Ecology of Freshwater Fish 2012: 21: 609–616
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ECOLOGY OF
FRESHWATER FISH

Behaviour and physiology of mountain whitefish (*Prosopium williamsoni*) relative to short-term changes in river flow

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Accepted for publication May 23, 2012

Abstract – Despite the growing recognition that river flow can have an effect on the growth, distribution and survival of fishes, little is known about the underlying mechanisms to explain this effect. Furthermore, there are few examples of integrated measures of behaviour and physiology to study the responses of fish to river hydrology. Here, axial swimming muscle electromyograms were logged as a sensitive indicator of activity from 19 mountain whitefish (*Prosopium williamsoni*) across a large range of hourly discharge magnitudes (mean = 621 $m^3 \cdot s^{-1}$, range = 0–1770 $m^3 \cdot s^{-1}$) in a hydropeaking reach of the Columbia River, Canada. Hourly mean discharge had a significant positive effect on swimming muscle activity. However, a large amount of the variance was unexplained, possibly due to social interactions, feeding and/or flow-refuging behaviours. Fluctuating flows were no more energetically costly than stable flows. Discharge magnitude had a significant positive effect on blood cortisol concentrations. Yet, cortisol concentrations were low overall (mean ± SD = 1.60 ± 0.09 $ng \cdot ml^{-1}$), suggesting that the small observed response could be the result of routine physiological processes rather than a stress response *per se*. Based on low blood lactate concentrations, mountain whitefish were not swimming exhaustively (i.e., anaerobic burst-type swimming) at high flows.

Key words: locomotor activity; electromyogram telemetry; fish; physiology; cortisol; fish swimming

Introduction

There is a growing recognition that altered flow regimes threaten the biodiversity and ecosystem functions of rivers on a global scale (Nilsson et al. 2005). The ecosystem response depends on how the components of flow (magnitude, rate of change, timing, frequency and duration) have changed relative to a river's natural flow regime (Poff et al. 1997). Indeed, there is a growing recognition that river flow (extreme low and peak flows) can affect growth (Weyers et al. 2003; Korman & Campana 2009), abundance (Liebig et al. 1999) and mortality (e.g., Weyers et al. 2003; Xu et al. 2010; Young et al. 2010) of fish. Yet, little is less known about the underlying mechanisms to explain this effect.

Beyond some threshold swimming speed, fish may perceive their environment as noxious and exhibit a stress response (e.g., Young & Cech 1994). Stress can affect an individual on all levels of organisation from rapid changes in blood physiology to whole-animal performance over the longer term. The primary stress response includes endocrine changes such as increases in glucocorticoid (GC) stress hormones (Barton 2002). Release of cortisol, the primary GC in fish, is delayed postcontact with the stressor. Therefore, if cortisol is sampled properly, obtained values are not influenced by capture stress and can provide baseline GC concentrations (Gamperl et al. 1994). Secondary responses include changes in metabolism, hydromineral balance, and cardiovascular, respiratory and immune functions. Finally, tertiary responses

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doi: 10.1111/j.1600-0633.2012.00582.x

include whole-animal changes in performance, such as in growth and behaviour (Barton 2002). If a stress response remains uncorrected for days or weeks, the result is chronic stress, which is detrimental to health and fitness (Busch & Hayward 2009).

Behavioural responses to stress could include changes in spontaneous swimming activity. Changes in activity (hypo- or hyperactivity) are among the first symptoms observed in the presence of a stressor (Scherer 1992). Frequently, these changes are reported nonquantitatively and secondary to other results (Scherer 1992), partly due to the lack of suitable recording techniques. Early work focussed on contaminant-induced changes in fish swimming behaviour (e.g., Bengtsson & Larsson 1981; Macfarlane & Livingston 1983). The use of biotelemetry has expanded the utility of activity studies to a variety of subtle stressors such as thermal pollution (e.g., Cooke & Schreer 2003) and fish stocking density (Cooke et al. 2000). One technique to measure swimming activity is electromyogram (EMG) telemetry. Electromyograms are bioelectric voltage changes that are proportional to the degree and duration of muscle tension (Sullivan et al. 1963). Undulatory (or body/caudal) fin swimming in fish is powered by the segmented body musculature of the myotomes. The body and tail of a fish must push against the water to generate forward thrust when swimming. Thus, EMGs recorded from electrodes embedded into myotomes of the red oxidative muscles can be used as quantitative indicators of swimming activity (Cooke et al. 2004).

