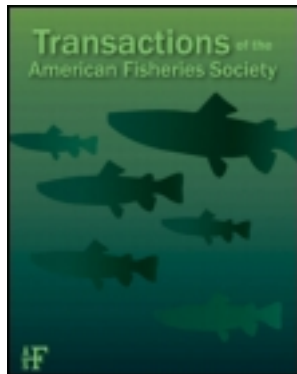


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Proximate Composition and Energy Density of Stream-Maturing Adult Steelhead during Upstream Migration, Sexual Maturity, and Kelt Emigration

Zachary L. Penney^a & Christine M. Moffitt^{b a}

^a Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Drive, Mail Stop 1141, Moscow, Idaho, 83844, USA

^b U.S. Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Drive, Mail Stop 1141, Moscow, Idaho, 83844, USA

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ARTICLE

Proximate Composition and Energy Density of Stream-Maturing Adult Steelhead during Upstream Migration, Sexual Maturity, and Kelt Emigration

Zachary L. Penney

Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Drive, Mail Stop 1141, Moscow, Idaho 83844, USA

Christine M. Moffitt*

U.S. Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Drive, Mail Stop 1141, Moscow, Idaho 83844, USA; and Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Drive, Mail Stop 1141, Moscow, Idaho 83844, USA

Abstract

In anadromous salmonids, muscle tissue provides the primary source of energy to support spawning migrations and spawning. We examined changes in the lipid content, protein content, and energy density of white muscle collected from stream-maturing Snake River–Columbia River steelhead *Oncorhynchus mykiss* during several phases of reproduction. At a basinwide scale, we estimated that between early freshwater entry and postspawning (kelt) emigration, the lipid content of white muscle was reduced by 94% to levels less than 1% of wet tissue weight. Lipid was depleted more rapidly than protein during the reproductive cycle and afterward provided the only remaining somatic energy source for the postspawning migration. We found that protein content was consistently higher in sexually mature male steelhead than in females, suggesting that energy allocation prior to reproduction varies between the sexes. In kelts, the lipid content, protein content, and energy density of white muscle were significantly higher in good-condition individuals than in poor-condition fish. Fork length was positively correlated with both protein content and energy density in kelts, suggesting that larger kelts have higher somatic energy than smaller kelts. We found no evidence of significant interannual variation in lipid, protein, and energy density of sexually mature steelhead and steelhead kelts. Postspawning survival of steelhead is likely limited by the low energy density of kelts, and our data lend support to observations of low iteroparity rates in this and other populations of inland stream-maturing steelhead.

‘Let us go down the river to finish our trip to the ocean. I shall recover there, regain my flesh. Part of my body, my fat and oil, is gone, but I shall live without it.’ He stayed in the ocean all that summer and returned the following winter, perfectly well. [Salish oral history of steelhead and spring salmon, transcribed by Adamson 2009.]

After re-entering freshwater to spawn, steelhead *Oncorhynchus mykiss* and Pacific salmon *Oncorhynchus* spp. undergo fasting and rely on stored lipid and protein to support

upstream migration, gonadal maturation, secondary sexual character development, redd construction, competition for mates, and spawning (Brett 1995; Hendry and Berg 1999; Hendry et al. 2000; Tocher 2003). The allocation and use of stored somatic energy during migration, sexual maturation, and reproduction have been reported for several semelparous species of anadromous Pacific salmon (Green 1916; Mommsen et al. 1980; Barrett and McKeown 1988; Hendry and Berg 1999; Crossin et al. 2004;

*Corresponding author: cmoffitt@uidaho.edu

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Kiessling et al. 2004; Magnoni et al. 2006) but not for steelhead. Unlike semelparous salmon that undergo rapid senescence and death after reproduction (Green 1916; Robertson and Wexler 1960; McPhee and Quinn 1998; Morbey et al. 2005), steelhead are iteroparous. Thus, in steelhead, lipid and protein are important not just for upstream migration and spawning but also for postspawning recovery.

Across stream- and ocean-maturing steelhead populations, the proportion of the population that repeats spawning varies from less than 1% to over 70% (Burgner et al. 1992; Lohr and Bryant 1999); however, ocean-maturing stocks generally exhibit higher frequencies of repeat spawning than do stream-maturing populations (Long and Griffin 1937; Withler 1966; Leider et al. 1986; Busby et al. 1996). Stream-maturing steelhead return to freshwater months before spawning (summer–fall) and undergo the bulk of their gonadal maturation while in freshwater, whereas ocean-maturing steelhead return to freshwater much closer to sexual maturity (winter–spring; Burgner et al. 1992). Busby et al. (1996) reviewed the spawning composition of 26 steelhead populations from California to British Columbia and found that on average, repeat-spawning individuals comprised 11% of ocean-maturing populations ($N = 19$) and 7% of stream-maturing populations ($N = 7$). In some ocean-maturing stocks located in southeast Alaska, repeat-spawning individuals constituted up to 70% of the overall spawning population (Lohr and Bryant 1999). Conversely, rates of repeat spawning in many stream-maturing stocks, such as those in the Columbia River, are so low (<2.0%) that the populations are often regarded as functionally semelparous (Burgner et al. 1992).

The higher frequency of repeat spawning in ocean-maturing steelhead has largely been attributed to their lower relative costs of reproduction compared with stream-maturing steelhead (Smith 1960; Withler 1966; Burgner et al. 1992; Busby et al. 1996). Ocean-maturing strategies are exclusive to coastal steelhead populations, whereas stream-maturing strategies are most common to inland stocks that migrate long distances (>400 km) to spawn. Because stream-maturing steelhead generally migrate farther and spend longer periods fasting than ocean-maturing steelhead, it is believed that stream-maturing individuals are more likely to experience energetic exhaustion (starvation) after spawning (Burgner et al. 1992; Keefer et al. 2008b). Although this hypothesis seems intuitive, little is known about the physiological and energetic factors that affect postspawning recovery in steelhead.

Enhancing iteroparity has become an important tool in the conservation and supplementation of threatened and endangered steelhead and Atlantic Salmon *Salmo salar* populations (Wingfield 1976; Gauthier et al. 1989; Brannon et al. 2004; Gephard and McMenemy 2004; Hatch et al. 2013). Improving repeat-spawning rates can increase natural production (Hatch et al. 2004), maintain localized genetic traits (Keefer et al. 2008b), and add genetic diversity to the spawning population (Crespi and Teo 2002; Narum et al. 2008). Reconditioning of kelts in hatcheries by re-initiating feeding, growth, and gonadal recrudescence

has been one method used to artificially increase the rates of repeat spawning in depressed populations (Johnston et al. 1987, 1990, 1992; Bosch 2004; Null et al. 2013). However, outside of controlled hatchery settings, the enhancement of natural iteroparity is more complex.

Many large river systems, such as the Columbia and Snake rivers, contain large hydroelectric impoundments with passage facilities for adults moving upstream (ladders) and smolts moving downstream (juvenile bypass facilities). River impoundments reduce downstream flow, add barriers that must be navigated during emigration, alter natural river cycles, and change environmental conditions (Larinier 2001). Although steelhead kelts are routinely intercepted in juvenile bypass systems (Evans et al. 2004; Wertheimer and Evans 2005; Buelow and Moffitt, in press), most of the downstream bypass facilities were not designed to accommodate emigrating kelts. Strategies to improve kelt passage by modifying dams (e.g., removable spillway weirs) and increasing spill during peak emigration periods have been implemented (Wertheimer and Evans 2005; Wertheimer 2007), but the overall effect on iteroparity is difficult to assess. The amount of somatic energy remaining for emigration is likely a critical factor for determining kelt recovery and survival in both natural and modified systems. Increasing our understanding of somatic energy use before and after reproduction is essential to the management and enhancement of repeat spawning in iteroparous anadromous salmonids.

