Physiological Responses of Free-Swimming Adult Coho Salmon to Simulated Predator and Fisheries Encounters

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ABSTRACT

The responses of free-swimming adult coho salmon (*Oncorhynchus kisutch*) to simulated predator and fisheries encounters were assessed by monitoring heart rate (f_H) with implanted data loggers and periodically taking caudal blood samples. A 10- or 30-min corralling treatment was conducted to simulate conspecifics being cornered by a predator or corralled by fisheries gear without physical contact. Corralling rapidly doubled f_H from ~31 beats min^{-1} to a maximum of ~60 beats min^{-1}, regardless of the duration of the corralling. However, recovery of f_H to precorralling levels was significantly faster after the 10-min corralling (7.6 h) than after the 30-min corralling (11.5 h). An exhaustive-exercise treatment (chasing for 3 min, with physical contact) to simulate a predator chasing a fish to exhaustion or a fish becoming exhausted after encountering fisheries gear resulted in increased f_H (to 60 beats min^{-1}), plasma lactate, glucose, sodium, osmolality, and cortisol (males only) and a significant decrease in mean corpuscular hemoglobin concentration. Recovery of f_H and most blood variables was complete about 16 h after exhaustive exercise and handling. The results illustrate a clear relationship between the intensity of exercise and the duration required for recovery of f_H. Changes in f_H were significantly correlated with those in plasma lactate, chloride, and sodium at 1 h after the exercise treatment protocols. Thus, measurements of f_H may provide an accurate indication of the general physiological response of salmonids to exhaustive exercise in the natural environment.

Introduction

The ability of an animal to recover from a stressful encounter is fundamental to the persistence of animal populations and represents a unique intersection of physiology, behavior, and life history (Zera and Harshman 2001; Ricklefs and Wikelski 2002; Romero 2004). This consideration is particularly important for adult Pacific salmonids (*Oncorhynchus* spp.) during their spawning migrations, when both natural and anthropogenic stressful encounters that presumably test their physiological limits are routine occurrences. Throughout their coastal approach and in-river migration, Pacific salmon are targeted by predators (e.g., sharks, seals, orcas, and bears) and fisheries (commercial, recreational, and First Nations fisheries sectors). These encounters can kill fish, or the acute stress, which may last just several seconds to minutes, can lead to sublethal physiological or behavioral impairments after either predation evasion (Lima and Dill 1990) or escape/release from fisheries gear (Chopin and Arimoto 1995).

Fish generally respond to a startle stimulus by engaging in brief burst-swimming activity that is powered predominately by white muscle tissue and supported by anaerobic metabolism (Milligan 1996). Burst activity triggers acute bradycardia (Stevens et al. 1972; Priede 1974), which may function to prevent the hypertension associated with constriction of peripheral blood vessels by skeletal muscle contractions (Farrell and Jones 1992). However, burst swimming cannot be sustained beyond a few minutes, after which avoidance behaviors rely on slower, steady swimming activity supported by slow-twitch, aerobic red muscle (Webb 1984). Exhaustive exercise pushes fish to their physiological limits, resulting in maximal endocrine disturbances, acidosis, and metabolite accumulation, responses that

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can temporarily impair physiological performance during recovery (Milligan and Wood 1986; Milligan 1996). In severe cases, delayed mortality is possible (Wood et al. 1983). During recovery, metabolic rate may increase to maximum levels (Reidy et al. 2000; Cheng and Farrell 2007), supported by increased cardiac output, heart rate (f_H), and cardiac stroke volume. Until routine cardiac output is restored, the circulatory capacity to respond to future encounters is likely compromised.

Technological limitations have made measuring cardiac output in free-swimming fish difficult, particularly for actively migrating species like Pacific salmonids. One study on Atlantic salmon (*Salmo salar* L.), using f_H telemetry, found that f_H increased by no more than 30% after fisheries capture (angling) and that f_H returned to precapture levels after ~16 h (Anderson et al. 1998). However, implantation of the electrocardiogram (ECG) electrodes had breached the pericardium (Anderson et al. 1998), which is known to impair normal cardiac performance in rainbow trout and sharks (Farrell et al. 1998). Largemouth bass (*Micropterus salmoides*) equipped with large external acoustic f_H transmitters increased f_H by 1.7-fold in response to fisheries capture (angling), but recovery profiles could not be quantified because of substantial variations in f_H and small sample sizes (Cooke et al. 2004).

Implantable data loggers (Clark et al. 2008*b*, 2009, 2010) can monitor f_H without disrupting the pericardium and therefore enable long-term, continuous assessment of the sublethal consequences of predator and fisheries encounters on fishes. Our study tested the hypothesis that varying intensities of exercise stress designed to simulate predator or fisheries encounters would differentially affect the response and recovery of f_H and hematological indices for free-swimming adult migratory coho salmon (*Oncorhyncus kisutch*). We predicted (1) that the magnitude of the f_H response would depend on the intensity and duration of the stressful encounter; (2) that the duration of recovery of f_H to prestress levels would depend on the intensity and duration of the stressful encounter; and (3) that f_H responses would correlate strongly with hematological indicators of physiological stress. Through an examination of these predictions, we aimed to determine whether f_H could act as a holistic indicator of the physiological response to and recovery from an ecologically relevant stressful encounter.