The purpose of this study was to determine whether there was a relationship between the hydrological parameters of a hydropeaking river, and mountain whitefish swimming energetics and physiological stress. Mountain whitefish are a lotic dwelling species, but also occupy lakes and reservoirs. River discharge at our study site, the upper Columbia River, is controlled by the release of water from the Revelstoke Dam (REV). Discharge peaks last less than a day and can occur multiple times per day depending on the demand for electricity.

Our first objective was to determine whether there was a statistical effect of peaking river flows on mountain whitefish swimming activity. Specifically, we regressed axial swimming muscle EMGs against hourly river discharge magnitude while controlling for the individual variation in mountain whitefish responses, as well as exogenous factors such as temperature and light. When discharge changes in a river, so does the spatial arrangement of energetically favourable positions and fish may shift from one position to another to compensate for the changing habitat quality (Kraft 1972). Given this, our second objective was to determine whether within-hour fluctuations in river flow had an effect on mountain whitefish swimming activity. Our third objective was to determine whether elevated (or reduced) river discharges were associated with GC release in mountain whitefish. And, although routine swimming would not be expected to increase lactic acid, exhaustive exercise would give rise to lactate production (Brown et al. 2008). Therefore, we also measured both baseline plasma cortisol and lactate concentrations relative to river discharge from a different set of free-swimming mountain whitefish.

Methods

Study site and fish surgery

A total of 31 fish (mean ± SD fork length = 628 ± 104 mm) were collected by boat pulsed DC electroshocker during 3 years: 2008 (16 fish; 24 September–8 October), 2009 (12 fish; 8–27 October) and 2010 (three fish; 26–27 October). Fish were captured at night using a large dip net, transferred into an aerated live well and relocated to the surgery site. Water temperatures at the time of capture ranged from 8 to 11 °C among all years. Fish were transferred again to a holding tank (diameter = 243 cm, depth = 90 cm and volume = 2839 l) on the shoreline of the Columbia River with aerated continuous flow-through water pumped from the river.

Fish were individually anaesthetised to stage 3–4 anaesthesia (Summerfelt & Smith 1990) in 60 ppm clove oil (emulsified in ETOH at a ratio of 1 part clove oil to 10 parts ETOH; Anderson et al. 1997) and transferred to a v-shaped plastic surgery trough in a supine position. Water was continuously pumped across their gills with a maintenance bath of 30 ppm clove oil. Using a scalpel (number 3 blade, rounded cutting point), a ~30-mm incision was made in the ventral body surface, posterior to the pelvic girdle, slightly off midline. A coded EMG transmitter was inserted (CEMG2-R11-12; dimensions: 11 × 48 mm, weight: 8.8 g in air). EMG electrodes were inserted ~10 mm apart, in the red axial musculature, using a rod and plunger. Electrode placement was standardised at 0.7 body length on the right side of the fish (Beddow & McKinley 1999). A 16 ½-gauge hypodermic needle was pushed through the body cavity wall and the antenna wire passed through to the outside. The incision was closed using four independent sutures (PDS II absorbable monofilament, 3/0, FSL needle). Additional information regarding EMG surgeries can be found in a review by Cooke et al. (2004). The entire procedure took approximately 5 min per fish, and the same surgeon performed all surgeries. Fish were then placed back into a holding tank until they recovered and exhibited 'normal' swimming behaviour at which time it was released back into the river.

Electromyogram data collection and analysis

Three fixed receiver stations (Lotek SRX600 telemetry receiver; Newmarket, ON, Canada) continuously logged EMG data from locations where fish were known to congregate (Fig. 1). Two antennas pointing both upstream and downstream were fixed to nearby trees at each fixed receiver station. Each receiver scanned eight channels in succession, 24 h·day^{-1}, and recorded any fish in the general vicinity of the station (estimated maximum distance of 400 m upstream and downstream). Coded EMG signals were converted to hourly averages; therefore, we approximated a value that corresponded to a prolonged period of swimming (Beamish 1978). Only hourly averaged values that were comprised of at least 100 instantaneous (2 s) EMG values were used in the analysis.

