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Physiological comparisons of steelhead kelts emigrating from the Situk River, AK and Clearwater River, ID

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Abstract The physiological status of migrating steelhead kelts (*Oncorhynchus mykiss*) from the Situk River, Alaska, and two tributaries of the Clearwater River, Idaho, was evaluated to explore potential differences in post-spawning survival related to energy reserves. Blood plasma samples were analyzed for metrics related to nutritional and osmotic status, and samples of white muscle tissue collected from recent mortalities at weirs were analyzed for proximate constituents. Female kelts from the Situk River had significantly higher plasma cholesterol, triglycerides, glucose and calcium concentrations, all of which suggested higher lipid and energy stores. Additional support for energy limitation in kelts was provided by evaluating the presence of detectable proteins in the plasma. Most all kelts sampled from the Situk River populations had detectable plasma proteins, in contrast to kelts sampled from the Clearwater River tributary populations where 27 % of kelts from one tributary, and 68 % of the second tributary were below the limits of detection. We found proximate constituents of kelt mortalities were similar between the Situk and Clearwater River populations, and the lipid fraction of white muscle averaged 0.1 and 0.2 %. Our findings lend support to the hypothesis that energetic limitations likely affect post-spawn survival in the Clearwater River kelts.

Keywords Steelhead · Kelt · Iteroparity · Plasma profiles · Repeat spawning · Physiology

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Introduction

Although all steelhead trout (*Oncorhynchus mykiss*) are iteroparous, the repeat spawning rates between and within populations are highly variable (<1 to >70 %) (Busby et al. 1996; Lohr and Bryant 1999). Busby et al. (1996) reviewed the life history characteristics of several steelhead populations along a latitudinal cline on the West coast of North America, and suggested that repeat-spawning rates decreased northward with California, Oregon, Washington, and British Columbia stocks exhibiting average repeat-spawning rates of 19 %, 15 %, 7 %, and 8 %, respectively. Busby et al. (1996) did not report repeat-spawning rates for inland populations of Snake River steelhead returning to tributaries in Idaho, Washington, and Oregon, where repeat-spawning rates are generally <2.0 % (Burgner et al. 1992). However, Lohr and Bryant (1999) observed

contrasting patterns as they documented repeat-spawning rates in Southeast Alaska systems, such as Peterson Creek, > 50 %.

Steelhead trout begin fasting during spawning migrations and rely on stored somatic and visceral energies (lipid and protein) to support upstream migration, gonadal maturation, redd construction, competition for mates, and post-spawn emigration as kelts (Hendry and Berg 1999). Quinn and Myers (2004) remarked that post-spawning mortality in anadromous salmonids was "presumably" related to the combined stresses of migration and rigors of spawning. In anadromous American Shad (*Alosa sapidissima*), Glebe and Leggett (1981) concluded that "interpopulation differences in energy allocation to migration versus reproduction" were a determining factor affecting repeat-spawning. Their hypothesis likely applies to steelhead that display differences in repeat-spawning by geographic group (e.g., coastal vs. inland) and maturation strategy (e.g., ocean vs. stream maturation. For iteroparous anadromous fish, the stress of migration occurs in upstream and downstream migrations, although freshwater residualism of kelts can occur, thus reducing the extent of post spawning migration (Null et al. 2013). While Busby et al. (1996) did discriminate between ocean- and stream-maturing populations of steelhead, they did not address the effect of variables such as migration distance, river conditions, fish size, and/or anthropogenic modification of the riverine habitat may have on successful repeat spawning.

Several authors hypothesize that because stream-maturing populations tend to migrate farther upstream and spend longer durations in freshwater, they are more susceptible to post-spawn mortality due to energetic exhaustion (Burgner et al. 1992; Brannon et al. 2004; Quinn and Myers 2004). Penney and Moffitt (2014a) hypothesized that inadequate energy reserves in Snake River steelhead kelts constrained their ability to repeat-spawn, especially with added downstream delays caused by the Federal Columbia River Power System (FCRPS). Although this hypothesis seems intuitive, no research has compared the physiological or energetic status of different steelhead populations with their estimated rates of iteroparity.

Blood plasma chemistry provides a suite of information on the physiological status of fish such as, nutrition (Patton et al. 1970; Congleton and Wagner 2006), stress (Woodward and Strange 1987; Barton et al. 1988; Raby et al. 2013), tissue damage (Wagner and Congleton 2004), metabolism (Simpkins et al. 2003), osmoregulation (Hasler et al. 2011; Buelow and Moffitt 2014), and exposure to pollutants (Shahsavani et al. 2010).