Material and Methods

Study Site and Animals

Experiments were conducted on adult Chehalis River coho salmon at the Chehalis River Hatchery, a Fisheries and Oceans Canada facility near Agassiz, British Columbia, during October and November 2008. Some fish showed early signs of secondary sexual characteristics, but most were silver in color, which is the preferred stage for many predators as well as fisheries. Mean ($\pm$SE) fork length was 67.9 $\pm$ 0.9 cm, and mean body mass was 3.6 $\pm$ 0.2 kg. Fish were individually removed from hatchery raceways by dip net after their normal 140-km river migration from the ocean. Thirteen fish (six males, seven females) were implanted with data loggers, and an additional 12 fish (seven males, five females) were surgery controls (subjected to identical handling treatments as the instrumented fish but without surgery to implant a data logger).

Implantation of Data Loggers and Blood Sampling

Fish were individually anesthetized in a bath containing 100 mg L^{-1} tricaine methanesulfonate (MS-222; Sigma, St. Louis, MO) buffered with 200 mg L^{-1} $NaHCO_3$. Upon loss of equilibrium (<1.5 min), a 2.5-mL blood sample was taken by caudal puncture with a heparinized vacutainer, which was then stored in ice-chilled water for <1 h until subsequent processing. A 3-mm-diameter lateral tissue biopsy and a small gill biopsy (1 mm from the tips of 5–8 gill filaments, ~0.03 g) were taken for another study. Very minor bleeding (<0.05 mL) was sometimes observed at the biopsy sites, but it ceased after 10–20 s. Similar biopsy procedures have been shown to have no detrimental effects on the health or behavior of fish (Cooke et al. 2005; Clark et al. 2009).

Once the fish were fully anesthetized (~5 min), body mass, length, girth, and height were measured, after which the fish were placed on a damp, plastic-lined foam surgery bench, where the gills were continuously irrigated with a maintenance dose of buffered anesthetic (70 mg L^{-1} MS-222, 140 mg L^{-1} $NaHCO_3$). Peterson disk identification tags were inserted into the dorsal tissue approximately 1 cm ventral and anterior of the dorsal fin. At this point, surgery control fish were recovered in one of two sections (L × W × D = 15 × 5 × 2 m) of a concrete, flow-through holding channel (9.0° $\pm$ 0.1°C) with a water depth of ~60 cm. For the remaining fish, a data logger (iLogR, mass 23 g in air; B. D. Taylor, La Trobe University, Melbourne, Australia) was implanted according to previously described surgical methods (Clark et al. 2009, 2010), with the fish supine. Briefly, a sterilized data logger was inserted through a 3–4-cm incision along the ventral midline of the fish, anterior of the ventral fins and associated cartilage. A pair of uterine forceps was used to position the electrocardiogram (ECG) leads ventral to the liver and close to the peritoneal membrane separating the pericardial and visceral cavities. The body of the data logger was loosely sutured to the peritoneal wall and associated ventral tissue, and the incision was closed with silk sutures. The data logger recorded the date, time, temperature, and ECG output at 200 Hz for a 10-s period at 10-min intervals. The entire ECG waveform was stored to memory for subsequent analysis of heart beats (see Clark et al. 2010 for a sample of raw ECG traces).

The entire surgical procedure lasted about 25 min, after which each fish recovered in a section of the holding channel detailed above. All fish resumed unassisted ventilation within several minutes, and within 2 h they exhibited behaviors indistinguishable from those of the surgery control fish and nonexperimental coho salmon at the hatchery. All surgical procedures were completed over a 2-d period.

Blood Processing and Analysis

Hemoglobin concentration ([Hb]) was assessed on whole, well-mixed blood with a handheld hemoglobin analyzer (HemoCue 201$^+$, Ängelholm, Sweden) calibrated for fish blood (Clark et al. 2008*a*). Hematocrit (Hct) was quantified with microcapillary tubes centrifuged at 10,000 *g* for 3 min. Mean corpuscular hemoglobin concentration (MCHC) was calculated as [Hb]/(Hct/100). The remaining blood sample was centrifuged at 7,000*g* for 5 min, and plasma was stored in liquid nitrogen before being frozen at −80°C until analysis. Plasma was subsequently analyzed for cortisol (Neogen ELISA with Molecular Devices Spectramax 240pc plate reader), lactate, glucose (YSI 2300 Stat Plus analyzer), osmolality (Advanced Instruments 3320 freezing-point osmometer), chloride (Haake Buchler digital chloridometer), sodium, and potassium (Cole-Parmer, model 410 single-channel flame photometer; Farrell et al. 2001).

Experimental Protocols

Corralling Protocol. After anesthesia, fish recovered undisturbed for at least 24 h before being guided into a 6 × 1 × 1.5-m raceway (L × W × D, water depth 0.6 m, water velocity ~0.2 m s^{-1}, water temperature 9.3° ± 0.1°C) attached to the recovery holding channel. To initiate fish movement, a large corralling fence, which spanned the width of the holding channel, was used to gently and slowly coax the fish into a 2 × 5-m section of the holding channel without physically contacting the fish. The fish were spontaneously active in the 2 × 5-m section. The corralling duration was set at 10 min ($N = 6$ with data loggers, $N = 6$ surgery controls) for the group in one raceway and at 30 min for the group in the other raceway ($N = 7$ with data loggers, $N = 6$ surgery controls). Movement into the raceway was completed with a smaller corralling net, after which each raceway was closed with a plastic mesh gate and half of the water surface was covered with high-density foam to provide shelter. The intent of this corralling procedure was to simulate conspecifics being cornered by either a predator or fisheries gear (e.g., crowding in a seine net) without direct physical contact for periods of 10 and 30 min. Thus, no biopsies were taken during the corralling procedure. Fish recovered undisturbed in the raceways for 4–6 d while data were logged continuously.