In the present paper, we provide a quantitative assessment of somatic energy and proximate constituents in stream-maturing Snake River–Columbia River steelhead. Stream-maturing steelhead of Snake River–Columbia River stocks were lethally sampled during five phases of the reproductive cycle to evaluate changes in white muscle tissue composition and energy density. Snake River–Columbia River steelhead begin their freshwater spawning migrations during late spring to early fall (June–October), overwinter in the main-stem Columbia River or Snake River (November–February), and spawn during the spring (March–June). Kelt emigration in the Snake and Columbia rivers occurs from early spring to summer (April–July), immediately after spawning. To account for the large number of individual steelhead stocks returning to the Snake River–Columbia River basin, we separated our analyses into broad, basinwide (all reproductive phases) and fine-scale (within-phase) evaluations.

Our first objective was to evaluate and describe broad-scale changes in lipid, protein, and energetic composition from early upstream migration to the kelt emigration. We hypothesized that somatic energy use by stream-maturing steelhead would be similar to that observed for other long-distance-migrating anadromous salmonids, in which lipid is prioritized over protein for energy. Our second objective was to examine fine-scale changes in sexually mature female and male steelhead to determine whether variations in proximate composition and energy content could be attributed to sample period, spawning year, FL, and sex. We hypothesized that white muscle content would differ between male and female steelhead due to differences in energy allocation for gonadal maturation and spawning

competition. Our final objective was to examine fine-scale variations in proximate composition and energy density of kelts during emigration. We hypothesized that differences related to external condition, spawning year, and fish length would be apparent.

METHODS

Location of Sampling

Stream-maturing Snake River–Columbia River steelhead were sampled in 2009, 2010, and 2011 across five phases of the freshwater reproductive cycle: (1) early migration, (2) fall migration, (3) overwintering, (4) sexual maturity, and (5) kelt emigration. These phases were differentiated in relation to the reproductive cycle of stream-maturing Snake River steelhead and were supported with visual assessments of gonad maturity. Early migrants and some fall migrants were opportunistically sampled from mixed stocks of hatchery-origin (adipose fin absent) and natural-origin (adipose fin present) steelhead. Early migrants were captured in the Columbia River Zone 6 tribal gill-net fishery (45°39'N, –120°57'W) that occurs between The Dalles and John Day dams on the Columbia River, Oregon–Washington. Fall migrants representing mixed stocks of hatchery- and natural-origin steelhead were sampled from tribal hook-and-line harvests at Mackay Bar (45°24'N, –115°28'W) on the Salmon River, Idaho; some fall migrants were also sampled from known-origin hatchery broodstock at Dworshak National Fish Hatchery (46°30'N, –116°19'W), which is located on the North Fork Clearwater River, Idaho. All overwintering and sexually mature steelhead were also collected from known-origin broodstock at Dworshak National Fish Hatchery. Mixed stocks of hatchery- and natural-origin emigrating kelts were intercepted at the Lower Granite Dam juvenile bypass facility (46°39'N, –117°26'W) located on the Snake River in Washington (Figure 1). Because these populations migrate into rivers

during the summer–fall but spawn in the spring, we categorized steelhead by spawning year (i.e., the year in which the steelhead spawned).

Tissue Collection and Processing

Fish sampled from tribal harvests were killed via blows to the skull; fish sampled at Dworshak National Fish Hatchery or at Lower Granite Dam were killed with lethal doses (200 mg/L) of tricaine methanesulfonate (MS-222; Finquel, Argent Laboratories, Redmond, Washington) buffered with NaHCO₃. Steelhead were measured for FL (nearest 0.5 cm), examined for marks and tags, and identified as being of hatchery or natural origin. External condition of kelts was rated as good, fair, or poor by using criteria we defined (Penney and Moffitt, in press). All prespawning and sexually mature steelhead were in good external condition. A fillet of muscle (skin on) was removed from a location posterior to the insertion of the dorsal fin in each fish. Fillets were weighed (0.05 g), stored in plastic bags on ice, and frozen (–20°C) until processing.

Proximate composition and energetic analyses.—In the laboratory, muscle fillets were partially thawed, and a knife was used to separate the white muscle from the skin and red muscle. White muscle samples were then minced, weighed (nearest 0.05 g), placed into individual dishes, and dried for 20 h at 70°C to a constant weight. The resulting dry tissue was cooled and weighed, and the percent moisture was determined ($100 - [\% \text{ dry tissue}] = \% \text{ moisture}$). Dried muscle tissue from each fish was pulverized in a coffee grinder. A subsample of tissue was gradually heated to incineration at 150°C for 1 h, 300°C for 1 h, 450°C for 1 h, and 550°C for 4 h to determine the inorganic (ash) content. The remaining portion of pulverized dry muscle was sealed with parafilm and stored at –20°C for lipid extraction and energetic analysis. To serve as a control, a pooled tissue sample was also analyzed at each ashing. The mean CV for pooled control tissue across all analyses ($N = 42$) was less than 1.0%.

Crude lipid content (neutral and polar lipids) was extracted from dried tissues by using an Ankom XT15 lipid extractor (Macedon, New York) at the Hagerman Fish Culture Experiment Station, Hagerman, Idaho. Dry protein content was calculated via subtraction of ash and lipid content ($100 - [\% \text{ ash} + \% \text{ lipid}] = \% \text{ protein}$). Carbohydrates were ignored because they comprise a negligible (<0.5%) proportion of muscle tissue in salmonids (Jonsson et al. 1991a; Brett 1995; Tocher 2003). The energy density of white muscle tissue was determined by using a Parr 6300 calorimeter (Moline, Illinois) and was expressed in kilojoules per gram of tissue (kJ/g). All lipid, protein, ash, and energy measures were expressed as a percentage of equivalent wet tissue weight with the same calculation described by Hendry et al. (2000). All methods were performed in accordance with standard operating procedures (AOAC 2000).

Validation of proximate composition and energy estimates.—Previous studies support the estimation of total-body proximate composition and energetic density from samples of muscle tissue as an alternative to homogenizing the entire body (i.e., skin,

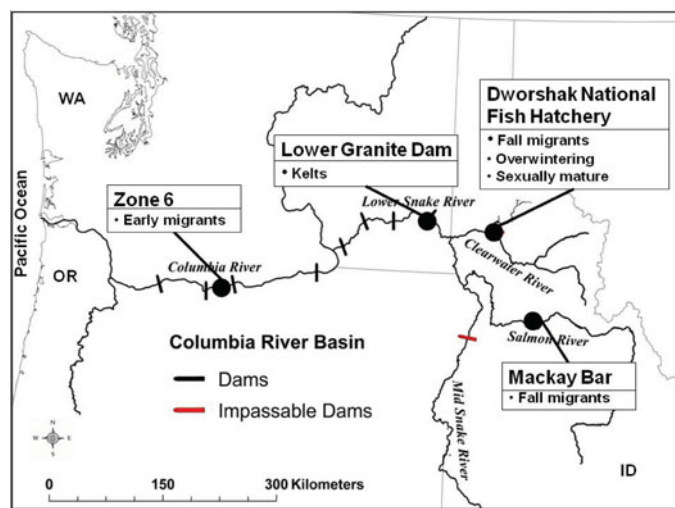


FIGURE 1. Map of sites where steelhead were lethally sampled in the Columbia River basin and Snake River subbasin.

skeleton, soma, viscera, and gonads; Brett 1995; Jobling et al. 1998; Hendry and Berg 1999). To validate this approach for our study, we examined 13 early migrants and 9 kelts to represent both high and low phases of somatic energy. Total-body composition of each fish was paired with the corresponding white muscle subsample, and the proximate constituents and energy density of the total body and subsample were compared with linear regression (Figure 2). The resulting models supported the use of empirical data from white muscle as an effective surrogate for total-body measures in steelhead. However, this two-point approach did not account for preferential energy use (viscera) from or deposition (gonads) into tissues other than the white muscle. Therefore, estimates from white muscle may not be appropriate for studies examining the specific costs of reproduction (e.g., upstream migration, gonadal maturation, secondary sexual character development, redd building and guarding, and competition for mates).

