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THE EFFECTIVENESS OF TISSUE BIOPSY AS A MEANS OF ASSESSING THE PHYSIOLOGICAL CONSEQUENCES OF FISHWAY PASSAGE

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ABSTRACT

Beyond assessing passage efficiency of fishway structures, there is a need to examine the sublethal impacts of passage on the physiological condition of fish. Muscle and plasma samples were collected from pink salmon (*Oncorhynchus gorbuscha*) at four sites along a fishway and were compared in order to assess the effectiveness of these methods as they apply to fishway studies. Both plasma and muscle tissue revealed changes consistent with anaerobic activity when fish sampled from within the fishway were compared with field baseline estimates taken from fish sampled 50 m downstream of the fishway entrance. Plasma Cl^-, osmolality and haematocrit increased significantly (all $p < 0.01$) during ascent, whereas muscle glycogen and phosphocreatine declined (both $p < 0.03$). Both plasma and muscle tissue collected from fish sampled at a site just upstream of the fishway showed physiological profiles that were consistent with metabolic recovery relative to physiological conditions during passage: plasma lactate, haematocrit and muscle lactate had decreased (all $p < 0.01$), and muscle phosphocreatine increased ($p = 0.01$). When examining the physiological changes that specifically occurred between the two sites within the fishway structure, we found no differences in plasma samples, but muscle lactate and water content both showed significant changes (both $p < 0.05$). These results are consistent with the greater sensitivity of muscle tissue than of blood to exercise-related physiological changes and highlight the usefulness of sampling muscle tissue for assessing fishways that ascended in a short time. Fishway studies could benefit from greater inclusion of physiological tools and approaches to identify the costs of passage and areas of difficulty within a fishway.

KEY WORDS: fish ladder; lactate; physiological stress; Pacific salmon; *Oncorhynchus gorbuscha*

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INTRODUCTION

Fishways can provide connectivity between habitats upstream and downstream of an otherwise impassable river reach and have become crucial to the persistence of many populations of Pacific salmon (*Oncorhynchus* spp.) in river systems such as the Columbia and Snake Rivers, USA, where dams have become ubiquitous (Nehlsen *et al.*, 1991; Lichatowich, 1999). Indeed, as the number of dams has proliferated on waterways throughout the world over the past century (Rosenberg *et al.*, 2000), the provision of an effective means of passage around these barriers remains an important issue in the management and conservation of numerous fish species. A key aspect of an effective fish bypass system is that it is designed to minimize 'undue effort' associated with passage (Powers *et al.*, 1985; Odeh, 1999; Bunt, 2001; Schilt, 2007; Castro-Santos *et al.*, 2008). In extreme cases, excessive exercise during passage attempts may result in passage failure and even death (Hinch and Bratty, 2000). The accumulation of physiological stress during passage may also be detrimental to the migration and reproductive success of Pacific salmon (Hinch *et al.*, 2006), as stress responses associated with exercise may impair reproductive development (Schreck *et al.*, 2001). Although many studies have been conducted to assess the passage efficiency of fishways at a population level, few efforts have been made to understand the physiological impacts of fish passage (Schilt, 2007) and to quantitatively assess and characterize the effects of passage on individual fish (Castro-Santos *et al.*, 2008; Roscoe and Hinch, 2010).

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Among the tools available to examine fishway passage impact is electromyogram (EMG) telemetry, which provides a means of estimating instantaneous swim speeds and metabolic costs of activity in free-swimming fish (Cooke *et al.*, 2004; Hasler *et al.*, 2009). EMG telemetry has been used to good effect to quantify the extent to which Pacific salmon invoke anaerobiosis during fishway passage events (Hinch and Bratty, 2000; Brown *et al.*, 2006; Pon *et al.*, 2009). Despite its effectiveness, the use of this technology can be expensive, which may enforce a limitation on potential sample sizes (e.g. Hinch and Bratty, 2000; Gowans *et al.*, 2003; Scruton *et al.*, 2007; Pon *et al.*, 2009; for exception, see Brown *et al.*, 2006). Additionally, a successful EMG study relies on the 'cooperation' of the target species. An examination of swimming energetics during fishway passage by pink salmon (*Oncorhynchus gorbuscha*) was unsuccessful as EMG-tagged fish showed a propensity to retreat downstream despite the observation of non-tagged pink salmon moving through the fishway (Pon *et al.*, 2006). In contrast, radio-tagged and EMG-tagged sockeye salmon (*Oncorhynchus nerka*) demonstrated much higher rates of successful passage at the same fishway (Roscoe and Hinch, 2008; Pon *et al.*, 2009), suggesting species-specific issues related to telemetry-based fishway analyses.

