality ($mosmol \cdot kg^{-1}$)	–0.298 (0.473)	8	0.101 (0.830)	7	–0.296 (0.629)	5
	Plasma glucose ($mmol \cdot L^{-1}$)	–0.458 (0.215)	9	–0.283 (0.497)	8	0.487 (0.405)	5
	Plasma lactate ($mmol \cdot L^{-1}$)	–0.434 (0.243)	9	–0.230 (0.585)	8	0.436 (0.464)	5
	Plasma cortisol ($ng \cdot mL^{-1}$)	–0.542 (0.132)	9	–0.003 (0.995)	8	0.467 (0.428)	5
	Plasma testosterone ($pg \cdot mL^{-1}$)	0.456 (0.217)	9	–0.162 (0.701)	8	0.672 (0.214)	5
	Plasma 11-ketotestosterone ($pg \cdot mL^{-1}$)	0.202 (0.603)	9	–0.462 (0.249)	8	0.256 (0.678)	5
	Gill Na^+,K^+-ATPase (μmol ADP·mg $protein^{-1} \cdot h^{-1}$)	–0.809 (0.008*)	9	–0.511 (0.195)	8	0.257 (0.677)	5
10 °C-treated males	Gross somatic energy ($MJ \cdot kg^{-1}$)	–0.238 (0.412)	14	–0.062 (0.848)	12	0.474 (0.141)	11
	Hematocrit (%)	–0.168 (0.643)	10	–0.229 (0.585)	8	–0.347 (0.446)	7
	Plasma Na^+ ($mmol \cdot L^{-1}$)	–0.098 (0.751)	13	–0.318 (0.341)	11	–0.340 (0.337)	10
	Plasma K^+ ($mmol \cdot L^{-1}$)	–0.050 (0.872)	13	0.139 (0.685)	11	–0.081 (0.825)	10
	Plasma Cl^- ($mmol \cdot L^{-1}$)	–0.088 (0.775)	13	–0.161 (0.637)	11	0.375 (0.285)	10
	Plasma osmolality ($mosmol \cdot kg^{-1}$)	–0.070 (0.821)	13	–0.086 (0.802)	11	–0.298 (0.403)	10
	Plasma glucose ($mmol \cdot L^{-1}$)	0.118 (0.701)	13	0.302 (0.367)	11	0.372 (0.289)	10
	Plasma lactate ($mmol \cdot L^{-1}$)	–0.120 (0.696)	13	–0.026 (0.940)	11	–0.173 (0.633)	10

Table 3 (*concluded*).

Treatment × sex	Physiological variable	Travel time to site 3 (~35 km upriver)	*N*	Travel time to site 4 (~70 km upriver)	*N*	Travel time to site 7 (~85 km upriver)	*N*
	Plasma cortisol ($ng \cdot mL^{-1}$)	0.807 (0.005)	10	0.830 (0.011*)	8	0.390 (0.388)	7
	Plasma testosterone ($pg \cdot mL^{-1}$)	–0.157 (0.686)	9	0.035 (0.942)	7	–0.813 (0.048*)	6
	Plasma 11-ketotestosterone ($pg \cdot mL^{-1}$)	–0.135 (0.730)	9	–0.341 (0.454)	7	–0.791 (0.061)	6
	Gill Na^+,K^+-ATPase (μmol ADP·mg protein$^{-1} \cdot h^{-1}$)	–0.007 (0.982)	14	–0.089 (0.785)	12	0.093 (0.783)	11

Note: The point of release was Derby Reach in Fort Langley, British Columbia. Values in parentheses are *P* values; *, significantly different a $\alpha = 0.05$; **, significantly different after Bonferroni correction (females, 13 variables, $P = 0.0038$; males, 12 variables, $P = 0.0042$).

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

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

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

Based on the literature, we did not expect, nor did we find, reproductive-hormone concentrations to differ between the treatment groups at the end of the holding period. The significant negative correlations that we observed between reproductive-hormone levels and migration travel times, however, are consistent with a wide body of literature. Testosterone for example has a potent affect on the migratory behaviour of salmon (Munakata et al. 2001; Onuma et al. 2003; Young et al. 2006; Cooke et al. 2008; Crossin et al. 2007), and is highly correlated with aggressiveness, restlessness, and overall migratory activities across taxa (reviewed by Dingle 1996). In 10 °C males and females, concentrations of testosterone and 17β-estradiol, respectively, were highest in the fish that arrived first at spawning areas. This trend was also observed in 11-ketotestosterone concentrations in 18 °C females, the group of fish that suffered the highest mortality. Though only a single 18 °C female survived to reach spawning areas, the driving influence of reproductive hormones on migration time in these animals was evident at multiple locales.