Exhaustive-Exercise Protocol. The exercise protocol was conducted on November 4 and 6, when water temperature was 8.1° ± 0.3°C and water velocity was ~0.2 m s^{-1}. Between 0845 and 1030 hours, six fish were rapidly (<3 s) removed from each raceway by dip net and placed in pairs in one of the three net pens (L × W × D = 1 × 1 × 1.5 m × 60 cm water depth) attached to each raceway. Fish recovered for 5–6 h before being randomly assigned to one of three protocols: (i) 3-min exhaustive exercise, (ii) 3-min exhaustive exercise plus 1-min air exposure, and (iii) untouched control. Exhaustive exercise involved individual fish being rapidly netted (<5 s), placed in a donut-shaped exercise tank (diameter 150 cm, inner diameter 50 cm, water depth 40 cm) containing aerated water, and coaxed manually to burst swim for 3 min (Milligan 1996) by three experimenters positioned around the exercise tank. After 3 min of exercise with continuous behavioral observations, the fish was either replaced directly into its respective net pen by dip net or held in air for 1 min in the dip net. A 60-min postexercise recovery period followed in the net pens (to anticipate the peak postexhaustion plasma cortisol and lactate concentrations, as measured in other salmonids [Barton 2000] and as used in other studies with adult coho salmon [e.g., Farrell et al. 2000, 2001]). At this time, biopsies were taken by individually removing all fish by dip net and placing them supine in a water-filled, V-shaped sampling trough. Within 2 min, each fish was returned to its respective net pen to recover overnight for a further 16–18 h until a final biopsy was taken. Thus, the three biopsies were (i) during initial anesthesia, (ii) 60 min postexhaustion, and (iii) 16–18 h postexhaustion. Control fish were similarly biopsied but without the exhaustive exercise. After the final biopsy, fish were released together into a central section of the large holding channel, where they were monitored for a further 2–3 d, during which there was no delayed mortality. The intent of this exhaustive-exercise procedure was to monitor f_H and blood physiology while simulating responses that might occur when wild fish are trapped but escape from a predator (e.g., a bear) or fishing gear (e.g., a gill net).

Data-Logger Removal, Data Handling, and Statistical Analyses

All fish were euthanized with a blow to the head, reweighed, and remeasured. The exact position of the ECG sensors within the body cavity was verified, and the data logger was removed for subsequent download.

The data file from each data logger was downloaded with a custom-built computer interface, and the text file was imported into LabChart software (ADInstruments, Sydney, Australia) for analysis. A rate-meter function was applied to the ECG data to calculate f_H in beats per minute, and calculations were verified by manual examination of all data.

All continuous variables were assessed for univariate and multivariate model assumptions and in certain cases were subject to transformations, as noted below. Student *t*-tests were used to test for relationships in the response of f_H to corralling and the time required for recovery to pretreatment values. Multiple analysis of variance (MANOVA), with sex as a main effect, was used to compare all blood physiological variables between sexes at each time interval separately. MANOVA, with surgery control as an effect, was used at each time interval during recovery from the exercise treatment to compare all blood physiological variables between the surgery control fish and fish that had been implanted with data loggers. Student *t*-tests were used to assess whether sex or exercise had an effect on f_H at each time interval after the exercise treatment. In cases where MANOVA whole models were significant, two-way ANOVAs were conducted on each variable to identify the variables driving the model. Two-way repeated-measures ANOVA (RM ANOVA), with treatment, time, and their interaction as effects,

was used to compare mean f_H and mean blood physiological indices at each time interval during recovery from the exercise treatment. Least squares regression analyses were used to compare each of the blood physiological values (dependent variables) and f_H (independent variable) for all fish combined from each treatment at both the 1-h and 16-h sampling intervals. The level of significance (α) was assessed at 0.05 for the linear regressions and MANOVAs. In cases of multiple comparisons (i.e., two-way ANOVAs and two-way RM ANOVAs), α was Bonferroni-corrected to 0.006. All values are means ± SE, unless otherwise noted. All statistical analyses were conducted using JMP, version 7.0 (SAS Institute, Cary, NC).

Results

Initial Blood Variables and Heart Rate

The initial blood variables were the same among surgery controls and instrumented fish (MANOVA: $F_{8,27} = 0.376$, $P = 0.301$) and therefore were pooled for further analysis. Sex-specific differences in blood variables (MANOVA: $F_{8,14} = 3.889$, $P = 0.013$) were limited to plasma cortisol (two-way ANOVAs: $P < 0.001$); cortisol was subsequently analyzed by sex, whereas all other variables were pooled (Table 1). Females randomly assigned to the subsequent exercise group had a significantly higher plasma cortisol than did exercise controls at the time of instrumentation; however, this was not significant after Bonferroni corrections.

After surgery, f_H reached a maximum of 58.7 ± 1.3 beats min^{-1} at 47 ± 8 min into recovery. After an 18.4 ± 1.5-h recovery, f_H had stabilized at 35.1 ± 1.0 beats min^{-1} (8.6°C). There were no significant differences in f_H between sexes.

Heart Rate Responses to Corralling

Heart rate before corralling was 31.5 ± 1.2 beats min^{-1} (8.0°C; $N = 13$; Fig. 1). Corralling induced short periods of burst swimming as fish attempted to avoid the net and experimenters. Even so, corralling was slow, and fish could remain inactive for periods of ~1 min before swimming to a different region of the holding channel to evade the net. The individual level of exercise was considered uniform because fish swam as an aggregate, regardless of whether they were corralled for 10 or 30 min.