An alternative approach to assessing the intensity of exercise is through the collection of tissues such as white muscle or through blood plasma collection. Whereas several studies have assessed the physiological condition of fish following fishway or channel ascent from plasma samples (e.g. Collins *et al.*, 1962; Connor *et al.*, 1964; Dominy, 1971; Schwalme *et al.*, 1985; Peake and Farrell, 2005; Pon *et al.*, 2009), the use of muscle tissue samples has seen much more limited field use within this context (but see Peake and Farrell, 2005). However, muscle tissue samples have been commonly used in laboratory-based studies of swimming physiology (e.g. Black *et al.*, 1962; Milligan and Wood, 1986; Wang *et al.*, 1994a), and the application of this method may be useful in the assessment of fishway ascent, particularly in identifying the extent to which fish may invoke anaerobiosis during passage. Anaerobic exercise in fish consumes energy and generates metabolic wastes, which can be measured from tissue samples (Milligan, 1996; Kieffer, 2000). For example, lactate, a metabolic by-product of anaerobic glycolysis, initially accumulates in muscle tissue but can also leak into the circulatory system (Kieffer *et al.*, 1994; Wang *et al.*, 1994a). Indeed, many exercise-related changes observed in blood samples are driven by changes within the musculature, and thus muscle tissue may provide a more direct and sensitive measure of anaerobic exercise in fish.

The objective of the present study was to compare the effectiveness of blood and muscle tissue samples in identifying physiological responses to exercise during fishway passage. To this end, we collected both tissue types from pink salmon at four successive sites along a fishway in order to obtain a sequential series of physiological profiles, which were used to compare the relative effectiveness of the two methods at detecting changes in these responses over common spatial and temporal scales. If fish were invoking anaerobiosis during fishway ascent, then we would predict to see corresponding evidence of metabolic activity including decreases in muscle energy stores [e.g. adenosine triphosphate (ATP), phosphocreatine (PCr), and glycogen] and increases in metabolic by-products in both blood and muscle tissues (e.g. lactate), as well as other indications of vigorous exercise such as the loss of ionic homeostasis and elevated haematocrit (Hct). We predicted that muscle tissue would provide a more sensitive metric for identifying physiological changes associated with exercise than blood samples, as evidenced by the occurrence of detectable changes between sample locations.

METHODS

Study site

The study was conducted at a fishway located on the Seton River, a tributary to the Fraser River located ~350 km upstream from the mouth of the Fraser and nearby Lillooet, B.C., Canada (50°41′N, 121°56′W; Figure 1). The fishway is a vertical-slot design and allows passage for resident and anadromous fish species around a water diversion dam spanning the width of Seton River. The fishway consists of 32 consecutive pools, which are separated by concrete baffles, and spans a total length of 107 m, with an elevation of 7.4 m, creating an effective slope of 6.9%. Maximum water discharge through the fishway is 1.3 $m^3 s^{-1}$, and water velocities can range from negligible values up to 2.1 $m s^{-1}$ as concrete baffles between pools create complex flow patterns. Water flow through the fishway is determined by the forebay head, which was at a consistent height throughout the study period. Water discharge downstream of the Seton dam is regulated and was set at 11.0 $m^3 s^{-1}$ throughout the study period. Mean daily water temperatures in the Seton River ranged from 13.5°C to 14.5°C during the 3 days of fish capture and sampling.