As an additional validation of our approach, we compared the white muscle energy density determined by bomb calorimetry with the energy density estimates determined by multiplying the mass-specific caloric equivalents of lipid (26.4 kJ/g) and protein (20.1 kJ/g; Brett 1995). The use of caloric equivalents (lipid + protein) has reportedly provided somatic energy estimates that are nearly identical to those from bomb calorimetry (Craig et al. 1978; Hendry and Berg 1999). Using the same early migrants ($N = 13$) and kelts ($N = 9$), we estimated energy density empirically and indirectly and compared the estimates by use of a two-sample t -test. The absolute difference between the two values ranged between 0.6% and 1.0%, and the difference was not statistically significant (t -test: $df = 42$, $t = 1.23$, $P > 0.20$). Energy estimates derived from bomb calorimetry are reported in the results.

Experimental Design and Data Analysis

Three separate analyses were conducted to evaluate broad- and fine-scale changes in the lipid percentage, protein percentage, and energy density (kJ/g) of stream-maturing Snake River–Columbia River steelhead: (1) broad, basin-scale changes in proximate content and energy density were evaluated from early migration to kelt emigration; (2) fine-scale variations in proximate composition and energy content were evaluated in sexually mature female and male steelhead among different sample periods, among spawning years, and between sexes; and (3) fine-scale variations in proximate composition and energy content in kelts were evaluated among sample periods, spawning years, and external condition categories.

Changes during freshwater residence.—To provide a sequential model of the changes in lipid, protein, and energy density of white muscle from the early migration phase to the kelt emigration phase, we used samples collected from one spawning cycle (spawning year 2011) to remove the possibility of interannual variations (Table 1). We selected good-condition females for this analysis (1) because few male steelhead were sampled during early migration or during kelt emigration and (2) to avoid

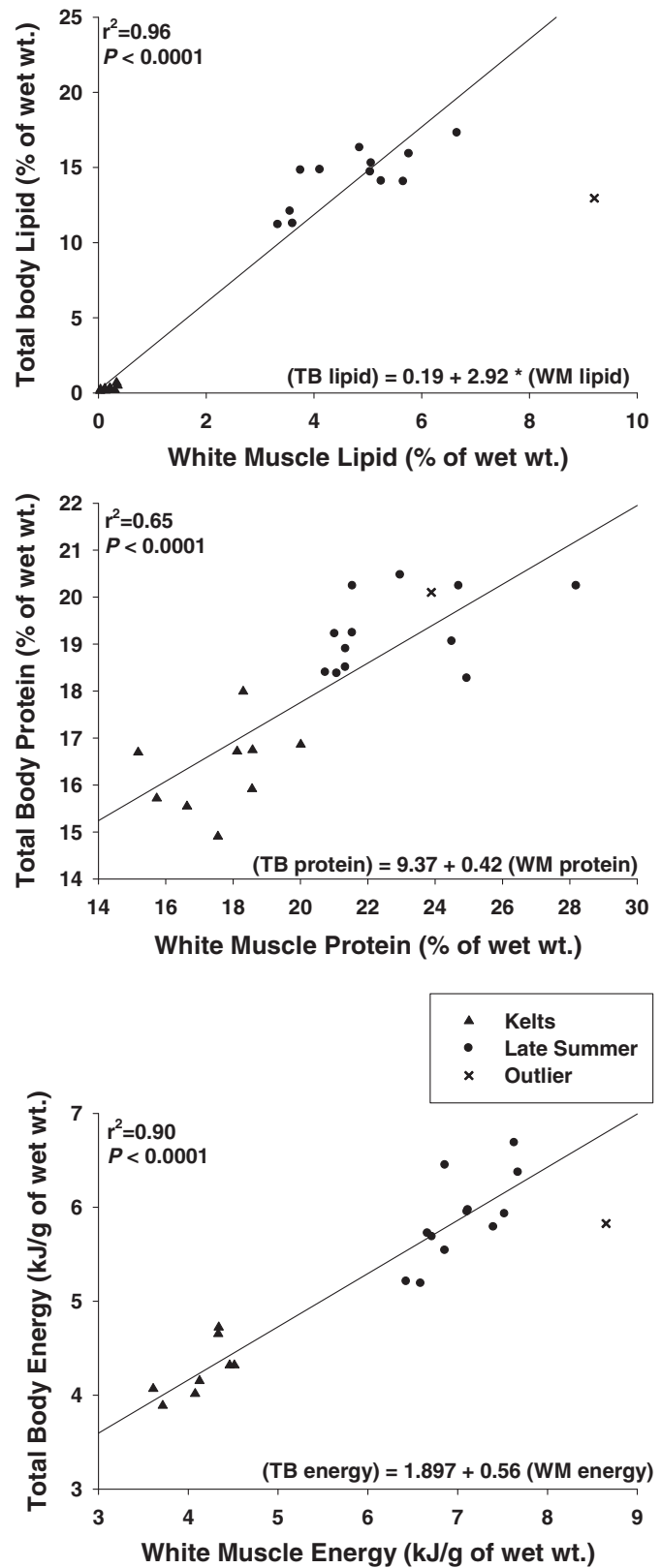


FIGURE 2. Linear regression results for comparisons between white muscle (WM) and total-body (TB) proximate composition and energy density of steelhead.

TABLE 1. Number of Snake River–Columbia River steelhead (separated by reproductive phase and sex) that were lethally sampled during spawning years 2009–2011. Not all locations were sampled in each spawning year. Only good-condition females sampled in spawning year 2011 were used for broad, basinwide comparisons.

Phase	Site and system	Spawning year	Sample date(s)	Sex	
				F	M
Early migration	Zone 6 tribal fishery, Columbia River, Washington and Oregon	2011	Aug 25, Sep 10	9	4
Fall migration	Mackay Bar tribal fishery, Salmon River, Idaho	2011	Oct 21–23	4	5
	Dworshak National Fish Hatchery, Clearwater River, Idaho	2011	Oct 28	6	9
Overwintering	Dworshak National Fish Hatchery	2010	Jan 5, Jan 12, Feb 23	34	20
		2011	Nov 10, Dec 8, Jan 6, Feb 1	46	15
Sexual maturity	Dworshak National Fish Hatchery	2009	Apr 21	30	0
		2010	Mar 23, Apr 20	39	20
		2011	Mar 8, Mar 28	20	10
Kelt emigration	Lower Granite Dam juvenile bypass facility, Snake River, Washington	2009	May 14–Jun 19	32	5
		2010	Apr 1–Jun 28	70	26
		2011	Apr 26–May 12	16	4

the possibility of condition-related variations in proximate composition and energetic density. We expressed our results in two ways. We first evaluated the effect of sampling period (5 phases) on percent lipid, percent protein, and energy density of white muscle in female steelhead. Significant differences attributed to reproductive phase were identified with Tukey’s studentized range (honestly significant difference [HSD]) post hoc test. Our second evaluation involved determining the change in lipid, protein, or energy density between the samples from early migration and each subsequent phase and expressing the change as a percentage of the value determined for the early migration phase. The median lipid percentage, protein percentage, and energy density values for male steelhead are reported but were not analyzed statistically.

Final stages of sexual maturation.—Sexually mature steelhead that were sampled at Dworshak National Fish Hatchery provided the only white muscle samples from a known stock in this study. We used general linear models and ANCOVA to evaluate variations in lipid, protein, and energy content across spawning years and between sexes at similar sampling periods (e.g., April 2009 versus April 2010), and we included FL as a covariate. Two separate between-year comparisons were made. In one comparison, we examined between-year variation in and the influence of fish FL on the dependent variables of lipid percentage, protein percentage, and energy density of tissues from mature female steelhead sampled in April 2009 and April 2010. We used the following model: $y_{ij} = \mu + \alpha_i + \beta X_{ij} + \varepsilon_{ij}$, where y_{ij} is the response variable (percent lipid, percent protein, or energy density), μ is the overall mean, α_i is the main effect of spawning year, βX_{ij} is the length covariate, and ε_{ij} is random

error. In the second comparison, we examined the effects of sex, spawning year, and fish FL between two sampling periods (late February–early March and late March) and two spawning years (2010 and 2011). We tested the following model: $y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta X_{ijkl} + \alpha\beta\gamma_{ijk} + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \varepsilon_{ijkl}$, where y_{ijkl} is the response variable (lipid, protein, or energy density); α_i is the main effect of spawning year; β_j is the main effect of sex; γ_k is the main effect of sampling period; δX_{ijkl} is the length covariate; $\alpha\beta\gamma_{ijk}$ is the three-way interaction among spawning year, sex, and sampling period; $\alpha\beta_{ij}$ is the two-way interaction between spawning year and sex; $\alpha\gamma_{ik}$ is the two-way interaction between spawning year and sampling period; $\beta\gamma_{jk}$ is the two-way interaction between sex and sampling period; and ε_{ijkl} is random error.