Heart rate increased to about 60 beats min^{-1} during corralling independent of its duration (Fig. 1; *t*-test: $P > 0.05$). However, the time taken for f_H to recover to pretreatment values was 456 ± 56 min for the 10-min corral and 50% longer, 691 ± 65 min, for the 30-min corral (*t*-test: $P = 0.021$). After 4–6 days of undisturbed recovery, minimum f_H was not significantly different among groups (25.1 ± 0.6 beats min^{-1}; 8.0°C; $N = 13$).

Physiological Responses to Exhaustive Exercise and Air Exposure

Heart rate increased significantly after the rapid transfer (<3 s) of fish by dip net from a raceway to a net pen (Fig. 2; Table 2). During manual chasing, burst swimming lasted for varying durations (minimum = 47 s; maximum = 170 s; mean = 95 s), but no fish maintained burst-swimming activity for the entire 3-min period. Other visual signs of exhaustion included slow righting response when fish were rolled supine and slow body movements during air exposure. On return to the net pen, all fish regained equilibrium immediately, except one air-exposed fish that took 20 s.

Air exposure had no significant effect on the recovery of heart rate and blood variables measured at 1 h (heart rate, *t*-test: $t_4 = 0.329$, $P = 0.759$; blood variables, MANOVA: $F_{8,7} = 0.489$, $P = 0.871$) and 16 h postexhaustion (heart rate, *t*-test: $t_4 = -1.06$, $P = 0.348$; blood variables, MANOVA: $F_{8,15} = 0.435$, $P = 0.865$). Thus, these two groups were pooled for subsequent analyses. There were no sex-specific differences in mean f_H at 1 h (*t*-test: $t_8 = -1.27$, $P = 0.238$) and 16 h (*t*-test: $t_8 = 1.15$, $P = 0.283$) after exhaustive exercise. Heart rate was significantly higher for exhaustively exercised fish (58.5 ± 1.8 beats min^{-1}) than for controls (40.5 ± 3.2 beats min^{-1}) at 1 h after treatment, before biopsy. Biopsy similarly elevated f_H in control fish, which remained higher than that in exercised fish for 8 h after biopsy (Fig. 2). Nevertheless, recovery of f_H in control fish had the same duration (926 ± 151 min) as that in exhaustively exercised fish (729 ± 68 min; *t*-test: $t_8 = -1.34$, $P = 0.217$).

Blood variables for surgery control fish and those implanted with data loggers did not differ at 1 h (MANOVA: $F_{8,14} = 0.843$, $P = 0.251$) and 16 h after exhaustive exercise (MANOVA: $F_{8,3} = 0.511$, $P = 0.973$) and thus were pooled for subsequent analyses. Sex-specific differences in blood physiology at 1 h (MANOVA: $F_{8,27} = 11.414$, $P < 0.001$) and 16 h after exhaustive exercise (MANOVA: $F_{8,3} = 13.594$; $P = 0.028$) were again solely due to plasma cortisol (two-way ANOVAs: $P < 0.001$). As expected, plasma lactate, glucose, sodium, osmolality, and cortisol (males) concentrations were all significantly higher 1 h after exhaustive exercise than in non-exercised controls, but baseline levels were restored after 16 h.

Relationships between Heart Rate and Blood Variables during Recovery

Significant relationships existed between f_H and several plasma variables at 1 h after treatment for pooled data (exercise and nonexercised controls; Fig. 3) but not at 16 h (i.e., all $P > 0.05$). A positive linear relationship existed between f_H and plasma sodium ($R^2 = 0.605$, $P = 0.008$) as well as plasma chloride ($R^2 = 0.596$, $P = 0.009$), and a logarithmic relationship existed between f_H and plasma lactate ($R^2 = 0.826$, $P < 0.001$; Fig. 3). A trend existed between f_H and plasma osmolality, but small sample sizes precluded statistical inferences.

Table 1: Heart rate and blood physiological variables for non exercised control and exhaustively exercised treatment groups of adult coho salmon (*Oncorhyncus kisutch*) at instrumentation (8–9 d before treatment) and at 1 and 16 h after the exercise treatment