Fish capture and sampling

Pink salmon were captured, sacrificed and sampled at four sites along a fishway between 20 September and 22 September 2005. In order of ascent, fish were sampled from sites including a location roughly 50 m downstream of the fishway entrance ($n = 8$), a second location at an approximate midpoint of the fishway structure ($n = 9$), a

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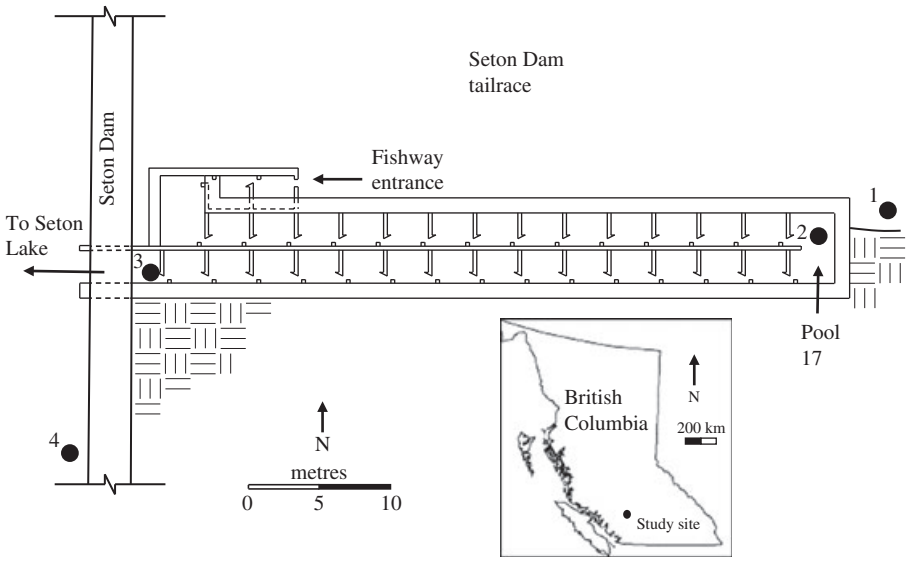

Figure 1. A schematic of the fishway structure at the Seton Dam. The fishway is situated against the south bank of Seton Creek. The four fish capture sites are indicated by the solid black circles, 1: downstream site, 2: midpoint of fishway, 3: top pool of fishway, 4: upstream site.

third location at the top pool of the fishway ($n = 11$) and, finally, at a site just upstream of the fishway (<10 m) where fish displayed milling behaviour prior to entering Seton Lake ($n = 8$) (Figure 1). Dip nets were used to capture fish as this method provided the most expedient means of landing fish (within 5–10 s). Fish were then immediately sacrificed by cerebral concussion, and tissue samples were quickly collected.

Within the first 20–30 s following death, a blood sample (3 mL) was drawn from the caudal vasculature, posterior to the anal fin using a Vacutainer syringe (1.5-in., 21-gauge needle and 3-mL lithium heparin Vacutainer, Becton Dickson, NJ). Blood samples were temporarily stored in an ice-water slurry for less than 10 min prior to centrifugation. Immediately following blood collection, a portion of white epaxial muscle (approximately 50–100 g) was excised from behind the left operculum and above the lateral line using a razorblade, freeze clamped with aluminium tongs pre-cooled in dry ice, wrapped in pre-labelled aluminium foil and immediately frozen in dry ice (Suski *et al.*, 2003). This procedure was conducted in less than 10 s. After returning to the laboratory, all collected samples were transferred to a −80°C freezer until processing. The entire sampling procedure from capture to tissue storage was typically conducted in less than 1 min per fish.

Because of logistical limitations associated with determining the amount of time that had elapsed between passage and sampling for the upstream group and whether fish sampled downstream would have actually passed through the fishway, data from these groups were not interpreted in terms of measuring the actual physiological impact of fishway passage at the Seton dam. Instead, the data collected from these two sample sites were included in the analyses as field baseline (i.e. pre-passage) and recovery (i.e. post-passage) groups to demonstrate the potential application of tissue sampling to understanding physiological changes associated with directed movements between habitats separated by a fishway.