Several studies have shown significant relationships between EMGs and metabolic rate (e.g., Hinch et al. 1996; Geist et al. 2005). However, the slopes and intercepts of these relationships can vary among individuals, suggesting that EMG transmitters behave differently in different fish (See Brown et al. 2007). These authors suggested that the only way to account for this variation in field studies is to have individual EMG metabolic rate calibrations for each subject before deployment in the field. However, in practice, this is difficult owing to the inability to transport the respirometers and the difficulty in transporting live fish without exposing them to harmful stress (Cooke et al. 2004). Considering this, we modelled the effect of discharge on uncalibrated EMGs and controlled for potential variation in the model intercepts and slopes. Because the intercepts varied among fish (Wald $Z = 2.348$, $P = 0.019$), we controlled for this between-subject variation using random factors under the linear mixed model framework (MIXED, Version 18; SPSS Inc., Chicago, IL, USA). Secondly, R^2 was used as the effect size, which is independent of individual differences in slopes and intercepts. Preliminary analysis using ordinary least-squares (OLS) regression suggested autocorrelation in the model residuals owing to the repeated EMG measurements on the same fish (Durbin–Watson statistic $\ll 2$). Autocorrelation plots confirmed this; therefore, the mixed model analysis started with an autoregressive (AR1) covariance structure to account for this autocorrelation. Also, EMGs showed different ranges for different fish; for example, a resting EMG for one fish may be '12', whereas for another fish, a resting EMG may be '5'. Standardised EMGs (SEMG) were then calculated by subtracting the resting value from all EMGs.

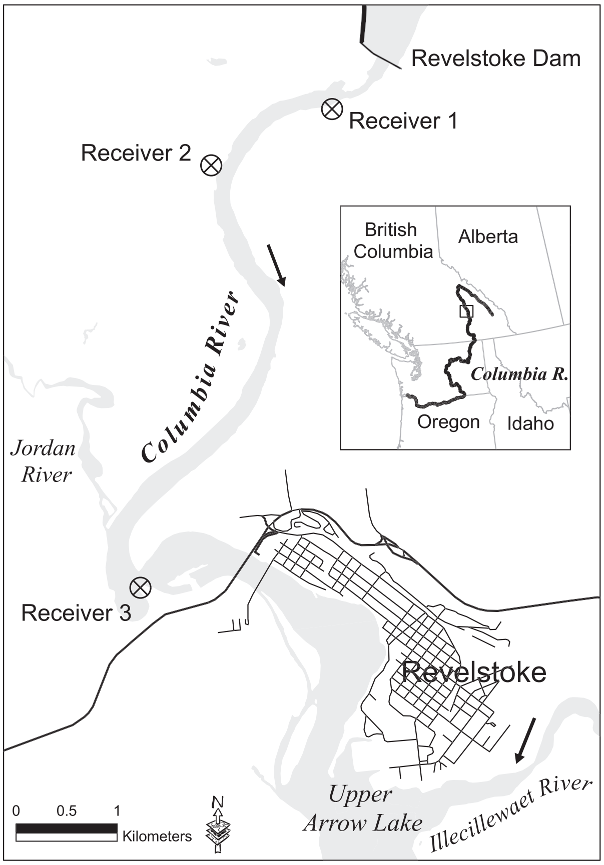

Fig. 1. Map of the Columbia River downstream of Revelstoke Dam, Revelstoke, British Columbia, Canada. Study area was a 10.6-km section from Revelstoke Dam downstream to the Illecillewaet River. Circle with X represents the fixed receiver locations.

We added predictors using a hierarchical technique of building successive models and accessing the behaviour of the model in response to each new predictor (Field 2009). Our two hydrological predictors were added first: hourly mean discharge magnitude (mean discharge across a given hour) and within-hour change in discharge (difference between instantaneous maximum and minimum discharges within a given hour). Control variables were then added (water temperature and diel period). Not all fish were logged at all receivers. Because of an imbalance of data among receivers, data from all receivers were pooled. We also tested for a time-lag effect of water flow (measured at the dam) to reach receiver 1 (~1 km), receiver 2 (~2.5 km) and receiver 3 (~7 km) downstream of the dam. A 30-min time delay for river flow to reach receiver 3 was accounted for. The interaction between hourly average discharge and diel period was tested. The interaction between the number of EMG observations for each fish and discharge was also tested to see whether the number of observations modulated the effect of discharge.