In this study, we compare biochemical metrics of blood plasma between female kelts migrating downstream from the Situk River, Alaska, with kelts from the Clearwater River, Idaho, a tributary to the Snake/Columbia River systems (Fig. 1). The Situk River is short river system that has stream-maturing and ocean-maturing steelhead populations with repeat spawning rates averaging 9 % (Marston et al. 2012) and habitat with little anthropogenic modification. The Clearwater River hosts stream-maturing populations, and the Columbia River watershed is a highly altered inland system with low rates of iteroparity (< 2 %) (Keefer et al. 2008; Bowersox et al. 2011; Copeland et al. 2013). We focused our sampling efforts on good condition female kelts due to the predominance of female kelts, a trait observed in steelhead and Atlantic salmon (Burgner et al. 1992; Fleming 1998; Quinn and Myers 2004). We hypothesized that nutritional and energy-related factors would be higher in Situk River kelts, and analytes related to stress and tissue damage would be higher in Clearwater River kelts due to a longer migration and time fasting in freshwater. We also hypothesized that white muscle lipid, protein, and total energy would be similar between Situk and Clearwater River kelts at death, regardless of location.

Methods

Study sites and population characteristics

Kelts from the Clearwater River, were sampled between March and June of spawning year 2010 (year of spawning) in two tributary systems: (1) Potlatch River (Lower Clearwater; ~ 838 rkm from ocean), and (2) Fish Creek, a tributary of the Lochsa River (Upper Clearwater; ~ 977 rkm from ocean). In the Potlatch River, kelts were intercepted at four weir sites which included Little Bear weir (46°38'N, −116°40'W), Big Bear weir (46°37'N, −116°39'W), West Fork weir (46°48'N, −116°25'W), and East Fork weir (46°47'N, −116°25'W). All kelts from Fish Creek were sampled from one weir (46°20'N, −115°21'W), (Fig. 1). Average daily water temperatures during blood sampling at the Potlatch River and Fish Creek ranged between 4 and 16 °C and 7–15 °C, respectively. Kelts were sampled from box traps, or occasionally with dip-nets above the

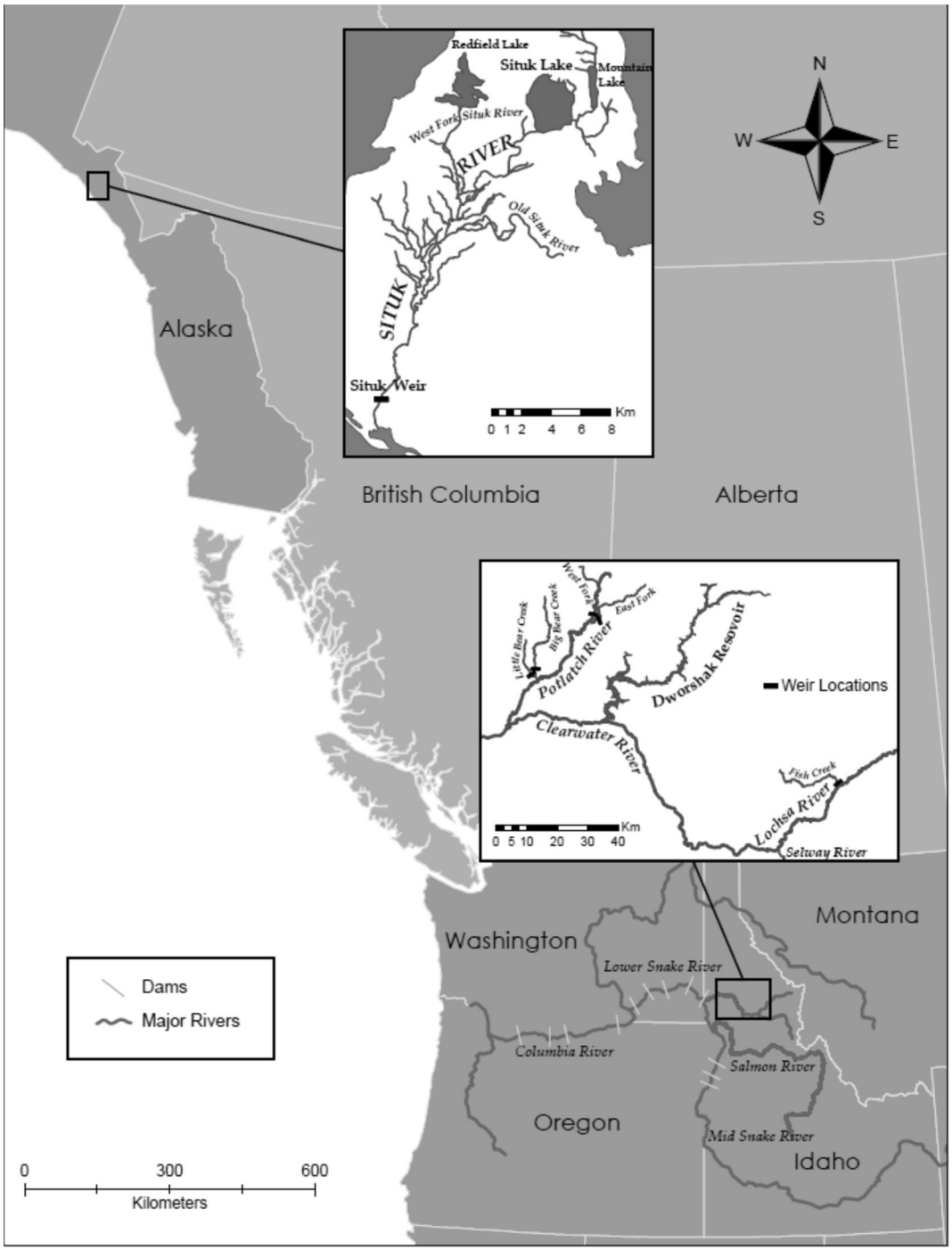

Fig. 1 Map of sample sites

weir. In total, 47 good condition female kelts were sampled from the Potlatch River and 22 good condition female kelts were sampled from Fish Creek.