Tissue assays

To assess the physiological impact of fishway passage on fish, we measured several variables from muscle and blood samples, which are commonly used in the study of physiological responses to exercise (e.g. Wang *et al.* 1994a; Wendelaar Bonga, 1997; Postlethwaite and McDonald, 1995; McDonald and Milligan, 1997). From plasma samples, lactate and glucose were measured, as elevated levels of these metabolites are indicative of anaerobic swimming and exhaustive exercise (Wood, 1991; Kieffer, 2000). Plasma ions (Na^+, K^+, Cl^-) and osmolality were measured to determine the osmoregulatory state of fish, a function that can be disturbed following prolonged or exhaustive swimming (Postlethwaite and McDonald, 1995; McDonald and Milligan, 1997). Similarly, Hct (the per cent of packed red cell volume) can quickly become elevated following vigorous aerobic exercise (Gallaugher *et al.*, 1992).

Immediately following blood collection in the field, a small amount of the sample was reserved in a capillary tube to determine Hct values using a Readacrit centrifuge (4.5 min at 5900 g; Clay Adams, New York, NY). The

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balance of the blood sample was centrifuged using a Compact II centrifuge (6 min at 1163 g; Clay Adams, New York, NY), and the separated plasma was temporarily stored on dry ice until vials could be transferred to a −80° C freezer for storage until analyses could be run. Blood plasma samples were analysed for ion concentrations of sodium (Na^+), chloride (Cl^-), potassium (K^+), osmolality, lactate and glucose following the methods detailed by Farrell *et al.* (2001).

Several commonly used metrics of anaerobic activity were assessed from muscle samples. White muscle PCr and ATP are both energy sources for anaerobic activity, which are used up within the first 10–15 s of burst activity, whereas glycogen is the primary energy source available to sustain exercise (Kieffer, 2000). Muscle lactate rapidly increases in muscle tissue as anaerobic fuels are converted to ATP during glycogenolysis (Wang *et al.*, 1994a). PCr and ATP concentrations were measured following the enzymatic methods of Lowry and Passonneau (1972) after first grinding the frozen muscle with a mortar and pestle under liquid nitrogen and extracting metabolites according to the procedure described in a study by Suski *et al.* (2003). An additional portion of muscle tissue was used to enzymatically quantify glycogen according to the method of Hassid and Abraham (1957). Muscle water content was quantified by drying tissue samples at 80°C for 48 h and by comparing wet mass with dried mass.

Data analysis

Each group of physiological metrics (i.e. muscle and blood) was compared among the four sampling locations using one-way analyses of variance (ANOVAs). Where data could not be transformed to meet parametric assumptions of normality and homogeneous variances, Wilcoxon–Kruskal–Wallis tests were used instead. The following data transformations were made, plasma lactate by square root, plasma Cl^- by $\log_{10}$, Hct by squared and muscle lactate by $\log_{10}$, to meet normality assumptions, which were assessed with Shapiro–Wilk tests. *Post hoc* pairwise Tukey tests were conducted where ANOVA found significant differences. Significance was assessed at $\alpha = 0.05$ with Bonferroni corrections applied to groupings of analyses (i.e. blood analyses, muscle tissue analyses). As Bonferroni corrections can be highly conservative, both the corrected and uncorrected results are provided (Cabin and Mitchell, 2000). All statistical analyses were conducted using SAS v9.1 (SAS Institute, Cary, NC).