Kelt emigration.—We evaluated the effects of external condition, spawning year, and fish length on the lipid percentage, protein percentage, and energy density of fish sampled during kelt emigration. Male kelts were excluded from these analyses because sample sizes of males were small across all spawning years. The effect of condition on female kelts sampled during May (peak of kelt emigration) of spawning years 2009 and 2010 was evaluated with the following model: $y_{ij} = \mu + \alpha_i + \beta X_{ij} + \varepsilon_{ij}$, where y_{ij} is the response variable (lipid, protein, or energy density), α_i is the main effect of external condition, βX_{ij} is the length covariate, and ε_{ij} is random error. To assess whether white muscle lipid, protein, and energy density varied between spawning years, good-condition kelts sampled in May 2009 and May 2010 were compared by use of the model $y_{ij} = \mu + \alpha_i + \beta X_{ij} + \alpha\beta_{ij} + \varepsilon_{ij}$, where y_{ij} is the response variable (lipid, protein, or energy density), α_i is the main effect of spawning

year, βX_{ij} is the length covariate, $\alpha\beta_{ij}$ is the interaction between spawning year and sampling period, and ε_{ij} is random error.

All data analyses were performed using the Statistical Analysis System version 9.2 (SAS Institute, Cary, North Carolina). Diagnostic plots were used to examine and confirm normality and homogeneity of variance for all models. For general linear models, interaction terms were removed hierarchically when no significant interactions ($\alpha = 0.05$) were found. Significant main effects were examined via pairwise comparisons using least-squares means. Any significant interactions were interpreted via additional analyses by reducing the interaction to simple main effects for comparing the levels of each factor. In cases where FL was a significant covariate in ANCOVA, we used Spearman's rank correlations to further examine the relationship between significant factors.

RESULTS

Changes during Freshwater Residence

From early freshwater entry to kelt emigration, the lipid percentage (ANOVA: $F_{4,85} = 31.1$, $P < 0.001$), protein percentage ($F_{4,85} = 37.4$, $P < 0.001$), and energy density ($F_{4,85} = 61.6$, $P < 0.001$) of white muscle significantly decreased in Snake River–Columbia River steelhead (Figure 3). Lipid was depleted more rapidly than protein; however, decreases in protein content were observed when lipid stores were nearly exhausted. From early migration to kelt emigration, we estimated that the median lipid (early migration: 4.9%; kelt emigration: 0.3%), protein (early migration: 23.0%; kelt emigration: 18.2%), and energy density (early migration: 6.9 kJ/g; kelt emigration: 4.4 kJ/g) of white muscle in females decreased by 94, 18, and 36%, respectively. Similar decreases in lipid (early migration: 5.7%; kelt emigration: 0.1%), protein (early migration: 21.3%; kelt emigration: 18.2%), and energy density (early migration: 7.5 kJ/g; kelt emigration: 4.1 kJ/g) in white muscle were also observed for males. Lipid content decreased by approximately 50% from the early migration phase to the fall migration phase, whereas decreases in protein (<7%) and energy density (<10%) were lower. Little to no change in lipid content, protein content, and energy density of white muscle was observed between the overwintering period and sexual maturation (Tukey's HSD test: all $df = 85$, $P > 0.05$). From late sexual maturation to kelt emigration, significant decreases were observed in lipid (35%; Tukey's HSD test: $df = 85$, $P < 0.05$) and energy density (14.9%; Tukey's HSD test: $df = 85$, $P < 0.05$) but not in protein (5.3%). White muscle lipid in all female and male steelhead kelts was nearly depleted, leaving protein as the only somatic energy source after spawning.

Final Stages of Sexual Maturation

Comparisons between sexually mature female and male steelhead across spawning years indicated that protein content was consistently higher in males (ANCOVA: $F_{1,83} = 3.86$, $P = 0.05$; Figure 4). We found little evidence of interannual

variation in lipid and energy density from comparisons across the spawning years 2009, 2010, and 2011. No significant interannual variation in lipid, protein, or energy density was detected between female steelhead sampled in April 2009 versus April 2010 (ANCOVA: $F_{1,46} = 0.68$, $P > 0.50$; Table 2). Lipid content for female and male steelhead was higher in late February–early March samples than in late-March samples (2.1% versus 1.8%; ANCOVA: $F_{1,83} = 4.8$, $P = 0.03$; Table 2). We detected a significant spawning year \times sampling period interaction effect on energy density (ANCOVA: $F_{1,80} = 4.18$, $P = 0.04$). We reduced the interaction to main effects of sampling period and spawning year and found that energy density during late February–early March was significantly lower in 2011 than in 2010. Fork length did not have any influence on lipid, protein, or energy density (ANCOVA: all $P > 0.05$) in any of the models.

Kelt Emigration

External condition and FL were significant factors associated with protein percentage and energy density in the white muscle of female kelts (Table 3). Protein content (ANCOVA: $F_{2,58} = 19.7$, $P < 0.001$) and energy density ($F_{2,58} = 15.5$, $P < 0.001$; Figure 5) in white muscle were consistently higher in good-condition kelts than in fair- and poor-condition kelts. Lipid content did not differ among external condition categories, but lipid values were depleted to low levels in all kelts regardless of condition, making protein the remaining energy source; thus, protein and energy density were essentially equivalent. Fork length of female kelts was a significant covariate with protein content (ANCOVA: $F_{1,58} = 17.4$, $P < 0.001$) and energy density ($F_{1,58} = 12.6$, $P < 0.001$; Figure 5). Positive relationships were found between FL and protein (Spearman's rank correlation coefficient $r = 0.37$, $P < 0.01$, $N = 63$) and between FL and energy density (Spearman's $r = 0.34$, $P < 0.01$, $N = 63$). However, our data set had few kelts larger than 70 cm FL ($N = 5$).

No significant differences in lipid, protein, or energy density were observed between good-condition female kelts sampled in spawning year 2009 and those sampled in spawning year 2010. Fork length was a significant covariate with protein percentage (ANCOVA: $F_{1,29} = 11.6$, $P = 0.002$) and energy density ($F_{1,29} = 6.86$, $P = 0.014$). Weak positive correlations were observed between FL and protein (Spearman's $r = 0.50$, $P < 0.01$, $N = 32$) and between FL and energy density (Spearman's $r = 0.41$, $P = 0.02$, $N = 32$), again suggesting that large kelts contained higher protein levels during emigration than did small kelts.