Kelts emigrating out of the Situk River were presumed to be both stream- and ocean-maturing stocks. Sampling occurred during May of spawning year 2011 at a weir (59°27'N, −139°34'W), ~ 2 rkm from the ocean (Fig. 1). The average daily water temperature at the Situk River weir during blood sampling was 9 °C. All collections of emigrating kelts were from the box

trap. In total, 24 good condition female kelts were sampled from the Situk River.

Blood plasma and white muscle tissue sampling

Steelhead were measured for fork length, examined for sex and condition to validate post-spawn phase (Fig. 2). A sample of blood was removed from the caudal vessel using a heparinized 3 mL syringe, fitted with a 21 gauge-3 cm needle. Blood samples were stored on ice for periods <1 h until centrifugation to separate the plasma fraction. The plasma fraction was transferred to a polypropylene screw cap tube and stored in dry ice until transfer to a − 80 °C freezer.

Kelts from the Clearwater River weirs were sedated with 100 mg/L tricaine methanesulfonate (MS 222, Argent Laboratories, Redmond, WA) buffered with 200 mg/L sodium bicarbonate. Sedated kelts were held briefly in a recovery area and then released to continue emigration downstream. Due to potential capture in a local subsistence fishery, kelts sampled at the Situk River were not sedated but immobilized on a sampling board by field technicians.

The body condition of each fish captured was rated as good, fair, or poor using the criteria defined by Penney and Moffitt (2014b). Only samples from good condition natural-origin (adipose fin intact) kelts were used in analysis to reduce bias related to physiological differences in condition (see Penney and Moffitt 2014b; Buelow and Moffitt 2014). In addition, statistical comparisons were made using only female kelts to minimize variation due to low samples of male kelts at all sites.

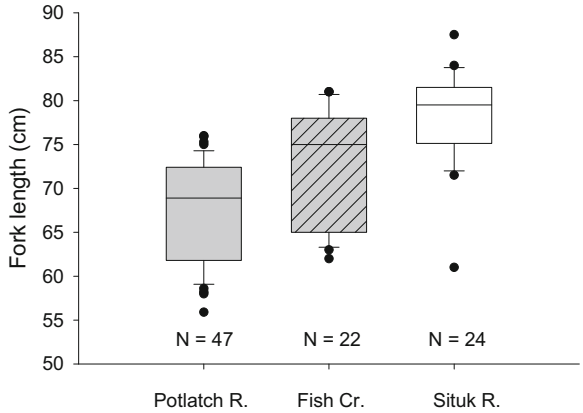

Fig. 2 Boxplot of kelt fork length at sample locations

At weirs on the Potlatch River and Situk River, a muscle fillet (skin on) was removed from a location posterior to the insertion of the dorsal fin from select kelts mortalities (< 6 h old) that were identified by field crews stationed on site. Fillets were weighed (0.05 g), placed in plastic bags on ice, and frozen (−20 °C) until processing.

Laboratory analysis

The plasma samples were analyzed for a suite of biochemical parameters by autoanalyzers (dimension AR-IMT, Dade Behring Inc. Newark, Delaware) at Gritman Medical Center, Moscow, Idaho; and (Beckman Coulter CX5 and 114 Olympus Au400e, Beckman Coulter Inc. Brea, California) at Panhandle Animal Lab, Coeur d' Alene, Idaho. Quality assurance followed guidelines established by the Joint Commission for Accreditation of Health Care Organizations or by the College of American Pathologists, Veterinary Laboratory Association, respectively. To serve as a control, we analyzed a pooled sample of plasma along with samples to assess precision and accuracy of each metric by the autoanalyzers. We omitted reporting of any biochemical metrics with control CV > 15 %.

The following 14 analytes were reported for plasma samples: cholesterol (mg dL^{-1}), triglycerides (mg dL^{-1}), total protein (g dL^{-1}), glucose (mg dl^{-1}), calcium (mg dl^{-1}), sodium (mmol L^{-1}), chloride (mmol L^{-1}), magnesium (mg dL^{-1}), potassium (mmol L^{-1}), phosphorus (mg dl^{-1}), alkaline phosphatase (ALP; IU L^{-1}), aspartate aminotransferase (AST; IU L^{-1}), alanine aminotransferase (ALT; IU L^{-1}), and lactate dehydrogenase (LDH; IU L^{-1}).