RESULTS

Comparisons of blood plasma samples among sample sites revealed several differences in physiological parameters including plasma lactate, Cl^-, osmolality and Hct (ANOVA, all $p < 0.01$; see Table I). Specific differences in fish physiology between the downstream site and the midpoint of the fishway included significant increases in plasma Cl^- ($p = 0.004$) and osmolality ($p = 0.012$). Both remained elevated through the group sampled upstream of the fishway, with no indication of recovery to pre-passage levels. A similar but non-significant trend in plasma Na^+ was also observed among these sample locations, corroborating an ionic response. Consistent with an anaerobic response, Hct increased significantly from the downstream site to the fishway midpoint ($p = 0.005$), remained at this level through the top of the fishway but then decreased significantly at the post-passage location ($p = 0.011$). Plasma lactate in the post-passage sample group was significantly lower than that observed in fish sampled from the top of the fishway ($p = 0.001$), suggesting that physiological recovery from anaerobiosis was occurring. Notably, we found no differences in blood parameters among the two sites located within the fishway structure.

Among the four sites, muscle samples showed significant differences in available energy resources (ANOVA, glycogen: $p < 0.001$; PCr: $p = 0.008$) and the accumulation of metabolic by-products during fishway ascent (ANOVA, lactate: $p = 0.007$; see Table II). Specifically, muscle glycogen reserves were significantly lower in the fishway midpoint than in the downstream site ($p = 0.002$) and remained at similar levels through the post-passage sample site. Muscle PCr levels decreased between the downstream site and the fishway midpoint ($p = 0.030$), remained depressed through the top of the fishway and then recovered to pre-passage levels by the post-passage group ($p = 0.010$). In contrast to the plasma results, we detected differences in muscle parameters between the two sites within the fishway. Muscle lactate increased between the downstream site and the top of the fishway ($p < 0.001$) and then began to show signs of recovery as levels in the post-passage group were lower ($p = 0.024$). Water content in white muscle was significantly higher in fish sampled at the downstream site fish than in fish sampled at the top of the fishway ($p = 0.008$) and upstream of the fishway ($p = 0.019$). Similarly, water content was higher in fish sampled at the midpoint of the fishway than at the top of the fishway ($p = 0.035$).

DISCUSSION

Comparisons among the four sample sites yielded trends in physiological measures that were consistent with previously reported changes associated with anaerobic exercise (e.g. Milligan and Wood, 1986; Wood, 1991; Wang *et al.*, 1994a). The declines in muscle glycogen and PCr between the downstream baseline site and the fishway midpoint were consistent with anaerobic activity, which is fuelled by white

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Table I. Summary of blood plasma parameters in pink salmon at different sites. *Post hoc* pairwise differences are indicated by letters in parentheses, following individual locations. The following data transformations were made: lactate by square root, Cl^- by $\log_{10}$, Hct by squared

Measure	Sample location	Mean ± SE	*n*	*F* or χ^2 stat.; *p*
Plasma lactate (mmol L^{-1})	Downstream (a)	5.44 ± 1.12	8	4.73
	FW midpoint (a)	5.89 ± 0.49	9	0.008[2]
	FW top (a)	6.44 ± 0.88	11	
	Post-passage (b)	2.84 ± 0.51	8	
Plasma glucose (mmol L^{-1})[1]	Downstream	5.39 ± 0.68	8	1.62
	FW midpoint	4.41 ± 0.10	9	0.656
	FW top	4.65 ± 0.19	11	
	Post-passage	4.38 ± 0.20	8	
Plasma (Na^+) (mmol L^{-1})	Downstream	134.38 ± 2.91	8	2.67
	FW midpoint	142.22 ± 2.29	9	0.064
	FW top	142.77 ± 2.00	11	
	Post-passage	137.81 ± 2.56	8	
Plasma (Cl^-) (mmol L^{-1})	Downstream (a)	123.78 ± 4.56	8	4.65
	FW midpoint (b)	135.31 ± 1.58	9	0.008[2]
	FW top (b)	135.87 ± 1.53	11	
	Post-passage (b)	133.74 ± 2.30	8	
Plasma (K^+) (mmol L^{-1})	Downstream	3.96 ± 0.31	8	1.99
	FW midpoint	4.21 ± 0.44	9	0.136
	FW top	4.05 ± 0.35	10	
	Post-passage	5.48 ± 0.78	8	
Osmolality (mosmol L^{-1})	Downstream (a)	284.25 ± 4.95	8	4.47
	FW midpoint (b)	299.44 ± 3.35	9	0.010[2]
	FW top (b)	302.95 ± 3.59	11	
	Post-passage (b)	301.00 ± 3.89	8	
Hct (%)	Downstream (a)	30.50 ± 0.82	8	5.56
	FW midpoint (b)	38.33 ± 1.55	9	0.004[2,3]
	FW top (b)	37.82 ± 1.89	11	
	Post-passage (a)	30.50 ± 3.09	8	