DISCUSSION

Our study is the first to report changes in the proximate constituents of somatic tissues in steelhead across their freshwater reproductive cycle. Previous scale analyses and more recent tagging studies of iteroparity in stream-maturing Snake River–Columbia River steelhead support low rates of iteroparity (<2%; Long and Griffin 1937; Whitt 1954; Keefer et al. 2008b). Our

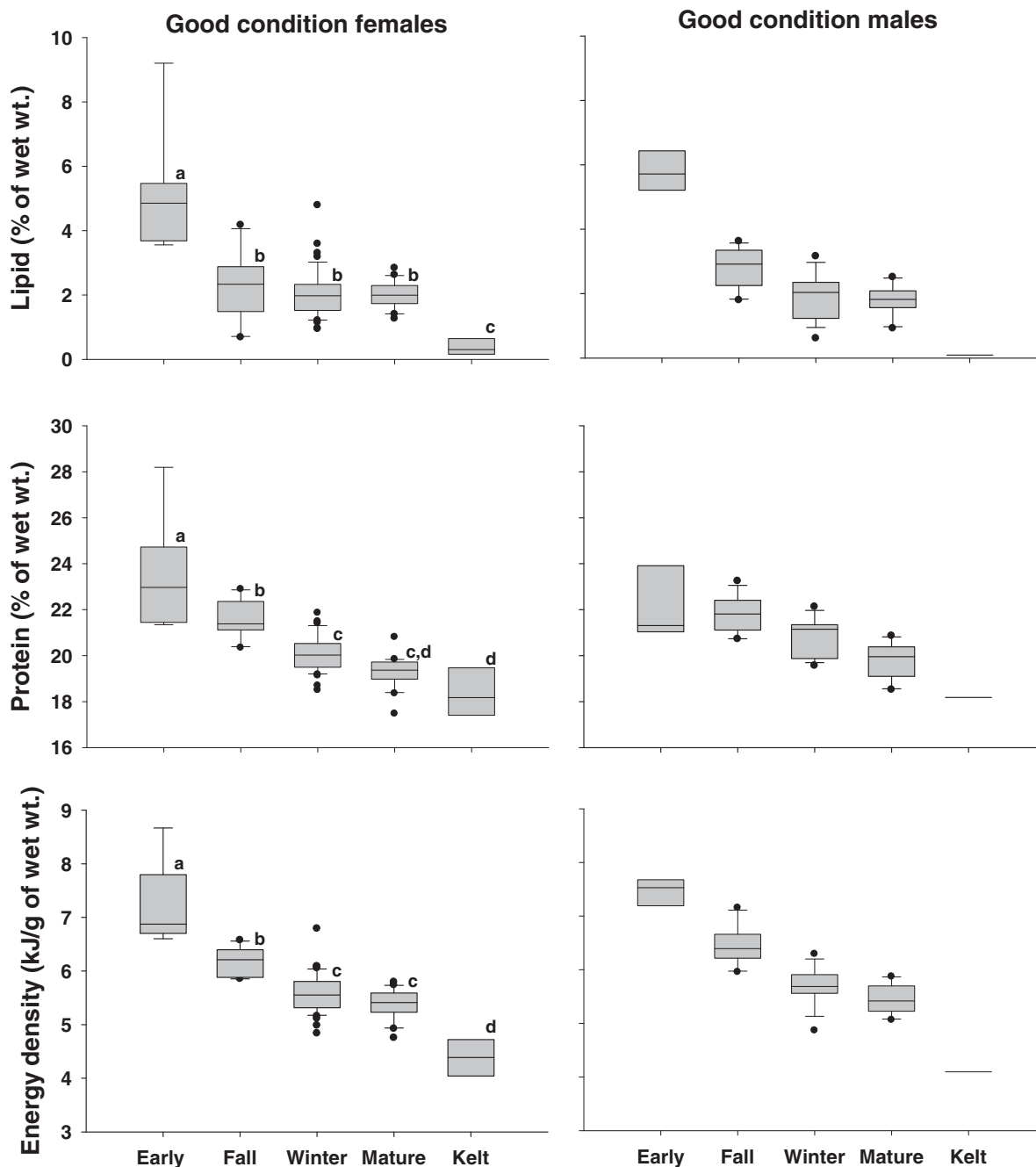


FIGURE 3. Box plots showing changes in lipid content, protein content, and energy density of white muscle from female and male steelhead sampled during spawning year 2011 (sample sizes for females: early migration [early], $N = 9$; fall migration [fall], $N = 10$; overwintering [winter], $N = 46$; sexual maturity [mature], $N = 20$; kelt emigration [kelt], $N = 5$; sample sizes for males: early, $N = 4$; fall, $N = 14$; winter, $N = 15$; mature, $N = 10$; kelt, $N = 2$). Groupings with the same letter are not significantly different (Tukey's honestly significant difference test: $P > 0.05$). Line within box = median; ends of box = 25th and 75th percentiles; ends of whiskers = 90th and 10th percentiles; and black circles = outliers.

findings provide empirical evidence that low postspawning somatic energy likely limits the survival of kelts during emigration.

We found that steelhead lost half of their white muscle lipid content between the early upstream migration and the fall migration. By the time of the kelt migration, the proportional depletion in lipid relative to that observed during early upstream

migration was 93–98%. The mass-specific energy of lipid (36.4 kJ/g) is higher than that of protein (20.1 kJ/g; Brett 1995) and can be exhausted without severely reducing physical performance (Jobling et al. 1998). High rates of lipid depletion in other anadromous species during upstream migration have been documented (Jonsson et al. 1991a; Crossin et al. 2004; Patterson

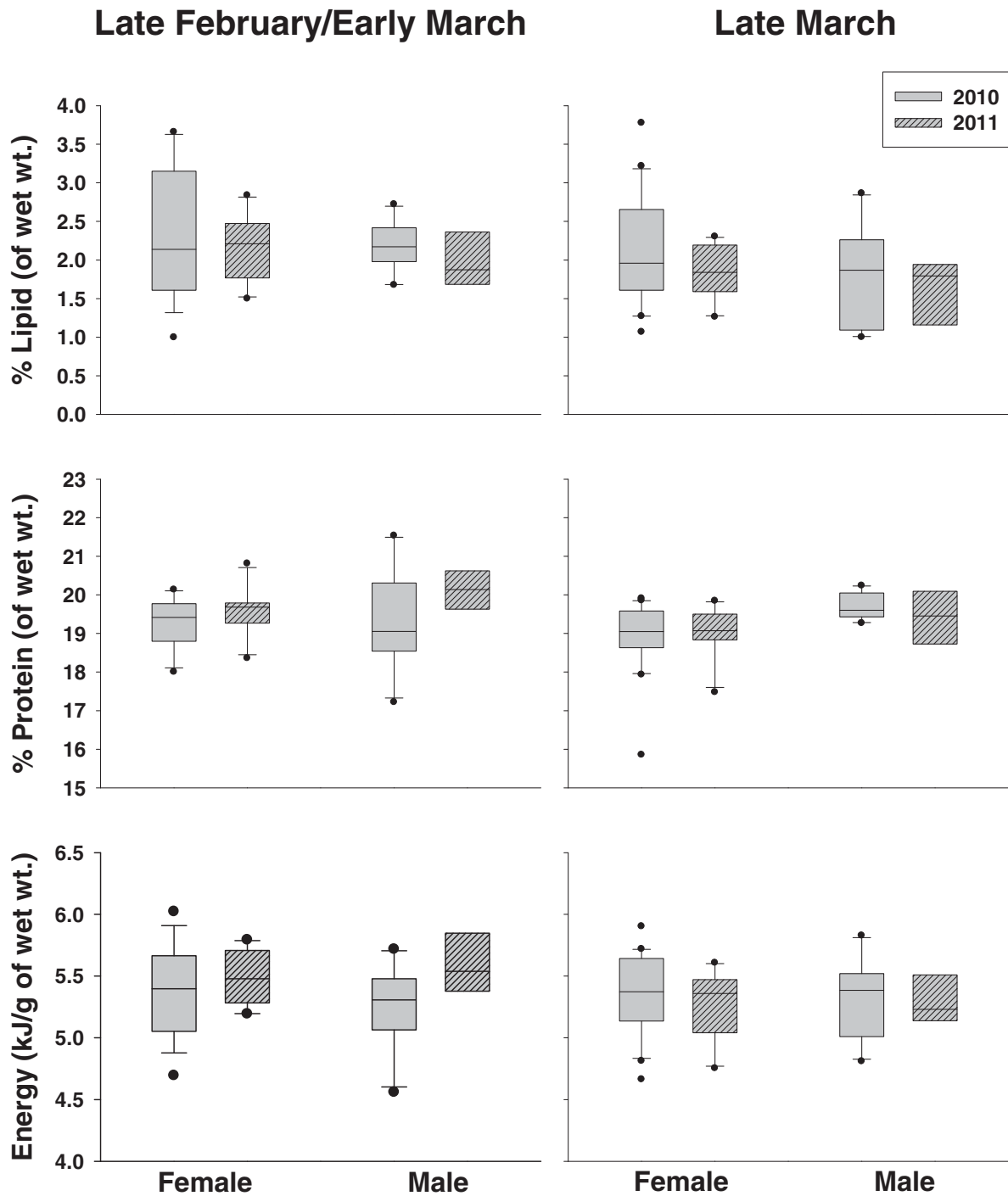


FIGURE 4. Lipid content, protein content, and energy density of sexually mature female and male steelhead sampled at Dworshak National Fish Hatchery in late February–early March and late March during spawning years 2010 and 2011. Line within box = median; ends of box = 25th and 75th percentiles; ends of whiskers = 90th and 10th percentiles; and black circles = outliers.