We determined proximate constituents and total energy content of samples of muscle tissue from mortalities, (% moisture, %lipid, % ash, % protein, kJ g^{-1}) following the procedures of Penney and Moffitt (2014a). Proximate constituents and energy density were expressed as a proportion (%) of equivalent wet tissue weight using the same calculation described by Hendry et al. (2000). All methods were performed in accordance to the standard operating procedures outlined by the Association of Official Analytical Chemists (AOAC 2000).

Statistical analysis

Descriptive statistics were used to summarize the plasma analyte concentrations across sample sites, and the

proximate constituents of mortalities. For analytes with a proportion of samples that were below the limits of detection (potassium and total protein), we used exact Chi-square analysis and Monte Carlo approximations (20,000 permutations) to compare the frequency of samples above and below the limits of detection. To compare the 12 analytes that were consistently detected (cholesterol, triglycerides, glucose, calcium, sodium, chloride, magnesium, phosphorus, ALP, AST, ALT, and LDH) across the sample sites, we used a combination of statistical tests. We modeled variation across sampling sites for multiple dependent variables with MANOVA and Wilks-lambda statistics and log transformed dependent variables to improve normality with the following general linear framework: $y_{i1} \ldots y_{i12} = \mu + \alpha_i + \varepsilon_i$, where $y_{i1} - y_{i12}$ refers to the set of response variables (12 plasma analytes with detectable measures), μ is the overall mean, α_i is sample site (Potlatch River, Fish Creek, Situk River), and ε_j is random error. After determining significant differences across multiple analytes, we assessed individual analytes across sample sites with the ANCOVA model: $y_{ij} = \mu + \alpha_i + X_{ij} + \varepsilon_{ij}$, where y_i refers to the response variable (plasma analyte), μ is the overall mean, α_i is sample site (Potlatch River, Fish Creek, Situk River), X_{ij} is the covariate fork length, and ε_j is random error. Pairwise single degree of freedom comparisons of least squared means were examined to discern variation between sample sites. All data analysis was performed using SAS 9.2 (SAS Institute, Cary, North Carolina) and tests were considered significant at $\alpha = 0.05$, but we reported differences when $\alpha < 0.10$.

Results

Of the 12 analytes that were consistently detected we determined significant variation across sample sites (MANOVA Wilks'$\lambda = 0.04$; $F_{26,\ 138} = 20.7$, $P < 0.001$). Sample location was significant for 10 of the individual variables analyzed separately (ANCOVA; all $P \leq 0.03$). Individual analyses of cholesterol, triglycerides, calcium, and glucose were significantly higher in Situk River kelts compared to Clearwater River kelts (Fig. 3), indicating higher nutritional status. Electrolytes sodium and chloride were variable across sample sites and did not exhibit any clear separation between Situk River and Clearwater River kelts. Fish Creek kelts had significantly lower sodium and chloride concentrations