FW, fishway.
[1]Non-parametric χ^2 test.
[2]*p*-values significant at $\alpha = 0.05$.
[3]Significance following Bonferroni correction ($\alpha = 0.007$).

muscle energy stores (Wood, 1991). PCr levels tend to recover relatively quickly following exercise, whereas ATP and glycogen can take longer (Wang *et al.*, 1994a). This may explain why there were significantly higher values of PCr in fish sampled at the upstream site than in those sampled from within the fishway, but no differences in either glycogen or ATP. Interestingly, ATP levels did not show any change among the four sample sites, suggesting that fish may have previously engaged in exhaustive exercise, possibly while swimming in the tailrace of the dam. Concurrent with energy depletion was an increase in white muscle lactate, a metabolic by-product of exhaustive exercise (Black *et al.*, 1962). Notably, an increase in muscle lactate was observable between the two sampling locations located within the fishway structure. Although muscle water content differed among the same two sites, the range of values were very small (i.e. all four sites were within 3%) and were comparable to those reported by Wang *et al.* (1994a). Thus, it is unlikely that the 5-mmol L^{-1} g^{-1} difference observed in muscle lactate could be simply attributed to the difference in water content, and this was in fact a physiological response to exhaustive exercise.

In contrast to muscle tissue parameters, no changes were observed among blood samples collected from the two sites within the fishway. For example, plasma lactate concentrations did not show a similar increasing trend to mirror those observed in muscle lactate. This is not surprising as peak lactate levels in white muscle tissue can be observed

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Table II. Summary of changes in muscle tissue parameters in pink salmon at different sites. *Post hoc* pairwise differences are indicated by letters in parentheses, following individual locations. Muscle lactate was $\log_{10}$-transformed to meet normality assumptions

Measure	Sample location	Mean ± SE	*n*	*F* stat.; *p*
Muscle ATP ($mmol\,L^{-1}\,g^{-1}$)	Downstream	2.92 ± 0.44	8	0.44
	FW midpoint	3.06 ± 0.38	9	0.725
	FW top	3.66 ± 0.49	11	
	Post-passage	3.37 ± 0.66	8	
Muscle PCr ($mmol\,L^{-1}\,g^{-1}$)	Downstream (a)	14.93 ± 1.58	8	4.71
	FW midpoint (b)	8.10 ± 0.84	9	0.008[1,2]
	FW top (b)	10.55 ± 2.45	11	
	Post-passage (a)	18.39 ± 2.59	8	
Muscle glycogen ($nmol\,mg^{-1}$)	Downstream (a)	23.37 ± 2.36	8	8.51
	FW midpoint (b)	14.37 ± 2.26	9	<0.001[1,2]
	FW top (b)	10.56 ± 1.35	11	
	Post-passage (b)	13.08 ± 1.37	8	
Muscle lactate ($mmol\,L^{-1}\,g^{-1}$)	Downstream (a)	11.63 ± 1.59	8	4.87
	FW midpoint (a)	14.80 ± 1.62	9	0.007[1,2]
	FW top (b)	19.96 ± 1.77	11	
	Post-passage (a)	14.59 ± 1.26	8	
Muscle water content (%)	Downstream (a)	81.40 ± 0.75	8	3.83
	FW midpoint (a)	80.72 ± 0.71	9	0.018[1]
	FW top (b)	78.62 ± 0.64	11	
	Post-passage (b)	78.78 ± 0.75	8	