				Two-way RM ANOVA					
				Treatment		Time		Interaction	
Variable, Group	At Instrumentation	1 h after Treatment	16 h after Treatment	*F*	*P*	*F*	*P*	*F*	*P*
Heart rate (beats min^{-1})				6.31	.036*	**27.73**	**<.001**	**33.63**	**<.001**
Control	...	40.5 ± 3.2^{A}	42.0 ± 4.0^{A}						
Treatment	...	58.5 ± 1.8^{B}	35.5 ± 1.5^{A}						
Hematocrit (%)				...		...		...	
Control	33.0 ± 1.0	36.0 ± 1.4	34.3 ± 1.5						
Treatment	33.4 ± 0.8	40.0 ± 1.5	34.8 ± 1.1						
Hemoglobin (g L^{-1})				...		...		...	
Control	99.4 ± 2.4	87.9 ± 2.9	78.8 ± 2.4						
Treatment	98.7 ± 1.4	87.1 ± 3.9	79.3 ± 2.2						
MCHC (g L^{-1})				4.42	.041*	**63.70**	**<.001**	2.01	.141
Control	302.9 ± 7.5^{X}	245.4 ± 3.6^{Y}	232.4 ± 8.7^{Y}						
Treatment	298.9 ± 7.4	216.2 ± 6.3	229.5 ± 4.9						
Lactate (mmol L^{-1})				**94.25**	**<.001**	**109.94**	**<.001**	**122.10**	**<.001**
Control	$1.9 \pm .1^{BY}$	$1.4 \pm .2^{BX}$	$1.5 \pm .2^{BY}$						
Treatment	$2.1 \pm .2^{B}$	$15.2 \pm .8^{A}$	$2.1 \pm .3^{B}$						
Glucose (g L^{-1})				2.65	.113	**102.89**	**<.001**	**5.63**	**.005**
Control	$5.6 \pm .3^{DX}$	$9.6 \pm .7^{CY}$	$10.8 \pm .7^{BCZ}$						
Treatment	$4.9 \pm .2^{D}$	$11.7 \pm .7^{D}$	$12.9 \pm .7^{AB}$						
Cortisol, females (ng mL^{-1})				5.95	.03*	**7.33**	**.002**	3.45	.04*
Control	94.1 ± 21.9^{X}	191.6 ± 32.1^{Y}	250.6 ± 22.7^{Y}						
Treatment	207.9 ± 33.3	327.9 ± 25.4	226.1 ± 34.0						
Cortisol, males (ng mL^{-1})				.72	.410	**13.07**	**<.001**	**6.05**	**.005**
Control	78.5 ± 19.8^{BCX}	102.2 ± 27.9^{BCY}	111.4 ± 16.6^{BCY}						
Treatment	58.5 ± 8.5^{B}	173.1 ± 19.8^{A}	111.2 ± 11.5^{C}						
Chloride (mmol L^{-1})				.05	.819	**51.99**	**<.001**	3.29	.04*
Control	$134.5 \pm .8^{X}$	$129.0 \pm .9^{Y}$	125.8 ± 1.2^{Z}						
Treatment	133.7 ± .5	131.4 ± 1.1	123.4 ± 1.2						
Sodium (mmol L^{-1})				4.65	.038*	**25.87**	**<.001**	**14.29**	**<.001**
Control	$155.2 \pm .9^{BX}$	153.5 ± 1.1^{BCY}	150.9 ± 1.1^{BCZ}						
Treatment	$155.2 \pm .7^{B}$	163.1 ± 1.5^{A}	150.2 ± 1.2^{C}						
Potassium (mmol L^{-1})				.02	.883	**7.42**	**.001**	.18	.835
Control	$1.9 \pm .2^{X}$	$1.6 \pm .2^{XY}$	$1.1 \pm .2^{Y}$						
Treatment	1.9 ± .2	1.4 ± .3	.9 ± .1						
Osmolality (mOsm kg^{-1})				**36.73**	**<.001**	**30.91**	**<.001**	**44.00**	**<.001**
Control	321.4 ± 1.3^{BCX}	312.1 ± 1.9^{CDY}	310.1 ± 3.0^{CDZ}						
Treatment	$322.6 \pm .8^{B}$	344.9 ± 1.9^{A}	309.5 ± 2.2^{D}						

Note. MCHC = mean corpuscular hemoglobin concentration. Cortisol differed between males and females and thus was analyzed independently for each sex. Values are means ± SEM. Superscript letters indicate the results of Tukey's post hoc tests: dissimilar letters indicate differences between values, where A–D denote differences with respect to the treatment and time interaction and X–Z denote differences with respect to the time main effect across treatments. Results are also given for two-way repeated-measures (RM) ANOVAs conducted on each variable. Two-way RM ANOVAs contained treatment and time as main effects and their interaction. Bold values denote significance at Bonferroni-corrected $\alpha = 0.006$.

*Significant at $\alpha = 0.05$.

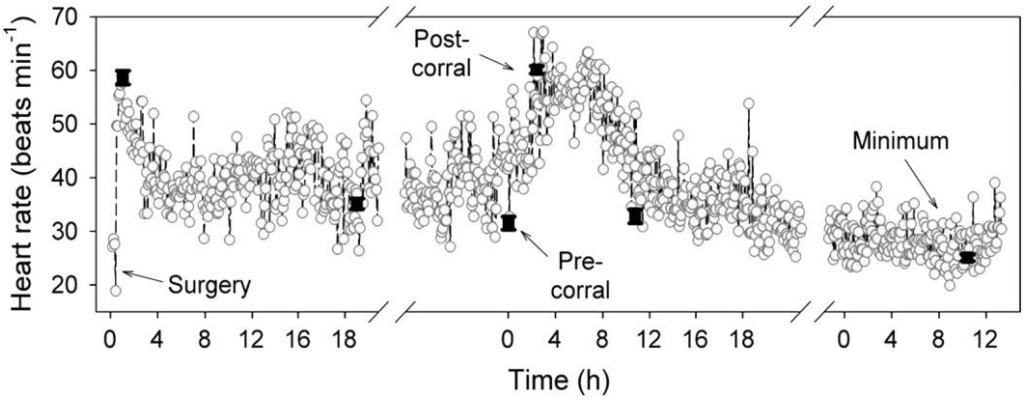

Figure 1. Heart rate (f_H) traces during recovery from data-logger implantation, for the short (10-min) corralling treatment, and for the period when f_H reached a minimum between the corralling treatment and the exercise treatment (~9°C). Circles represent raw data from an individual fish, while squares represent mean (±SEM) values from all fish that underwent the short corralling treatment ($N = 6$). Fish had 24–48 h recovery from surgery before the corralling treatment. Heart rate profiles from the fish that underwent the long (30-min) corralling treatment were identical, except that f_H took longer to reach precorralling levels after the corralling treatment (see "Results").

Discussion

Responses of Fish to Corralling with No Physical Handling

This is the first study on free-swimming adult Pacific salmonids to measure the response and recovery of f_H when subjected to varying stress intensities. In addition, no study has concurrently measured the f_H of a group of conspecifics (with or without corralling), and so the data from the present study have relevance to capture attempts on salmon aggregations, which are common during Pacific salmon migrations. Routine f_H was stable and low (e.g., ~30 beats min^{-1}) relative to findings of previous studies on adult salmonids (Anderson et al. 1998; Gallaugher et al. 2001; Steinhausen et al. 2008), particularly those engaging in prespawning behaviors (Clark et al. 2009; Makiguchi et al. 2009).