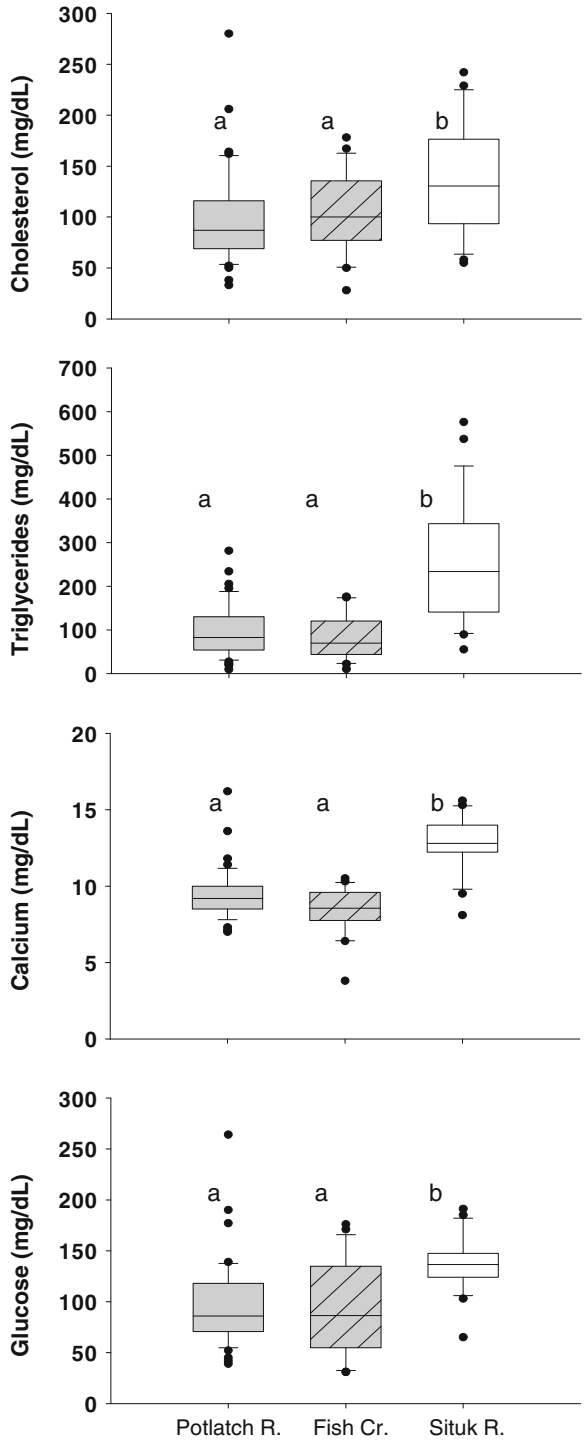

Fig. 3 Box plot summary of nutritional analytes in plasma samples from steelhead kelts from three locations. Plots with the same letters (*a*, *b*, *c*) were not significantly different in pairwise tests of least squared means

than the other two sites (ANCOVA; $P < 0.001$). We detected no significant differences between sodium and chloride in the Potlatch and Situk River kelts. Plasma magnesium was significantly lower in kelts from the Situk River than those from the Clearwater River. We found no differences in the phosphorous levels in plasma across all sites (Fig. 4).

Total plasma protein concentrations in kelts showed significantly different patterns across sampling locations. The majority (68 %) of kelts from the Potlatch River had plasma protein levels below the detection limits of our analysis (2.5 g dL^{-1}). Only 27 % of samples from Fish Creek were below detection, and only 8 % of plasma samples from the Situk River kelts were below detection (Table 1). The proportions below detection were significantly different between Potlatch River kelts compared with Fish Creek or Situk River kelts, however the proportional comparisons between Fish Creek and Situk River were not significantly different.

Plasma enzymes ALT and AST were elevated in kelts from the Situk River. Concentrations of ALT were significantly higher in the Situk River over those from the Clearwater River kelts (ANOVA; $P < 0.001$). We found AST was highest in samples from the Situk River, and lowest in the Potlatch River kelts (Fig. 5). Although the fork lengths were significantly different across sites (Fig. 2), AST was the only plasma variable that showed significant covariance with fork length (ANCOVA; df = 2; F = 8.18; $P = 0.005$). Measures of AST appeared to increase with fork length, but these also appeared to be associated with length of kelts in the Potlatch and Situk Rivers ($P = 0.08$) (Fig. 5). Plasma LDH was lowest for the Situk River kelts, but was different across all sites (all ANOVA $P < 0.01$). The concentrations of ALP were significantly higher for kelts from Fish Creek over the two other sites (ANOVA all $P < 0.03$; Fig. 5).

Plasma potassium concentrations in kelts in the Situk River were lower than for kelts from the Clearwater River. In the Situk River, 46 % of samples were below limits of detection, compared with samples from Fish Creek kelts (32 %), and the Potlatch River (17 %). The proportion below detection between the Situk River versus the Potlatch River kelts was significant ($\chi 2 = 6.49$; $P = 0.013$) (Table 1).

The proximate constituents of white muscle tissues from mortalities were similar between the Situk and Clearwater Rivers. Median white muscle lipid content and energy density from the Potlatch River were =0.2 % and 3.8 kJ/g, respectively. The muscle lipid and energy density of mortalities from the Situk River were 0.1 % and 4.0 kJ/g respectively (Table 2).

Discussion

The steelhead we studied were from river systems with very different characteristics. The Clearwater River is the largest tributary to the Snake River, and the Snake River is the largest tributary in the Columbia River system. Historically, the Columbia River and its tributaries were considered to be the center of steelhead abundance in the northeast Pacific Ocean (Brannon et al. 2004). Steelhead returning to the Clearwater River must navigate eight hydroelectric dams in an altered ecosystem and swim more than 700 km in prespawn and post spawn migrations (Leonard et al. 2015). In contrast, the Situk River contains no substantial migration barriers or anthropogenic habitat alterations but is only 35.2 km long (Marston et al. 2012).

Our study provides evidence that higher energy stores exist in steelhead emigrating downstream from the Situk River over those from the Clearwater River system, and supports our hypothesis that migration distance and duration of freshwater-residence (fasting) can be reflected in blood plasma chemistry. We were not able to differentiate between ocean maturing and stream-maturing stocks sampled in the Situk River, but all samples from Situk River kelts showed more elevated energy stores. Cholesterol is considered to be the most important simple lipid, which can exist unesterified in cellular membranes or in neutral lipid (Tocher 2003). Likewise, triglycerides are neutral lipids comprised of glycerol triacylglycerols that provide the primary lipid-derived energy in salmonids (Jobling et al. 1998). The presence of circulating cholesterol and triglycerides in blood plasma of steelhead kelts indicates that lipid stores are being mobilized for energy during downstream migration. Depletions of plasma cholesterol and triglycerides over the freshwater re-entry, spawning, and post-spawning senescence have also been reported in fasting semelparous Pacific salmon (Hutton 1967; Patton et al. 1970; Ballantyne et al. 1996; Kiessling et al. 2004). Given that plasma cholesterol and triglycerides are reflective of lipid storage and metabolism, we deduced that Situk River kelts likely began emigration with higher levels of