FW, fishway.
[1]*p*-values significant at $\alpha = 0.05$.
[2]Significance following Bonferroni correction ($\alpha = 0.01$).

immediately following exercise, whereas peak plasma lactate may not occur until several hours after exercise (Wood, 1991). When plasma samples were compared among all four sites however, there was clear evidence of an exercise-related physiological response. Lactate concentrations were significantly lower in fish sampled upstream of the fishway than in any of the other sample sites, with values similar to those of resting fish reported elsewhere (i.e. under $2\,mmol\,L^{-1}$ in salmonids; Milligan, 1996; Wagner *et al.*, 2006). That plasma lactate levels were elevated in fish downstream of the fishway further suggested that fish may have engaged in exhaustive exercise in the tailrace of the dam prior to entering the fishway.

Plasma ion concentrations taken from fish within the fishway were elevated relative to those in fish sampled at the site downstream of the fishway. This observation was consistent with previously documented responses to anaerobic exercise (e.g. Wood *et al.*, 1983; Farrell *et al.*, 2000) and can be explained by transcellular osmotic imbalances associated with elevated levels of muscle lactate (Milligan and Wood, 1986). Ion measures remained elevated at the upstream site, suggesting that fish sampled here were still in the process of recovery as ion concentrations may remain elevated for several hours following anaerobic activity (McDonald and Milligan, 1997). Haematocrit values in pink salmon ascending the fishway were elevated relative to those in fish downstream and upstream of the fishway and were similar to those previously reported for pinks captured from the hydropower tailrace located just downstream of the Seton–Fraser confluence (i.e. 37%; Williams *et al.*, 1986), a location characterized by high water velocities and complex flow patterns. Wang *et al.* (1994a) reported a similar increase in Hct following 6 min of exhaustive exercise, which returned halfway to baseline values after 4 h of recovery. Taken together, the physiological response of pink salmon to fishway ascent, as assessed from both blood and muscle tissue samples, strongly suggested that anaerobic efforts were required in order for fish to successfully pass through.

Whereas both muscle and blood samples are capable of providing relevant and accurate biological information on physiological responses to exercise, they differ in terms of the magnitude and the timing of these responses. Based on the findings of the present study, muscle tissue appeared to be a more sensitive indicator of physiological changes than blood and thus may be a more effective metric in fishway assessments, particularly where passage occurs in a relatively brief period. At the Seton dam, a previous

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radio-tagging study found that pink salmon could successfully pass through the fishway in as little as 31 min (Pon *et al.*, 2006), which may partially explain why no differences were observed among plasma samples between the fishway midpoint and the top pool of the fishway. However, for longer ascent periods, blood samples may be suitable for detecting changes in fish condition and should not be ignored as a useful measure. For example, Chinook salmon (*Oncorhynchus tshawytscha*) spent several hours passing through the Bonneville Dam fishway on the Columbia River (Brown *et al.*, 2006), an adequate period in which plasma variables may be expected to show detectable changes. Plasma sampling may also be useful in cases where baseline physiological profiles can be established at sites downstream of the fishway. There are also several methodological considerations that should be taken into account when weighing the advantages and disadvantages of each sample type. For one, it is important to consider logistical constraints associated with the fish capture method. To obtain a representative sample, it is important to minimize stressors associated with capture and handling, which may impart additional changes to the fish's physiology (Mazeaud *et al.*, 1977; Wang *et al.*, 1994b). Although relevant for both plasma and muscle tissue, the comparatively rapid response of muscle energy levels and metabolites to additional physiological disturbances may become a methodological hindrance to collecting accurate data if fish cannot be sampled and tissue stored in an expedient manner. Blood sampling, though certainly time dependant, may be the preferred method if more accurate estimates of physiological condition are required in cases where fish cannot be immediately sampled after initial capture stressors are applied.

