ARTICLE

Levels of stored energy but not marine foraging patterns differentiate seasonal ecotypes of wild and hatchery steelhead (*Oncorhynchus mykiss*) returning to the Kalama River, Washington

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Abstract: Anadromous fishes need to store sufficient energy at sea to migrate and reproduce, but the energetic demands of freshwater migration distance have been difficult to distinguish from the demands of fasting in fresh water prior to spawning. In addition, differences in stored energy may result from differences in metabolic storage or marine diet. We estimated somatic lipids (SL) and stable isotopes of N and C (as indices of marine trophic position and feeding location) in adult wild and hatchery steelhead (*Oncorhynchus mykiss*) of summer (stream-maturing) and winter (ocean-maturing) runs that migrate similar distances to spawn. We found that SL at return depended on several factors. Summer steelhead had twice the SL as winter steelhead, and within each run SL decreased with arrival date, converging on about 1% SL. In summer steelhead, females had 19% more SL than males, and wild fish had 21% more SL than hatchery fish. In winter steelhead, females had 27% less SL than males, and there was no difference between rearing origins. No differences were detected in $\delta^{15}N$ or $\delta^{13}C$ between ecotypes or rearing origins. Taken together, the results indicated different patterns of energy storage among and within ecotypes despite no apparent differences in marine trophic position or foraging area.

Résumé : Les poissons anadromes doivent stocker suffisamment d'énergie en mer pour migrer et se reproduire, mais les demandes énergétiques imposées par la distance de migration en eau douce se sont avérées difficiles à distinguer des demandes associées au jeûne en eau douce précédant le frai. Les variations de la quantité d'énergie stockée peuvent en outre découler de variations du stockage métabolique ou du régime alimentaire en mer. Nous avons estimé les lipides somatiques (LS) et les isotopes stables de N et de C (comme indices de la position trophique en mer et du lieu d'approvisionnement) chez des truites arc-en-ciel (*Oncorhynchus mykiss*) anadromes sauvages et issues d'écloserie de migrations estivale (maturation en rivière) et hivernale (maturation en mer), dont la migration pour frayer couvre des distances semblables. Nous avons constaté que les LS au retour dépendaient de plusieurs facteurs. Les poissons de la migration estivale présentaient deux fois plus de LS que les poissons de la migration hivernale et, pour chaque migration, les LS diminuaient en fonction de la date d'arrivée, convergeant vers environ 1 % de LS. Chez les truites de la migration estivale, les femelles présentaient 19 % plus de LS que les mâles et les poissons sauvages présentaient 21 % plus de LS que les poissons d'écloserie. Chez les truites arc-en-ciel anadromes de la migration hivernale, les femelles avaient 27 % moins de LS que les mâles et il n'y avait aucune différence entre les poissons sauvages et d'écloserie. Aucune différence n'a été décelée dans les $\delta^{15}N$ et $\delta^{13}C$ entre écotypes et entre poissons sauvages et d'écloserie. Collectivement, les résultats indiquent différents motifs de stockage d'énergie selon l'écotype et au sein des écotypes, malgré l'absence de variation apparente de la position trophique marine ou du lieu d'approvisionnement. [Traduit par la Rédaction]

Introduction

Anadromous fishes achieve most of their overall growth while feeding at sea prior to returning to spawn in freshwater habitats (McDowall 1988), apparently an adaptation to the typically superior growing conditions in high-latitude marine systems compared with freshwater habitats (Baker 1978; Northcote 1978; Gross et al. 1988). High densities of adult fishes (e.g., alosines, salmonids, and petromyzontids) could not be supported by the limited productivity of many of the freshwater systems in which they spawn. Anadromous fishes often enter fresh water just prior to spawning, maximizing growth and minimizing their prespawning freshwater residency, which is typically a period of fasting or limited feeding. However, in some populations of Atlantic salmon (*Salmo salar*; Shearer 1990; Quinn et al. 2006), sockeye salmon (*Oncorhynchus nerka*; Hodgson and Quinn 2002), Chinook salmon (*Oncorhynchus tshawytscha*; Healey 1991), steelhead (*Oncorhynchus mykiss*; Withler

1966), and other salmonids, the fish return to and reside in fresh water many months to a year prior to spawning, termed "premature migration" (reviewed by Quinn et al. 2016). Populations entering fresh water long before spawning tend to enter with more stored energy than populations spawning shortly after they leave the ocean (e.g., Chinook salmon; O'Neill et al. 2014; Hearsey and Kinziger 2015). In addition, populations that migrate long distances to spawning grounds enter fresh water with higher energy stores than populations with shorter migrations (Gilhousen 1980; Brett 1995; Crossin et al. 2004; Quinn 2005). However, populations that differ in timing often migrate different distances, confounding the ability to differentiate the importance of each factor on energy storage needs.