The model structure was evaluated in terms of random slopes and covariance structures using Akaike's information criterion (AICs) (Field 2009; Heck et al. 2010). A variance components covariance type for the random subject coefficients was found to be the best structure. Maximum likelihood estimation was used. Because multilevel models are an extension of regression, all of the assumptions of OLS regression were tested as well as some additional assumptions pertaining to the random coefficients. OLS regression was used to fit linear, cubic and quadratic models for each fish independently, and then correlation coefficients were compared using ANOVA to test for linearity between the dependent variable and the main independent variables; the linear model fit best. Scatterplots of all combinations of predictors were inspected to find correlations (i.e., collinearity) that may affect the model's ability to address the main question. A significant positive correlation existed between hourly mean discharge and within-hour discharge rate of change ($r = 0.30$). Furthermore, discharge was significantly greater during the day (mean = 879 $m^3{\cdot}s^{-1}$) than at night (mean = 450 $m^3{\cdot}s^{-1}$). These pairs of predictors were added both alone and together so as to look for suppressor/synergistic effects. We compared the activity between day and night using estimated marginal means, thus controlling for the difference in discharges. Model residuals were positively skewed; therefore, SEMGs (+1) and predictors were log-transformed. Residuals were plotted across the range of predicted values and met the assumption of homoscedasticity. Random intercepts were normal about their means.

Blood sampling and analysis

During each survey, 8–12 mountain whitefish (mean ± SD fork length = 261 ± 34 mm) were captured and sampled one at a time. Surveys were conducted every 4 h over five 24-h periods (4 November 2008; 12 November 2009; 3 June 2010; and 29 October 2010) coinciding with daily stable low, stable high, rising and falling river flows. Fish were captured using boat shocking from multiple reaches of a 10.6-km segment downstream of REV. The same river segment was not resampled more than once in the same day. Once netted, a blood sample was taken via caudal puncture using a Vaccutainer (3 ml with lithium heparin, 21-g needle; Becton Dickson, Franklin Lakes, NJ, USA) within 2 min of capture. Laboratory studies have demonstrated that capture-related stress will not significantly influence cortisol concentrations if the sample is taken quickly (<3 min; Sumpter 1997). Samples were held on ice slurry for no more than 100 min before blood was centrifuged for 6 min at 10,000 ***g***. Plasma was then flash-frozen in liquid nitrogen and later stored frozen in a −80 °C freezer until analysis. Samples were analysed using a commercial radioimmunoassay kit (immunoChem Cortsiol I25I RIA Kit; MP Biomedicals, Orangeburg, NY, USA) routinely used to quantify cortisol concentrations in fish plasma (Gamperl et al. 1994) and a Cobra Auto-Gammer counter (Hewlett-Packard, Palo Alto, CA, USA). Detailed methods can be found in the study of Gamperl et al. (1994). All samples were assayed together, and intra-assay variability was 3.4% as quantified from 16 samples run in duplicate. Lactate was measured on blood plasma by adding 10 ml of blood to a hand-held lactate meter (Lactate Pro LT-1710 portable lactate analyser; Arkray Inc., Kyoto, Japan). Appropriate standards and calibrations were used with meters prior to analysis as per manufacturer guidelines. The Lactate Pro has previously been validated as a reliable tool for field physiology of fishes (Brown et al. 2008). Because only one sample was taken from independent fish, a general linear model (GLM) was used to test for a relationship between river discharge on cortisol and lactate concentrations. An average discharge for each of 1–6 h before blood was sampled was tested against cortisol concentrations to determine which timescale was the best at predicting cortisol; mean discharge from the preceding 1 h before sampling produced the best model. We repeated this step with a within-hour change in discharge. The mean temperature during the same 1-h sampling period was also controlled for. Temperatures never varied by more than 0.4 °C during any one sampling period; therefore, temperatures were most different between seasons. The diel effect of day and night was also controlled for. Dawn and dusk were considered to be 1 h before and after sunrise and sunset. However, because of the relatively small amounts of data collected during the dawn/dusk period, these data were removed from the analysis. The reciprocal of cortisol was used to achieve normality of model residuals – more traditional transformations (e.g., log and square root) were not effective. Residuals conformed to the assumption of homoscedasticity.