et al. 2004). Hendry and Berg (1999) reported that stored lipid was the primary energy source for upstream migration and egg production in Sockeye Salmon *O. nerka*, whereas protein was conserved for secondary sexual development and metabolism during spawning. Gilhausen (1980) reported that some stocks

of Fraser River Sockeye Salmon used over 70% of their somatic lipid stores during upstream migration and spawning. Pinson (2005) found that spring/summer-run Chinook Salmon *O. tshawytscha* in the Snake River used 67% of their muscle lipid from upstream migration to arrival on the spawning grounds.

TABLE 2. Sample size, median FL, proximate composition (moisture, lipid, ash, and protein), and energy density of female and male steelhead sampled during the final stages of sexual maturation at Dworshak National Fish Hatchery in spawning years 2009–2011. Values in parentheses below medians represent range of all data.

Sample date	Sex	N	FL (cm)	Proximate component				Energy density (kJ/g)
				Moisture (%)	Lipid (%)	Ash (%)	Protein (%)	
Spawning year 2009								
Apr 21	F	30	84.0 (72.0–88.0)	77.0 (72.3–79.2)	1.7 (0.8–3.5)	1.4 (1.3–2.6)	20.0 (18.1–24.5)	5.3 (4.7–6.0)
Spawning year 2010								
Feb 23	F	18	79.5 (72.0–91.0)	76.6 (75.1–79.0)	2.1 (1.0–3.7)	1.6 (1.4–2.1)	19.4 (18.0–20.1)	5.4 (4.7–6.0)
	M	10	81.0 (66.0–87.0)	77.0 (74.2–78.5)	2.2 (1.7–2.7)	1.7 (1.4–2.4)	19.1 (17.2–21.5)	5.3 (4.6–5.7)
Mar 28	F	20	80.5 (73.0–87.0)	76.9 (75.5–80.2)	2.0 (1.1–3.8)	2.0 (1.6–2.4)	19.1 (15.9–19.9)	5.4 (4.7–5.9)
	M	10	85.8 (80.0–89.0)	76.8 (75.5–78.3)	1.9 (1.0–2.9)	1.6 (1.32–2.02)	19.6 (19.3–20.2)	5.4 (4.8–5.8)
Apr 20	F	19	80.0 (74.0–86.0)	77.4 (76.1–78.8)	1.7 (0.9–2.6)	1.5 (1.3–1.8)	19.6 (18.4–20.3)	5.2 (4.8–5.5)
	M	10	85.0 (76.0–90.0)	77.4 (76.0–79.8)	1.3 (0.6–1.8)	1.5 (1.4–1.8)	19.5 (18.2–20.5)	5.1 (4.4–5.4)
Spawning year 2011								
Mar 8	F	10	81.5 (75.0–86.0)	76.3 (74.8–77.7)	2.2 (1.5–2.8)	2.0 (1.8–2.2)	19.7 (18.4–20.8)	5.5 (5.2–5.8)
	M	5	88.0 (81.0–89.0)	76.0 (74.6–77.1)	1.9 (1.6–2.5)	2.1 (1.9–2.3)	20.1 (19.2–20.9)	5.5 (5.3–5.9)
Mar 28	F	10	84.0 (76.0–86.0)	76.9 (76.2–79.3)	1.8 (1.3–2.3)	1.9 (1.6–2.1)	19.1 (17.5–19.8)	5.4 (4.8–5.6)
	M	5	89.0 (83.0–91.0)	77.0 (75.6–78.2)	1.8 (0.9–2.0)	1.9 (1.7–2.2)	19.5 (18.5–20.4)	5.2 (5.1–5.7)

Atlantic Salmon returning to the River Drammen, Norway, were estimated to use approximately 70% of muscle lipid during their upstream migration, whereas the protein losses were lower (<20%; Jonsson et al. 1997). Our results also indicate that lipid is the primary energy source used by stream-maturing Snake River–Columbia River steelhead during upstream migration and spawning.

We did not differentiate between polar and neutral lipids in this study. Neutral lipids (e.g., triacylglycerols) provide the primary source of lipid-derived energy for growth, reproduction, and spawning migrations in fish (Jobling et al. 1998), whereas polar lipids (e.g., phospholipid) are important for the structural integrity and function of cellular membranes (Tocher 2003). It is possible that the small amount of lipid (0.1–0.9%) remaining in the white muscle of kelts consisted of polar lipids. Future analysis of neutral and polar lipids as well as fatty acid content in kelt tissues is warranted.

The observed changes in the energy density of Snake River–Columbia River steelhead (decreases of 36.1–45.6%) were similar to values for Pacific salmon and Atlantic Salmon during

upstream migration. Crossin et al. (2004) reported that various stocks of Fraser River Sockeye Salmon used between 30% and 53% of their total somatic energy reserves during spawning migrations. Hendry and Berg (1999) found that Sockeye Salmon migrating 98 km to spawn used between 28% and 34% of their somatic energy reserves. Energetic investments into gonadal maturation can constitute a substantial portion of energy use (>30%) during upstream migration, especially in females (Fleming 1998). Our estimates for early migrating steelhead do not represent somatic energy at the “true” start of migration. Freshwater re-entry is widely accepted as the period during which most anadromous Pacific salmon cease feeding and storing energy for reproduction (Mommensen et al. 1980; Hendry and Beall 2004), although fasting can begin before freshwater re-entry (Kadri et al. 1995). We were not able to collect tissues from Snake River–Columbia River steelhead at freshwater re-entry, as steelhead of mixed stocks were first sampled after they had migrated over 300 km. We adopted the same approach used by Hendry and Berg (1999), wherein fish were sampled from a “complex of populations” migrating at similar times and sites

TABLE 3. Sample size, median FL, proximate composition (moisture, lipid, ash, and protein), and energy density of female and male steelhead kelts sampled at the Lower Granite Dam juvenile bypass facility during May in spawning years 2009–2010. No male kelts were sampled in May 2009. Values in parentheses below medians represent range of all data.