The immediate response to corralling (a near doubling of f_H) was uniform and independent of the duration of the event. Even so, it is likely that the longer period of corralling was more stressful, since f_H took 1.5 times as long to recover. Therefore, a threefold increase in corralling duration increased the duration of recovery from the corralling treatment by 50% without affecting the maximum f_H response. Cooke et al. (2003) found that a 30-s presentation of avian predator models to largemouth bass without physical contact increased f_H by 30%–50% at ~24°C, which is half the increase in f_H documented in our study. The bass recovered more rapidly (20–40 min) from the short-duration stressor (Cooke et al. 2003) than did the corralled coho salmon studied here.

Responses of Fish to Exhaustive Exercise and Handling

To our knowledge, this is the first study with free-swimming fish to combine long-term measurements of f_H with repeat blood sampling to examine the interrelations between f_H and blood indices of physiological stress. As with the corralling protocol, exhaustive exercise resulted in a rapid doubling of f_H, and additional air exposure did not further elevate f_H. Furthermore, the capture, handling, and biopsy procedure induced a similar doubling of f_H in the control fish 1 h after the treatment protocols. These data confirm that the magnitude of the f_H response is independent of the magnitude of the stress, and thus our first prediction is rejected. That is, even minor stressful encounters initiate a maximal f_H response, possibly resulting from a rapid release of vagal tone (e.g., Sandblom et al. 2009). At similar temperatures, Atlantic salmon increased f_H by only 20% in response to brief angling (Anderson et al. 1998). Furthermore, the relative increase in f_H was unaffected by temperature (i.e., 30% at 16.5°C and 20% at 20°C; Anderson et al. 1998). Cooke et al. (2004), using similar methodology with largemouth bass in the laboratory, found that manual chasing doubled f_H at a range of temperatures from 13° to 25°C.

As per our second prediction, recovery of f_H depended on the intensity of the stress, taking up to 16 h after exhaustive exercise and either 7.6 or 11.5 h after corralling, depending on the duration. After angling stress in the field, Atlantic salmon required up to 16 h to recover f_H (Anderson et al. 1998). At 1 h after exercise in our study, plasma cortisol, lactate, sodium, and osmolality were elevated, which was to be expected, given the time course for these variables (Milligan 1996; Barton 2000). Males and females, respectively, had a 3-fold and a 1.5-fold increase in plasma cortisol, which is comparable to the 2.7-fold increase measured 30 min after coho salmon were exhaustively exercised in saltwater (Cech et al. 2004) and the 3.3-fold increase that occurred during the first hour after capture of coho salmon by a commercial gill net in saltwater (Farrell et al. 2001). Plasma cortisol was the only sex-specific plasma variable measured here, and this difference may reflect physiological differences in reproductive development between sexes of Pacific salmon (Carruth et al. 2002; Clark et al. 2009; Sandblom et al. 2009).