Results

Data collection

Of 31 mountain whitefish tagged over 3 years, 19 were recorded at fixed stations and yielded sufficient information for analysis of axial swimming muscle EMGs. The remainder either (i) were never located in the 10.6-km study area, (ii) were located in the study area, but were not located near the three fixed receivers or (iii) were located within range of the receivers, but for a relatively short time. On average, 113 ± 116 (mean ± SD) hourly mean EMG values were logged for each whitefish during a mean hourly discharge of 621 $m^3 \cdot s^{-1}$ (range = 0–1770 $m^3 \cdot s^{-1}$). Zero flow release from REV did occur, but residual river water, tributary inflow and back-up from the downstream Arrow Lakes prevent the river from completely dewatering. The mean difference between within-hour maximum and minimum discharge was 176 $m^3 \cdot s^{-1}$ (range = 0–920 $m^3 \cdot s^{-1}$). The mean temperature was 10.2 °C (range = 7.5–12.2 °C).

Two hundred and one blood samples were collected over five 24-h survey periods during an overall mean discharge of 900 $m^3 \cdot s^{-1}$ (range = 566–1524 $m^3 \cdot s^{-1}$) and a mean temperature of 8.6 °C (range = 5.3–10.7 °C). All blood samples were analysed for cortisol, and a subset of ninety samples were analysed for lactate.

Electromyogram data

Hourly mean discharge magnitude had a significant positive effect on swimming muscle activity (MIXED, $F_{1,2130} = 126.80$, $P < 0.001$). Upon adding within-hour change in discharge, hourly mean temperature, diel period and sampling year, only discharge magnitude (MIXED, $F_{1,2134} = 108.71$, $P < 0.001$) and diel period (MIXED, $F_{1,2128} = 44.82$, $P < 0.001$) were significant predictors and remained in the final model (Table 1). The number of hourly average SEMG values logged per fish did not modulate the effect of discharge (MIXED, $F_{1,370} = 0.94$, $P = 0.33$). Finally, the addition of a random discharge slope was not significant (Wald $Z = 1.51$, $P = 0.13$). In the final model, hourly mean discharge and diel period explained 14% and 4%, respectively, of the variation in swimming muscle activity (Table 2). Pairwise comparisons, based on estimated marginal means of log (SEMG + 1), suggested that muscle activity during the day was 1.11 times that of muscle activity during the night ($P < 0.001$) when controlling for discharge.

Table 1. Summary of steps to fit linear mixed model for mountain whitefish log(SEMG + 1) in the Columbia River, Revelstoke, BC.

Model	Fixed effect	ΔEffect size (ΔR^2)†	Sig$^{(\text{new term})}$
1	**D_{MAG}**	0.14	<0.001
2	D_{MAG}, **D_{CHANGE}**	0.00	0.234
3	D_{MAG}, **Temp**	0.00	0.075
4	D_{MAG}, **Diel**	0.04	<0.001
5	D_{MAG}, Diel, **Year**	0.00	0.447
6	D_{MAG}, Diel, **D_{MAG}*Diel**	0.00	0.141
7	D_{MAG}, Diel, **D_{MAG}*#Observ**	0.00	0.334

SEMG, standardised EMG.

One fixed effect was added at a time and is in bold face. D_{MAG} is the hourly average discharge; D_{CHANGE} is the difference between within-hour instantaneous maximum and minimum discharges; Temp is the hourly average temperature; Diel is a categorical value of daytime or night-time; Year is the year in which data were collected (2008, 2009 or 2010). #Observ is the number of electromyogram values logged/fish. Sig$^{(\text{new term})}$ refers to the *P* value of the new term added to each successive model. Continuous predictors were log-transformed.

†Δ from previous model.