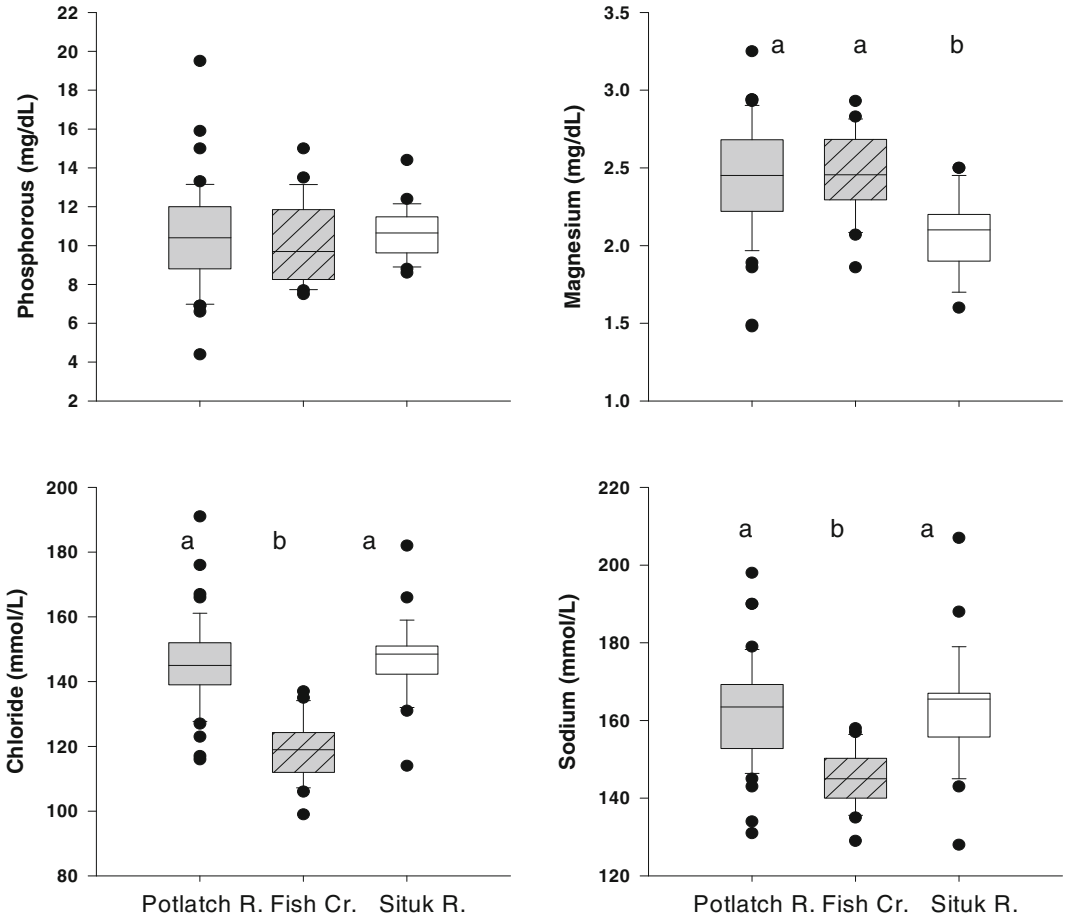

Fig. 4 Box plot summary of plasma electrolytes in samples from steelhead kelts from three locations. Plots with the same letters (*a*, *b*, *c*) were not significantly different in pairwise tests of least squared means

lipid than Clearwater River kelts, thus appear more energetically fit to complete emigration for recovery.

We found evidence that elevated plasma glucose was likely also a nutritional factor in contrast to observations of migrating juvenile salmonid smolts where glucose has been attributed to stress (Wagner and Congleton 2004). Similar evidence supporting glucose as a nutritional factor in steelhead kelts was reported by Buelow and Moffitt (2014) who found plasma glucose was higher in good condition kelts over poor condition kelts. Penney and Moffitt (2014b) also documented glycogen stores (vacuolization) were evident in livers of spawning

Table 1 Summary of number of plasma samples below detection by sample location. Sample sizes, likelihood ratio chi-square values and permuted *P* values are provided for single degree of comparisons between locations

	Number of samples below detection			Likelihood ratio Chi-Square and permuted *P*-value		
Plasma metric	Potlatch River $N = 47$	Fish Creek $N = 22$	Situk River $N = 24$	Potlatch River vs Fish Creek	Potlatch River vs Situk River	Fish Creek vs Situk River
Protein (g L^{-1})	32	6	2	11.44	27.39	2.96
				(0.002)	(< 0.001)	(0.128)
Potassium (mmol $L^{-1)}$	8	7	11	1.85	6.49	0.95
				(0.216)	(0.013)	(0.379)