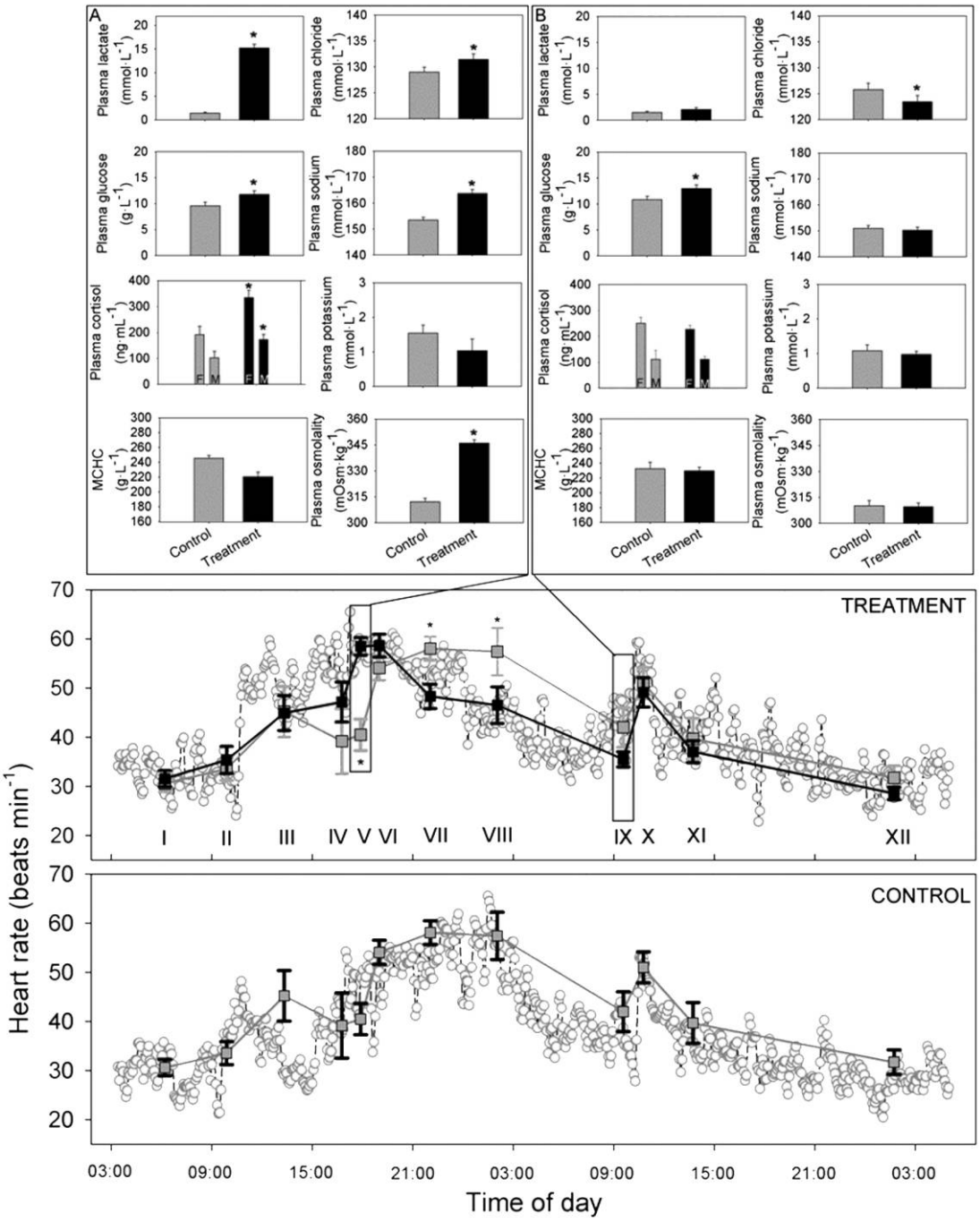

Figure 2. Heart rate (f_H) traces for fish in the exercise treatment group and those in the exercise control group (~8°C). Circles represent raw data from an individual fish (one treatment fish and one control fish). Squares represent mean (±SE) values from all fish that underwent the exhaustive-exercise treatment ($N = 6$; *black squares*), or the control group ($N = 5$; *gray squares*). The "treatment" panel shows mean f_H for the exercise treatment group (*black squares*) and overlays mean heart rate for the control group (*gray squares*) for easy comparison. The top panels display the blood physiological data at the time intervals indicated by boxes in the treatment panel. Descriptions of the time periods marked with Roman numerals are given in Table 2. MCHC = mean corpuscular hemoglobin concentration.

In accordance with the third prediction, strong, positive relationships existed for f_H and certain plasma variables (lactate, sodium, and chloride) at 1 h after treatment (pooled data for control and exercised fish). Plasma osmolality exhibited a similar pattern, but insufficient sample sizes precluded statistical analysis. Lactate production during glycolysis is known to decrease muscle and blood pH (Wang et al. 1994), which can lead to a disruption of ion osmoregulatory balance as water shifts

Table 2: Description of events that correspond to Figure 2

Time	Description	Response
I	4 h before transfer to holding pen (fish housed with 11–12 conspecifics)	Resting heart rate ~30 beats min^{-1} for both the exercise treatment group and the exercise control group
II	30 min before transfer to holding pen (fish housed with 11–12 conspecifics)	Resting heart rate ~34 beats min^{-1} for both the exercise treatment group and the exercise control group
III	3 h after transfer to holding pen	Heart rate elevated above resting values in both the exercise group and the control group
IV	Immediately before exhaustive-exercise treatment	Heart rate remained elevated and not significantly different between the exercise and control groups
V	Immediately before 1-h-posttreatment biopsy	Heart rate significantly elevated in exercise group compared with control group
VI	1 h after 1-h-posttreatment biopsy	Heart rate similarly elevated in both treatment and control groups in response to biopsy
VII	4 h after 1-h-posttreatment biopsy	Heart rate significantly elevated in control group compared with exercise group
VIII	8 h after 1-h-posttreatment biopsy	Heart rate remained significantly elevated in control group compared with exercise group
IX	Immediately before 16-h-posttreatment biopsy	Heart rate of both groups had returned to pre exercise levels (i.e., IV)
X	1 h after 1-h-posttreatment biopsy (fish housed with 24 conspecifics)	Heart rate similarly elevated in both treatment and control groups in response to biopsy
XI	4 h after 16-h-posttreatment biopsy (fish housed with 24 conspecifics)	Heart rate of both groups had returned to values recorded immediately before the 16-h-post treatment biopsy (i.e., IX) but was still above resting levels
XII	16 h after 16-h-posttreatment biopsy (fish housed with 24 conspecifics)	Heart rate had returned to resting levels (i.e., I)

from blood to muscle tissue. This leads to temporary increases in concentrations of some plasma ions in freshwater, followed by depressed ion concentrations over the longer term (Wood 1991). Temporary increases in plasma ions were observed at 1 h after treatment for both plasma sodium and osmolality. However, plasma ion concentrations were significantly depressed relative to pretreatment values when measured 16 h after treatment, consistent with the longer-term decreases in ion concentrations after a stressful event. Similar physiological disturbances have been observed in the blood and muscle of salmonids captured by angling (Booth et al. 1995; Brobbel et al. 1996; Wilkie et al. 1996, 1997) and commercial-fisheries gear (Parker et al. 1959; Farrell et al. 2000; Skomal 2007). Relationships between f_H and other variables may not have been detected because of the timing of blood sampling after the treatment. For example, blood glucose generally decreases and recovers rapidly (minutes) after exhaustive exercise, which may explain why we failed to detect relationships between f_H and plasma glucose at 1 h postexercise (Pagnotta and Milligan 1991; Milligan et al. 2000).