We had predicted that as a reflection of stress in the broadest sense, plasma cortisol, lactate, and glucose levels (Pickering et al. 1982) and Hsp70 levels might be elevated in salmon held at 18 °C. Previous studies of Hsp70 expression in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) erythrocytes show that individuals mount a heat-shock response when temperatures approach acute lethal levels at 25–26 °C, but not at 20 °C (Currie and Tufts 1997). Thus, exposure of adult sockeye salmon to 18 °C may not have been stressful enough to elicit a Hsp70 response, indicating that this holding temperature represented not an acute but rather a chronic temperature stress. Plasma cortisol and lactate concentrations did not differ between treatments, nor did they differ from normal background levels. Plasma glucose was within normal levels, 4–6 $mmol \cdot L^{-1}$, for all but the 18 °C females, which had levels that were significantly low but still within physiological norms. This small change may reflect a subtle but reduced nutritional and energetic state induced by the exposure to high temperature, especially since the migration success of these females was the lowest of all sex–treatment combinations, at only 13%. Migration was also slower in 18 °C females, although this was also true of 18 °C males. Interestingly, though, travel times for successful 18 °C males and the one successful 18 °C female did not differ from those of successful 10 °C fish. This suggests some individual variation in response to thermal stress.

Stress responses are known to differ between male and female salmon (Afonso et al. 2003). Perhaps because females

are investing significantly more energy in reproductive development during upriver migration (nearly 50% of their GSE stores vs. just 4% for males; Crossin et al. 2004), the metabolic stress imposed by high temperature may put females at greater risk of mortality. The greater energetic flexibility of males may allow them to buffer their response to stress.

In summary, we found that high temperature negatively affected migratory performance and survival in homing sockeye salmon. Physiologically, it is difficult to ascribe mechanisms to this mortality, though thermally mediated parasitic infections were certainly involved. The only clear physiological difference between treatment groups after treatment was in gill Na^+,K^+-ATPase activity, which was depressed in 18 °C males and females. Ultimately, the levels of reproductive hormones were the only significant correlates of migration time to reach spawning areas. To the best of our knowledge, this is the first experiment to directly manipulate the temperature experience of a wild, migrating adult fish and effect a change in subsequent migration success, and because sockeye are semelparous, in lifetime fitness.

There is a growing body of evidence that temperature is a key determinant of mortality in sockeye salmon that migrate into fresh water earlier than historic averages (Hinch et al. 2006). Our results clearly demonstrate that river temperatures only modestly above long-term averages, yet below acute thermal limits, can deleteriously affect individual migratory performance and survival and increase the likelihood of infections. In years to come, salmon are likely to encounter temperatures well above average, owing to climate change (Morrison et al. 2002; Rand et al. 2006). We should thus anticipate elevated levels of en-route mortality in adult Fraser River sockeye each year and make risk-averse conservation and management decisions that reflect the variable levels of temperature-related risk.

Acknowledgements

The authors thank Rick Stitt and Wayne Charlie at the Weaver Creek Spawning Channel for logistic support, and the Chehalis First Nation for access to their land and field assistance from their Fisheries Council. Thanks are also extended to Fisheries and Oceans Canada's Cultus Lake Laboratory and Chehalis Hatchery for use of their facilities, to the Fisheries and Oceans Canada E-Watch program, and to the Pacific Salmon Commission, especially Mike Lapointe and Steve Latham. Additional thanks are extended to those who helped with fish biopsy and telemetry: Lina Crossin, Erika Eliason, Kim Hruska, Stefan Larsson, Trevor Nowak, and Glenn Wagner. This work was funded by a Southern Endowment Fund Grant from the Pacific Salmon Commission to S.G.H., by a Strategic Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to S.G.H., A.P.F., and G.V.D.K., and by NSERC Discovery Grants to S.G.H. and A.P.F. G.T.C. was supported by a NSERC Canada Graduate Scholarship (CGS-D3).

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