Sex	External condition	N	FL (cm)	Proximate component				Energy density (kJ/g)
				Moisture (%)	Lipid (%)	Ash (%)	Protein (%)	
Spawning year 2009								
F	Good	12	57.3 (52.0–82.0)	81.3 (78.2–83.9)	0.3 (0.1–1.5)	1.5 (1.3–1.5)	16.8 (14.5–19.3)	3.9 (3.4–4.9)
	Fair	6	59.0 (55.0–78.5)	82.7 (80.3–83.5)	0.3 (0.1–0.5)	1.3 (1.2–1.4)	15.7 (14.8–18.4)	3.7 (3.6–4.1)
	Poor	6	66.0 (54.0–88.0)	83.6 (78.8–86.2)	0.2 (0.1–0.9)	1.4 (1.1–1.5)	14.8 (12.5–19.0)	3.4 (2.9–4.7)
Spawning year 2010								
F	Good	20	58.5 (52.0–78.0)	82.1 (78.6–84.9)	0.3 (0.1–1.0)	1.2 (1.0–1.4)	16.4 (13.7–19.5)	3.8 (3.2–4.6)
	Fair	9	57.0 (55.0–72.0)	84.1 (80.8–86.1)	0.3 (0.1–0.4)	1.2 (1.1–1.4)	14.5 (12.6–17.4)	3.4 (2.9–4.1)
	Poor	10	58.5 (48.0–71.0)	84.6 (83.1–86.5)	0.3 (0.2–0.5)	1.1 (1.0–1.4)	13.8 (12.4–15.2)	3.3 (2.9–3.6)
M	Good	2	57.5 (56.0–59.0)	82.2 (81.9–82.5)	0.2 (0.1–0.3)	1.3 (1.3)	16.3 (16.1–16.6)	3.7 (3.7–3.8)
	Fair	3	59.0 (59.0)	84.2 (80.5–84.7)	0.4 (0.2–0.6)	1.3 (1.0–1.3)	14.6 (13.7–17.6)	3.3 (3.2–4.1)
	Poor	1	58.0	86.1	0.2	1.1	12.6	3.0
Spawning year 2011								
F	Good	5	74.0 (69.0–79.0)	80.0 (78.3–81.4)	0.3 (0.1–0.9)	1.4 (1.2–1.4)	18.2 (17.3–20.1)	4.4 (4.0–4.7)
	Fair	5	67.0 (52.0–80.0)	82.2 (79.4–83.9)	0.1 (0.1–0.4)	1.3 (1.2–1.3)	16.4 (14.7–19.2)	3.8 (3.4–4.4)
	Poor	0						
M	Good	2	55.0 (54.0–56.0)	80.5 (80.3–80.6)	0.1 (<0.1–0.1)	1.3 (1.2–1.4)	18.2 (18.2)	4.1 (4.1)
	Fair	2	62.0 (56.0–68.0)	81.9 (81.0–82.9)	0.1 (<0.1–0.1)	1.4 (1.4)	16.6 (15.8–17.5)	3.8 (3.6–3.9)
	Poor	0						

and with nearly equivalent migration distances. Inferences derived from broad-scale analysis are not meant to reflect changes within any specific Snake River–Columbia River steelhead stock or to reflect the total costs of reproduction (i.e., investments into gonads, secondary sexual characters, and competition) but rather provide a general, basinwide depiction of the somatic energy changes that occur in stream-maturing steelhead.

In addition to the lipid in white muscle, lipid that is stored in visceral tissues also supplies some energy for upstream migration and possibly gonadal maturation (Jonsson et al. 1997; Hendry and Berg 1999; Pinson 2005; Mesa and Magie 2006). Idler and Clemens (1959) estimated that 2–4% of the energy required for reproduction in certain stocks of Fraser River Sockeye Salmon was derived from visceral stores of lipid. Idler and Bit-

ners (1960) found that the majority of visceral lipid and protein stores in Sockeye Salmon were used within the first 402.34 km (250 mi) of migration. We noted that samples of early migrants had lipid stores surrounding the gastrointestinal tract, but visceral stores were absent from sexually mature steelhead and kelts. Similar observations have also been reported for Atlantic Salmon (Belding 1934; Jonsson et al. 1997). In our validation of white muscle as a proxy for total-body proximate composition and energy content, we found that lipid content was higher in the total body than in the white muscle. It is possible that lipid contained in the viscera, gonads, and other lipid depots (e.g., skin) accounted for this difference.

We measured no statistically significant changes in the proximate content or energy density of the white muscle tissues

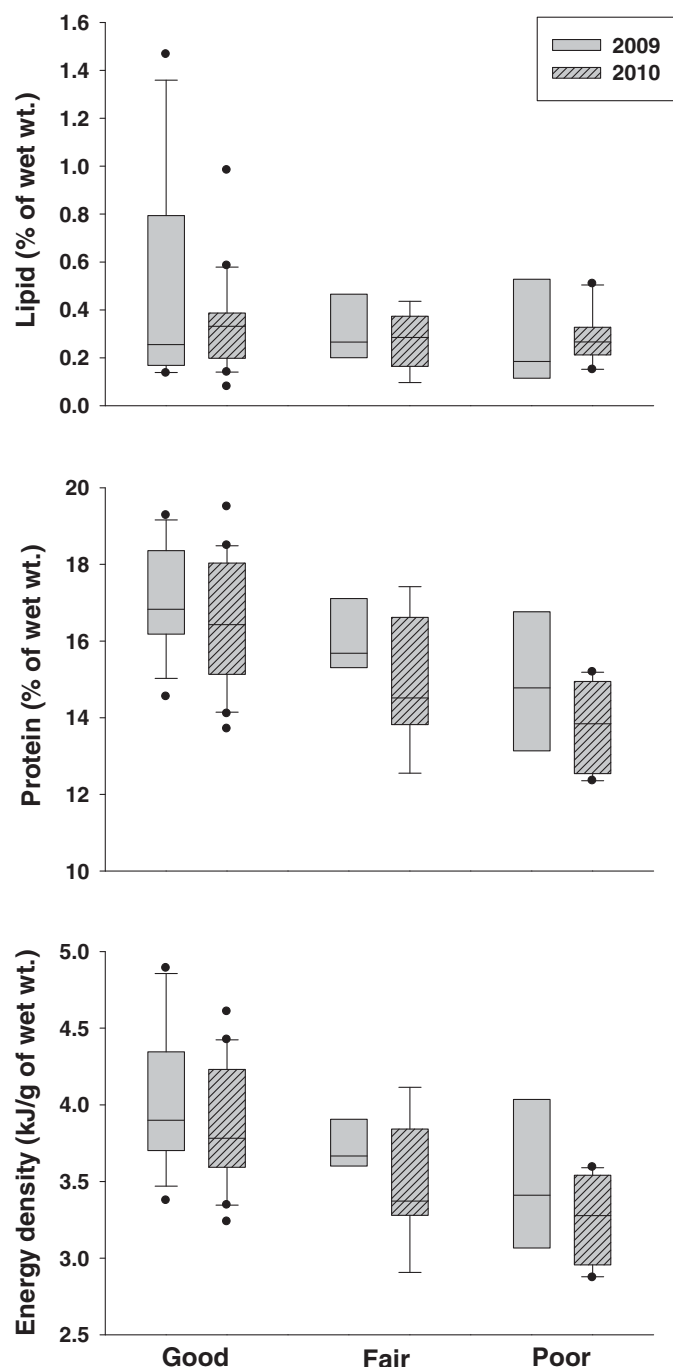


FIGURE 5. Lipid content, protein content, and energy density of good-, fair-, and poor-condition female steelhead kelts sampled at the Lower Granite Dam juvenile bypass facility during May in spawning years 2009 and 2010. Line within box = median; ends of box = 25th and 75th percentiles; ends of whiskers = 90th and 10th percentiles; and black circles = outliers.

during the overwintering period. We believe that the cold water temperatures encountered by steelhead slowed energy depletion and transfer. Direct observations of fish behavior in the region lend support to our findings. Thompson et al. (1958) reported that stream-maturing Snake River steelhead stopped

migrating when water temperatures dropped below 3°C, and Keefer et al. (2008a) found that over 97% of stream-maturing Snake River–Columbia River steelhead ceased their upstream migration when temperatures were below 4°C. Similar overwintering patterns have been observed in stream-maturing steelhead from the Skeena and Chilcotin rivers, British Columbia (Spence 1981; Robards and Quinn 2002).

For our study, the majority of fall-migrating, overwintering, and sexually mature steelhead were obtained from Dworshak National Fish Hatchery, where the 10-year average monthly stream temperatures for November, December, January, February, and March were 9.1, 7.4, 5.6, 4.6, and 4.9°C, respectively (www.cbr.washington.edu/dart/query/river_graph_text). After November and December, all monthly average temperatures were below 6°C. Behnke (1992) reported that most trout decrease feeding activity in water temperatures below 6–7°C and stop growing at temperatures less than 4°C. Metabolic costs of somatic growth decrease when water temperatures approach 4°C. Although the timing of entry into the hatchery was variable, most of the steelhead were held for less than 7 d. It therefore seems plausible that the lowered energy depletion during the winter period is related to the reduction in metabolism that accompanies low winter temperatures.