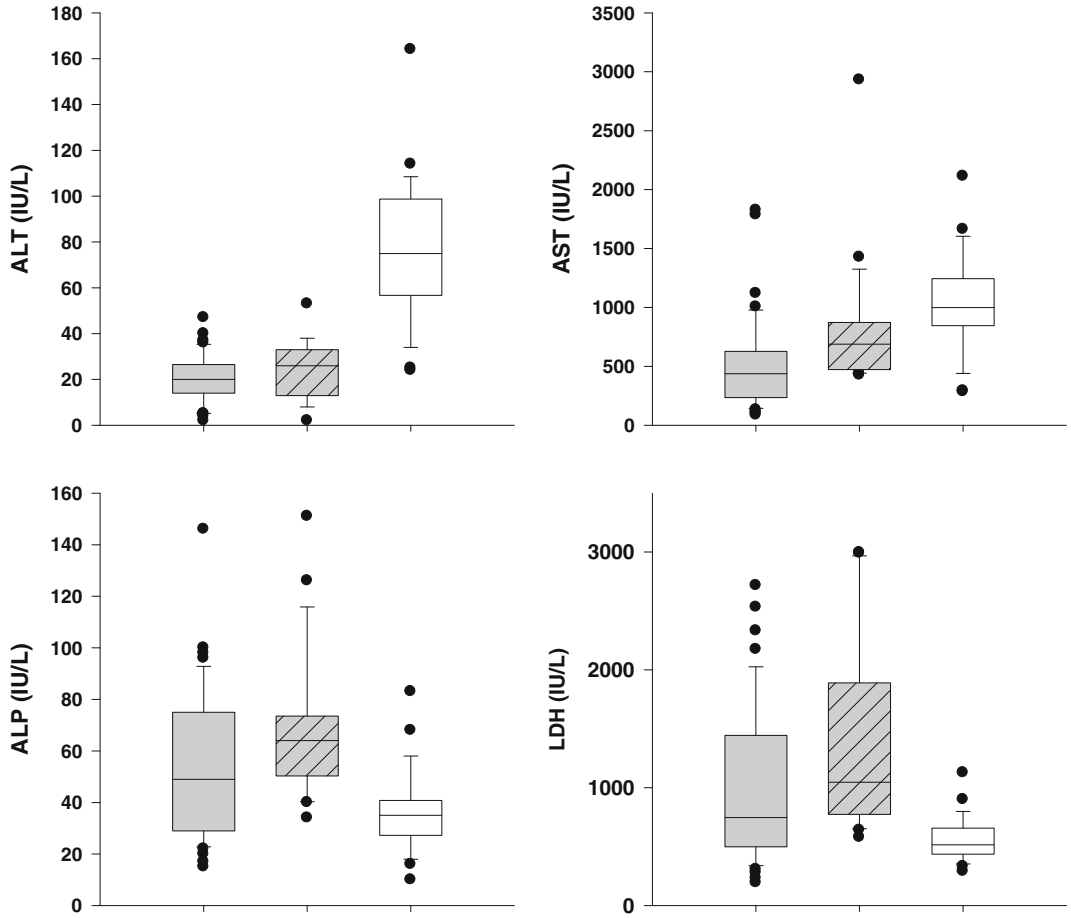

Fig. 5 Box plot summary of selected plasma enzymes in samples from steelhead kelts from three locations. Plots with the same letters (*a*, *b*, *c*) were not significantly different in pairwise tests of least squared means

steelhead of the Snake River sub basin, but were absent in migrating kelts. Future work using factor analysis to examine correlation structure among plasma analytes would be beneficial in determining if plasma glucose is more closely aligned with nutritional or stress factors in steelhead kelts.

Proteins represent the last available source of energy for kelts that have exhausted their lipid reserves. Total plasma proteins were below detection in 68 % of samples from Potlatch River kelts and 28 % of Fish Creek kelts, whereas only 8 % of Situk River kelts had plasma protein levels below detection. Plasma protein decreases during periods of prolonged fasting as somatic protein is used for energy (Triplett and Calaprice 1974; Fletcher et al. 1975; Congleton and Wagner 2006). However, unlike lipid depletion, the severe catabolism of protein from muscle tissues can degrade swimming ability (Hendry et al. 2000) and lead to starvation unless

Table 2 Summary of white muscle lipid, protein, and energy density in kelt mortalities from the Potlatch and Situk Rivers. The samples from Potlatch River were composed of 3 females and 2 males. All samples from the Situk River were from males

Site	N		Lipid (%)	Protein (%)	Energy density (kJ g^{-1})
Potlatch River	5	Median	0.2	16.3	3.8
		Range	0.1–0.5	15.1–18.4	3.6–4.2
Situk River	11	Median	0.1	17.1	4.0
		Range	0.1–0.3	16.1–19.9	3.7–4.4

feeding resumes (Belding 1934; Penney and Moffitt 2014a). Penney and Moffitt (2014b) hypothesized that low repeat-spawning rates in inland stream-maturing Snake River kelts were likely limited, due to their reliance on somatic proteins to support emigration. Our data from plasma protein in Clearwater River kelts supports this hypothesis, and suggests that kelts exhibiting plasma protein levels below detection may be nearing energetic exhaustion.