At 16 h after treatment, some parameters had returned to pretreatment levels (i.e., mean f_H, plasma lactate, sodium, osmolality, and cortisol [males]), yet mean MCHC, plasma glucose, chloride, potassium, and plasma cortisol (females) had not completely recovered. The time required to clear metabolites from the blood and restore muscle energy stores may limit performance, since this recovery rate will determine the frequency of maximal performance (Milligan 1996). While prolonged swimming performance can be repeated with relatively short recovery times in adult Pacific salmon (e.g., 40–45 min; Farrell et al. 1998; Jain et al. 1998), the duration of complete physiological recovery after the stressor may be prolonged (Tang and Boutilier 1991; Milligan et al. 2000; Farrell et al. 2001), potentially leaving the individual more susceptible to secondary predation or fisheries capture. Milligan et al. (2000) found that rainbow trout (*Oncorhyncus mykiss*) that recovered in flowing water and were able to swim at a constant low velocity (i.e., 0.9 body lengths s^{-1}) after exhaustive exercise had a complete metabolic recovery in ~2 h. Similarly, plasma cortisol levels remained relatively low in exhausted individuals that swam at constant velocity, compared with those held in static water. Given that the fish in our study recovered in a water velocity of only ~0.3 body lengths s^{-1}, the differences in recovery times between studies may be partly a consequence of differences in water velocity. However, we cannot rule out that because the fish in our study were reproductively mature and undergoing senescence, the series of stressors imposed during the study prevented them from a complete physiological recovery.

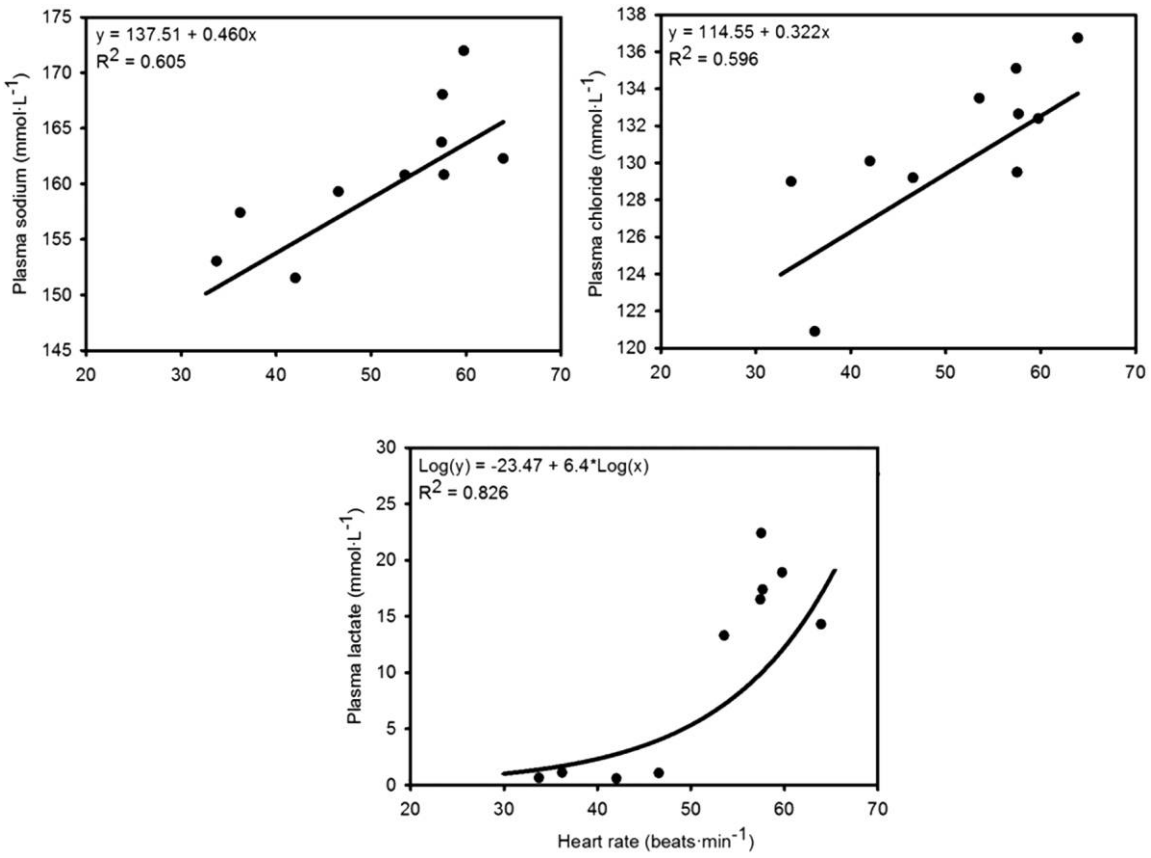

Figure 3. Linear regressions between plasma variables and heart rate (f_H) for pooled data (exhaustively exercised fish and nonexercised controls) from blood samples collected 1 h after treatment (~8°C).

Conclusions

Together, the biopsy and f_H data provide a better understanding of the consequences of simulated predator and fisheries encounters on free-swimming adult Pacific salmonids. Biopsy provides only a snapshot at various time intervals and, as our results show, can induce a rapid increase in f_H, but the continuous f_H data provide a complete characterization of the recovery profile. Clearly, while the level of elevation in f_H conveys little as to the magnitude of the stress, the intensity of the stress is reflected in the duration of recovery of f_H. Even without physical contact, a transient predator or fisheries encounter could maximally elevate f_H and result in a prolonged recovery period. The correlations observed between f_H and several plasma variables warrant further exploration under varying conditions of exercise and stress, but they suggest that biologging or biotelemetry of f_H (Donaldson et al. 2008; Clark et al. 2009, 2010) might be able to provide a general indication of the physiological responses of fish to stressful encounters. With growing conservation concerns over Pacific salmonids, including their survival to reach spawning grounds (e.g., Donaldson et al. 2010), this study provides valuable insight into how adult migratory salmon respond to and recover from acute, ecologically relevant stressors during the freshwater phase of their migrations.

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