Protein levels in male steelhead at sexual maturity were significantly higher than those in females, but the differences were small (~0.4%). Jonsson et al. (1991a) found that somatic lipid, protein, and energy density did not significantly vary between female and male Atlantic Salmon at sexual maturity. It is unclear whether such a small difference in protein content is biologically significant; however, our results were consistent between sampling periods (late February–early March and late March) and between spawning years (2010 and 2011).

There is evidence supporting differences in somatic energy allocation between female and male salmonids during reproduction. Female salmonids invest higher amounts of somatic energy into gonadal maturation, while males invest more energy into activities related to competition on the spawning grounds, such as secondary sexual character development and fighting (Fleming and Reynolds 2004). It is possible that the higher protein content in sexually mature male steelhead was due to preparation for competition on the spawning grounds. Alternatively, most of the secondary sexual characteristics in male salmonids are derived from cartilage deposition that is generated from protein reserves, and this might explain the higher protein content in males at sexual maturity (Hendry and Berg 1999). Further energetic analysis of sexually mature steelhead in the natural environment will be needed to address this question.

We hypothesized that variations in environmental conditions among spawning years could affect energy expenditure during upstream migration and overwintering, as is supported in the literature (Murphy 1985; Bjornn and Peery 1992; Brett 1995; Thorstad et al. 2008). However, we found no consistent variation in white muscle lipid, protein, or energy density attributable to spawning year (2009, 2010, and 2011). The lack of

interannual variability in steelhead may be linked to their behavior of seeking coldwater areas to reduce energy expenditure during migration and overwintering (Berman and Quinn 1991; Robards and Quinn 2002). High et al. (2006) determined that 61% of stream-maturing Columbia River steelhead staged in nonnatal systems for durations of up to 237 d. Keefer et al. (2008a) observed that steelhead did not immediately migrate into natal tributaries and that many (12.4%) stayed in the main stem or nonnatal tributaries to overwinter. These migration tactics may help steelhead to offset additional energy expenditures when environmental conditions are unfavorable.

Quantifying the specific costs of spawning (e.g., migration into spawning tributaries, redd construction, and competition for mates) was beyond the scope of this study, and fish sampled at maturity were from captive populations that were not inhabiting stream environments. However, in our study, the second-highest change in white muscle energy density was observed between samples obtained at sexual maturity and those obtained from naturally spawned fish at kelt emigration. White muscle lipid content was nearly exhausted in all kelts (<1.0%). With lipids depleted, protein remained as the only somatic energy source for emigration. Protein is a less efficient energy resource than lipid, especially when mobilized from muscle tissues. Extensive mobilization of protein without energetic replacement via feeding will cause atrophy in muscle tissues, eventually leading to death (Hendry et al. 2000).

The lipid content in the steelhead kelts we studied was two to three times lower than values reported for Atlantic Salmon kelts from two locations in Norway. Somatic lipids in Atlantic Salmon kelts from the River Isma ranged between 0.83% and 2.32%, and lipids in Atlantic Salmon kelts from the River Drammen ranged between 1.9% and 2.1% (Jonsson et al. 1991a, 1997). Only dead semelparous Pacific salmon exhibited somatic lipid content (<1%) similar to that of stream-maturing Snake River steelhead kelts (Hendry and Berg 1999; Pinson 2005; Mesa and Magie 2006).

Our findings provide further evidence that external condition is reflective of energetic status and that poor-condition kelts are likely to be physically and energetically more at risk of postspawn mortality than fair- and good-condition kelts. We documented that protein content and energy density were consistently higher in good- and fair-condition kelts than in poor-condition kelts. Halttunen et al. (2013) found that poor-condition Atlantic Salmon kelts in the Alta River attempted their migrations back to the ocean before (winter) good-condition kelts (spring), presumably to begin restoring somatic energy sooner. Keefer et al. (2008b) found that good-condition steelhead kelts were more likely to spawn repeatedly than poor-condition kelts. Hatch et al. (2013) reported higher mortality in poor-condition steelhead kelts compared with fair- and good-condition kelts in reconditioning studies. We (Penney and Moffitt, in press) determined that severe cellular necrosis of the liver, spleen, and gastrointestinal tissues in steelhead kelts was related to poor external condition.

Fork length was positively correlated with protein percentage and energy density in female kelts, suggesting that larger kelts had proportionately more protein in white muscle tissue. Increases in fish size generally correspond to a lower probability of repeat breeding (Dutil 1986; Jonsson et al. 1991b, 1997; Keefer et al. 2008b), which Fleming (1998) noted was likely due to an increased energetic investment into reproduction. Crespi and Teo (2002) showed that increased body length in salmonids was positively correlated with fecundity, egg weight, and gonadosomatic indices, whereas iteroparity was negatively correlated with gonadosomatic indices. In our study, the white muscle energy content of large Snake River steelhead kelts did not indicate that large fish used more somatic energy. Despite the similarity in postspawn somatic stores between large and small kelts, large kelts may be more vulnerable to exhaustion due to higher maintenance metabolism (Narum et al. 2008). Fleming and Reynolds (2004) hypothesized that larger fish may have more difficulty replacing somatic energy after spawning and thus may be more prone to energetic exhaustion.

Empirically, the relationship between energy and iteroparity in many anadromous fish species is poorly understood, especially considering the influence of fish condition on somatic energy. Glebe and Leggett (1981) hypothesized that anadromous American Shad *Alosa sapidissima* using more than 60% of their total somatic energy reserves during reproduction would not survive to spawn a second time. Jonsson et al. (1997) considered the 60% limit somewhat arbitrary, noting that postreproductive survival was more likely dependent on the amount of remaining somatic energy than on the total amount of energy spent. Individual-based modeling by Castro-Santos and Letcher (2010) on Connecticut River American Shad highlighted how variability in behavior, physiology, and migration delays (upstream and downstream) can affect reproductive effort and emigration success. We would further contend that the capacity for repeat spawning is not just dependent on how much somatic energy remains but also what type of somatic energy (lipid versus protein) remains.

Successful repeat spawning in steelhead is dependent on the kelts' ability to survive emigration, replenish somatic energy, and undergo gonadal recrudescence. Energetic recovery typically requires successful emigration back to the ocean, although exceptions to this have been observed (Null et al. 2013). The levels of somatic lipid and protein that must be maintained in steelhead kelts to avoid starvation are not yet known. Our results indicate that stream-maturing Snake River–Columbia River steelhead can exhaust white muscle lipid during reproduction, leaving protein as the primary somatic energy source. Evaluation of Snake River steelhead kelts in poor condition (nearest to death) revealed lipid (0.1–0.9%) and protein (13.6–14.8%) contents similar to those of dead postspawn semelparous Pacific salmon (Hendry and Berg 1999; Pinson 2005; Mesa and Magie 2006). In spawning year 2011, we opportunistically sampled the white muscle tissues from 11 male steelhead kelts that were in-stream mortalities (our unpublished data) from the

coastal Situk River, Alaska (~30 km in length); this stock has a repeat-spawning rate of approximately 9% (Marston et al. 2011). The median values of lipid and protein in white muscle were 0.1% and 17.1%, respectively. Thus, Snake River steelhead kelts and dead Situk River steelhead kelts displayed similar levels of white muscle lipid and protein. We speculate that when lipid is exhausted and protein content decreases below 18.0% (as a conservative estimate), steelhead may be approaching physiological starvation.

Summary

Our results showed that patterns of somatic energy in stream-maturing Snake River–Columbia River steelhead were similar to energy use and allocation in other anadromous salmonids during migration and reproduction. We found that upstream migration and spawning accounted for the largest changes in white muscle energy reserves. Lipid usage was prioritized over protein usage during migration and sexual maturation. Our findings may help to explain the low proportion of repeat spawning in stream-maturing steelhead populations. We found little evidence for interannual variations in white muscle energy content at sexual maturity or kelt emigration. Fork length was positively correlated with protein content and energy density in kelts, but the scarcity of large kelts in the samples limited our inferences. Future work examining the specific costs of upstream migration, gonadal investments, and spawning will increase our understanding of energy use in steelhead.

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