Blood physiology

All seasons combined, the mean cortisol concentration was 1.60 ± 0.09 $ng \cdot ml^{-1}$. There was a significant negative effect of hourly mean discharge on 1/cortisol (GLM, $F_{1,196} = 20.99$, $P < 0.001$, $R^2 = 0.10$). Within-hour change in discharge also had a significant negative effect on 1/cortisol (GLM, $F_{1,196} = 20.99$, $P < 0.001$, $R^2 = 0.05$). Water temperature had no effect on 1/cortisol (GLM, $F_{1,196} = 1.18$, $P = 0.28$). There was also no difference between 1/cortisol values from whitefish at night versus day (GLM, $F_{1,196} = 3.71$, $P = 0.08$).

All seasons combined, the mean plasma lactate concentration was 2.05 ± 2.11 mM. There was no significant effect of discharge on log(lactate) (GLM, $F_{1,83} = 3.16$, $P = 0.08$). Mean daily water temperature did have a significant negative effect on log(lactate) (GLM, $F_{1,83} = 15.06$, $P < 0.001$, $R^2 = 0.15$). There was no difference between log(lactate) at night versus day (GLM, $F_{1,83} = 0.26$, $P = 0.61$).

Discussion

Our first objective was to determine whether peaking river flows had an effect on mountain whitefish swimming activity. There was a significant positive correlation between hourly mean discharge magnitude and hourly mean swimming muscle activity. This may be explained by the fact that for a fish to hold station, it must maintain a constant position relative to the substrate, despite the flow of water exerting force to displace the fish downstream (Gee 1983). The energetic demand of swimming can be substantial and is a direct function of the fish's drag multiplied by the water's current velocity (Webb 1988). However, it was surprising that a large amount of variance in mountain whitefish muscle activity was unexplained by discharge. There are a number of behaviours associated with energy economy in high flows including 'flow refuging' – avoiding the high costs of swimming by positioning themselves in low flow microhabitats – which may explain the presence of some hours of relatively low muscle activity during high discharge. Also, some prolonged feeding attempts or social interactions may have occurred at opportunistic times when discharges were not necessarily high, thus resulting in greater-than-average activity at a given discharge. This highlights one of

Table 2. Parameter estimates for linear mixed model of mountain whitefish Log(SEMG + 1) in the Columbia River, Revelstoke, BC.

Parameter	Estimate	SE	d.f.	*t*	95% Confidence int. Lower	95% Confidence int. Upper	Sig
Intercept	0.346	0.028	26	12.314	0.288	0.404	<0.001
Log(D_{MAG})	0.050	0.005	2133	10.437	0.041	0.594	<0.001
Day	0.052	0.008	2128	6.695	0.036	0.067	<0.001
Night	Ref	–	–	–	–	–	–

SEMG, standardised EMG.

D_{MAG} is the hourly average discharge and Diel is a categorical value of daytime or night-time. 'Night' was used as the reference category; therefore, the 'Night' parameter estimate is redundant in the presence of the intercept parameter.

the critical differences between studying fish swimming behaviours in a laboratory flume, whereby fish are forced to swim, versus wild free-swimming fish. For some species, river discharge does not appear to exert control over swimming activity. For example, Geist et al. (2005) found that light levels and temperature, but not discharge, were related to swim speeds and oxygen consumption of juvenile white sturgeon (*Acipenser transmontanus*) in the Snake River, Idaho.

A diversity of responses to discharge was seen among individual fish as demonstrated by the random intercepts to the SEMG model. While individual variation in locomotor performance is a topic of interest to fish physiologists (see Kolok 1999), individual variation of intercepts in our uncalibrated SEMG model is difficult to interpret because we are not sure whether it is attributable to the transmitters and/or the actual performance of the individual (Brown et al. 2007). Therefore, we controlled for this individual variation using random coefficients while focusing on the main effects of hydrology by using R^2 as our effect size, which is independent of the intercepts.

Our second objective was to determine whether within-hour fluctuations in river flow had an effect on mountain whitefish swimming activity. Large within-hour changes in discharge occurred (range = 0–920 $m^3{\cdot}s^{-1}$), which is unique to hydropeaking systems. However, no significant effect of within-hour change in discharge was found. This was surprising considering changes in microhabitat use by fishes, as influenced by changing flows, have been documented by snorkelling observations (Pert & Erman 1994) and electrofishing (Valentin et al. 1994). Further, Cocherell et al. (2011) found that rainbow trout (*Oncorhynchus mykiss*) had significantly elevated swimming activity during the initial peaking phase of flow release in the American River, California.