Although fish were terminally sampled in the present study (because of collections of tissues unrelated to the present research), non-lethal sampling of both blood and muscle tissue is often desired. Live blood samples, with the use of the same procedure as described here, have been demonstrated to have no discernable impact on the migration success of sockeye salmon captured at sea (Cooke *et al.*, 2005). Similarly, white muscle samples can be collected in a non-lethal manner by using needles or dermal punches (Wang *et al.*, 1994b; Tyus *et al.*, 1999), although very rapid sampling would be needed to minimize handling impacts on physiological response variables. For non-lethal sampling protocols, it is important to consider the invasiveness of the sampling method. Muscle plugs collected by using a dermal punch would leave larger wounds than by using blood collection needles, and therefore post-sampling infection should be considered. These and other site-specific issues should be taken into consideration in studies intending to assess physiological impacts of fishway passage.

It is also important to assess multiple species that use the same fishway, as variations in swim performance and behaviour may influence passage success (Castro-Santos *et al.*, 2008). A separate study conducted on sockeye salmon at the Seton dam measured the same variables in the plasma of fish at the top of the fishway (i.e. FW_{top}) and concluded that fishway passage was not a particularly stressful event for sockeye salmon (Pon *et al.*, 2009). The fact that pink salmon in the present study clearly showed a physiological response consistent with anaerobic exercise highlights the variability among species in terms of swimming performance. Though pink salmon have previously been found to have similar swim speeds and energy use rates as sockeye salmon, differences in swimming behaviour and strategies have been reported (Hinch *et al.*, 2002; Standen *et al.*, 2002), and pink salmon have previously been described as poorer swimmers than sockeye salmon (Williams and Brett, 1987; Heard, 1991). However, this observation may stem from an apparent aversion among pink salmon to exceed critical swim speeds (U_{crit}) during upriver migration (Hinch *et al.*, 2002) and the minimization of anaerobic efforts because of the fact that locomotory costs are relatively high for this species (MacNutt *et al.*, 2006). These types of inter-specific differences in swimming performance highlight the importance of assessing the physiological impact of passage on all species that can potentially use the fishway, as passage assumptions based on assessments of stronger species may not apply to weaker ones.

Physiological tools are increasingly being used in hydropower assessments (Hasler *et al.*, 2009) and more generally to provide information on organismal health and condition to inform conservation and management initiatives (i.e. conservation physiology; Wikelski and Cooke, 2006; Young *et al.*, 2006). A single sample site assessment using either tissue type assessed in the present study can provide a snapshot of the physiological condition of fish following passage but may prove difficult to interpret without additional baseline information. Ideally, several sample sites (e.g. bottom and top of fishway) should be used to identify the magnitude of the physiological disturbance specifically associated with passage and to separate background 'noise'. Moreover, sampling at various sites within a fishway could help to identify areas of passage difficulty. The physiological assessments that we suggest here would also benefit from being done in parallel to field telemetry studies such that physiological findings can be put in the context of fish behaviour. Other tools, such as EMG telemetry may offer advantages such as continuous data, but there may be other costs or logistical restrictions that must be considered in their use. Ultimately, the investigative approach or combination of approaches depends on the question that is being addressed by the researchers.

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If fishways are to be constructed following guidelines associated with minimizing the physiological impact of passage on fish as advocated elsewhere (e.g. Powers *et al.*, 1985; Bunt, 2001; Schilt, 2007; Castro-Santos *et al.*, 2008), then there is a clear need for a means to assess this impact. This is particularly true as many fishway assessments have only been conducted in terms of a simple measure of passage success or efficiency (Roscoe and Hinch, 2010). Biopsy techniques using either blood or muscle tissue have hereby been demonstrated as a useful tool in providing data to assess physical effort associated with fishway passage and thus may have applications in fish management and conservation efforts. The results of the present study refine our knowledge of the potential use of blood and muscle biopsy to understand the immediate physiological consequences of fish passage and to identify areas of passage difficulty within fishways. Future studies may be able to expand on these findings by investigating the longer-term consequences of fishway passage in terms of the survival or reproductive fate of fish following passage.

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