Penney and Moffitt (2014a) reported that white muscle protein in emigrating inland stream-maturing Snake River steelhead kelts was positively correlated to fork length. Fork lengths of kelts sampled in the Potlatch River were significantly smaller than kelts sampled in Fish Creek or the Situk River (Fig. 2). It is possible that lower somatic protein in smaller kelts corresponds to low plasma protein; however, greater fork length in Atlantic salmon (*Salmo salar*) has generally been associated with a lower likelihood of repeat-spawning (Fleming 1998). Further comparisons between plasma protein and fork length, especially small steelhead kelts (< 70 cm) from coastal and inland populations are needed to further evaluate this relationship.

Our study supports that plasma calcium was also likely a nutritional factor as it was elevated in Situk River kelts. Studies of migrating brown trout (Boel et al. 2014) and juvenile salmon (Wagner and Congleton 2004) support plasma calcium as a nutritional factor. Approximately one-half of plasma calcium is bound to protein (Andreasen 1985) and a higher proportion of Situk kelts had detectable plasma protein. Calcium also binds to vitellogenin during gonadal maturation, and Nagler et al. (2012) reported that plasma vitellogenin levels remained elevated in female rainbow trout even after the completion of spawning.

In contrast to calcium, we found few clear differences in plasma sodium, chloride, magnesium, potassium, and phosphorus concentrations between Situk and Clearwater kelts. Kelts sampled at Fish Creek showed lower plasma sodium and chloride indicative of osmotic stress, but plasma sodium and chloride concentrations of Potlatch River and Situk River kelts were within the normal range expected for migrating kelts or smolts (e.g., Kennedy et al. 2007; Hanson et al. 2011; Hayes et al. 2012; Buelow and Moffitt 2014). In contrast to plasma electrolytes, the amylase, LDH, and ALP levels were significantly lower in Situk River kelts compared to Clearwater River kelts. Akinrotimi et al. (2013) found elevated levels of these enzymes in brood African sharptooth catfish *Clarias gariepinus* exposed to metomidate. We used tricane methanesulfonate anesthetic in sampling fish from the Clearwater River, but no anesthetic was used in the Situk River. The lower ALP levels are curious, as studies of Chinook salmon smolts by Wagner and Congleton (2004) linked ALP to nutritional parameters. ALP is a cell-membrane-associated glycoprotein found in all tissues and believed to be important to the movement of ions and water across cell membranes (Congleton and Wagner 2006). Penney and Moffitt (2014c) reported that many polyunsaturated fatty acids important to the structural membranes (C22:6n3) of cells were catabolized in Snake River steelhead kelts.

Interpretation of these trends is limited because most previous studies have been conducted on fed not fasting fish (Lemaire et al. 1991; Wagner and Congleton 2004; Šegvić-Bubić et al. 2013). It is possible that the leakage of specific enzymes follows different patterns due to the physiological and energetic stress of prolonged fasting. Situk River steelhead exhibit both stream- and ocean-maturing strategies, and we could not differentiate fish at sampling. Even so, Kadri et al. (1995) found anadromous salmonids may begin fasting before re-entering freshwater. Water temperatures could have affected the enzyme profiles, as activity generally increases with temperature to some maximum and then is sustained or declines (Lahnsteiner and Mansur 2012) depending on the physiological phase of a fish (e.g. sexual maturation versus post-spawn). Future examinations of plasma enzymes throughout migration and fasting in steelhead will be needed to answer this question.

We found surprising similarity in the proximate composition and energy density of white muscle tissues collected from mortalities in the Situk River and Clearwater River. Lipid content of white muscle was effectively exhausted (<1.0 %) in all samples. Penney and Moffitt (2014a), reported that lipid and protein content of poor condition kelts from mixed stocks migrating downstream in the Snake River (Columbia River system) ranged from (0.1–0.9 % lipid and 13.6–14.8 % protein), values similar to dead post-spawning semelparous Pacific salmon (Hendry and Berg 1999; Mesa and Magie 2006). These direct measures of muscle composition are, to our knowledge, the only data currently available for naturally spawning steelhead trout.

Conclusion and management implications

Our comparisons of Situk River and Clearwater River kelts provide evidence that Situk River kelts are nutritionally and energetically more robust than Clearwater River kelts during early emigration from spawning tributaries. Considering the difference in migration distance and difficulty between steelhead migrating in the Situk and Clearwater River, it seems apparent that Situk River steelhead would be more likely to return to the ocean and recover to spawn again. However, future research evaluating the energetic investment and potential for repeat-spawning between multiple populations of stream- and ocean maturing steelhead is warranted. Increasing steelhead iteroparity via natural or artificial means is a potential option for improving or conserving threatened and endangered populations (Brannon et al. 2004; Gephard and McMenemy 2004; Hatch et al. 2013). By improving our understanding of the physiological constraints on steelhead iteroparity, especially from different geographic and reproductive strategy perspectives, we will be better able to apply best management practices for this phase of steelhead life history.

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