The third objective of this study was to determine whether short-term changes in river flow are perceived as a physiological stressor, as measured by circulating baseline cortisol concentrations. Cortisol did show a significant positive relationship with hourly mean discharge and within-hour change in discharge when controlling for temperature and light. However, despite these correlations, overall mean (±SD) cortisol levels were only 1.60 ± 0.09 $ng{\cdot}ml^{-1}$. These values are in the range of 'prestress' values reported for a number of salmonids (see Barton 2002). For example, baseline cortisol concentrations were 1.0 ± 0.3, 1.1 ± 0.3 and 1.7 ± 0.5 $ng{\cdot}ml^{-1}$ for brown trout (*Salmo trutta*), arctic grayling (*Thymallus arcticus*) and rainbow trout, respectively. These fish had corresponding poststress concentrations of 94 ± 11, 46 ± 4.4 and 43 ± 3.5 $ng{\cdot}ml^{-1}$, respectively, after a standard 30-s handling. Mountain whitefish plasma cortisol concentrations can be >100 $ng{\cdot}ml^{-1}$ following prolonged capture and holding (held in beach seine for >20 min; Quinn et al. 2010). Flodmark et al. 2002 examined the effects of simulated hydropeaking on baseline cortisol concentrations of brown trout in an artificial stream channel and found that when fish were exposed to daily cyclical fluctuations of water flow, plasma cortisol levels were elevated on the first day compared to undisturbed fish. However, by the fourth day, cortisol remained at control levels, leading to the conclusion that these fish showed rapid habituation to the stressor. This may be the scenario in the Columbia River whereby mountain whitefish are habituated to repeatedly fluctuating discharges. Krimmer et al. (2011) examined the stress response of brook trout to experimental water withdrawal from a small mountain stream and found no differences between treatment and control reaches. However, blood samples were taken from anaesthetised fish at least 5 min postcapture, and cortisol values were likely influenced by the sampling protocol. Erickson et al. (2005) also found no difference in baseline cortisol concentrations of brown trout between urban storm runoff and base flow events, which included both significant changes in river flow and physical water quality parameters such as suspended solids and metals. Other than these three examples, we are not aware of any research examining the relationship between water flow and GC stress response.

Measuring blood lactate is a common method to assess the physiological consequences of swimming and fatigue (e.g., Gustaveson et al. 1991) as increased blood lactate reflects increased oxygen consumption during swimming. When oxygen availability for tissue function is impaired, glycolysis gives rise to increased lactate production. In this study, discharge showed no relationship with lactate, suggesting that study fish are not performing exhaustive anaerobic exercise during high flows. Prolonged recovery times of free-swimming fish to periods of peak discharge would leave fish vulnerable to predation as their capacity for anaerobic (i.e., 'burst') swimming would be impaired. Daily mean water temperature had a significant negative effect on lactate. Mean lactate concentrations from this study (2.05 ± 2.11 mM) were less than those reported for rainbow trout following exhaustive catch and release scenarios (6.26 ± 0.4 mM; Meka & McCormick 2005).

In summary, our results suggest that while axial swimming muscle activity increased at elevated discharges, the majority of the variation in activity was not explained by hydrology. Furthermore, physiology results reveal that discharge did not appear to be a stressor to mountain whitefish or cause exhaustive exercise. Our approach of integrating measures of both behaviour and physiology for the study of hydropower impacts on free-swimming fish is novel. We demonstrated that using mixed-modelling approaches, one can control for individual variation in biotelemetry data as well as account for the inherent violations of independence that occur when analysing data that were repeatedly measured from the same fish. We suggest that future research on fish responses to flow dynamics incorporate measures of both fish behaviour and physiology in an effort to further elucidate the complexity of animal–environment relationships, particularly in the context of water resource management.

Acknowledgements

We would like to thank BC Hydro (especially K. Bray and G. Martel) as this work was performed under contract from the BC Hydro Water Licence Requirements. SJC was supported by the Canada Research Chairs Program and the Natural Sciences and Engineering Research Council of Canada. All experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care. We would also like to thank B. Lewis, A. Nagrodski, N. Stafl, K. Mai and D. Ford for their help in the field. We would also like to thank E. Thorstad and anonymous reviewers for their helpful comments on the manuscript.

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