Steelhead, the anadromous form of rainbow trout, present an opportunity to investigate patterns of energy acquisition, storage, and use for migration and stream residence prior to spawning because this species varies greatly in the duration of prespawning

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158

Can. J. Fish. Aquat. Sci. Vol. 74, 2017

freshwater residency. Most steelhead spend 1–4 years in fresh water before migrating to the North Pacific Ocean, where they achieve most of their final body size during another 1–3 years of growth (Burgner et al. 1992; Busby et al. 1996). They return to fresh water during all months of the year but spawn in winter and spring. In much of their range, the migration timing of adult steelhead is bimodal, and populations or runs are categorized as summer or winter based on the time of year when they return. Summer steelhead return to fresh water from spring through fall in a relatively immature reproductive condition, overwinter in rivers, and spawn the following spring. In contrast, winter steelhead return from late fall through early spring in relatively mature reproductive condition and spawn weeks to a few months thereafter (Withler 1966; Smith 1969). Because the seasons of return can vary among regions, summer and winter runs are often referred to as stream-maturing and ocean-maturing, respectively (Busby et al. 1996). Unlike semelparous Pacific salmon, which inevitably die soon after spawning, iteroparous steelhead may survive the breeding season, return to the ocean, and return to fresh water to spawn up to four times in subsequent years (Leider et al. 1986). Consequently, steelhead need to reserve sufficient energy for postspawning survival. In many populations few adults return to spawn a second time (Jones 1973; Busby et al. 1996; Keefer and Caudill 2014), and energy depletion likely contributes to the low survival rate of populations with arduous migrations (Penney and Moffitt 2014a).

In addition to the diversity in migration timing among wild populations, some hatchery steelhead stocks in Washington were intentionally selected for earlier adult return timing than the wild fish (Crawford 1979), and one might expect correlated changes in energetics. Hatchery operations may also inadvertently select for reduced energy storage because artificial spawning eliminates selection for dominance displays, nest preparation, and other energetically demanding behavior patterns associated with spawning. In addition, hatchery fish are typically killed prior to spawning, so there is no selection to store energy to spawn multiple times in a single year or spawn in multiple years as occurs in wild fish (Hendry and Beall 2004). Additionally, many winter hatchery steelhead populations experience high in-river angling exploitation rates, which may disproportionately remove individuals that arrive early and hold longer prior to spawning (Hooton and Lirette 1986). For summer-run hatchery stocks, angling-induced selection on energy storage is less likely, since all individuals have a long in-river holding period.

Energy storage levels of anadromous fish upon freshwater entry may be affected by the quantity and quality of feeding opportunities in the ocean. Steelhead feed heavily on fish and squid while at sea, and their diets reflect environmental variation from year to year, fish size, and location (Atcheson et al. 2012a, 2012b). The relative composition of stable isotopes in fish tissues may be used to indirectly compare ocean feeding location and trophic position. Nitrogen isotope composition ($\delta^{15}N$) provides information on trophic level, whereas carbon ($\delta^{13}C$) provides information on how close to shore an organism is foraging (Johnson and Schindler 2009). Stable isotopes have been used to describe differences in ocean feeding among Pacific salmon species (Welch and Parsons 1993; Satterfield and Finney 2002; Kaeriyama et al. 2004; Johnson and Schindler 2009), intraspecific differences in Atlantic salmon (MacKenzie et al. 2012), and differences between wild and hatchery steelhead from a single river (Quinn et al. 2012). Such differences in ocean feeding and growth might also be associated with seasonal runs of steelhead differing in energy storage levels.

The overall goal of this study was to compare energy storage, as indicated by somatic lipids (SL), and marine foraging, as indicated by stable isotopes of N and C, between seasonal runs of steelhead of wild and hatchery origin. We did so by sampling steelhead returning to the Kalama River in southwestern Washington, including summer and winter fish of hatchery and wild origins. We

hypothesized that (1) winter steelhead would arrive at the vicinity of the spawning area in their natal river with lower SL than summer-run steelhead because winter-run fish have already incurred the cost of gonadal maturation prior to freshwater entry and will spend less time in the river prior to spawning; (2) hatchery steelhead would enter their natal river with less SL than wild steelhead within each run; (3) the relationship between female and male SL would depend on run type (i.e., a run × sex interaction); winter females should arrive with less SL than males, and summer females and males should arrive with similar SL owing to different energetic requirements for gonadal development between the sexes (Hendry and Berg 1999) and different stages of maturity of each run type at natal river entry; and (4) steelhead arriving earlier in each run would have higher SL than those arriving later in the run because earlier returning steelhead have a higher energetic demand during the prespawning freshwater residency period than later arriving steelhead (Jonsson et al. 1997; Crossin et al. 2004; Penney and Moffitt 2014a; Hearsey and Kinziger 2015). Our primary interest related to ocean feeding ecology was to test for differences in stable isotope ratios between the seasonal runs and between wild and hatchery steelhead, which would reflect variation in trophic position and (or) region of the ocean where they foraged. We hypothesized that trophic position and region of the ocean would differ between wild and hatchery steelhead based on a previous study (Quinn et al. 2012) and between seasonal runs based on the difference in time of year when they leave the ocean.

Materials and methods

Study site

The Kalama River watershed (537 km²) is a tributary of the Columbia River approximately 118 km from the Pacific Ocean (Fig. 1) that enters the Columbia River below the lowermost hydroelectric dam (i.e., Bonneville Dam). The Kalama River contains native, wild runs of summer and winter steelhead and also has hatchery-produced winter and summer runs that are derived from out-of-basin source populations that have been planted in the Kalama River since the mid-1950s (Crawford 1979; Leider et al. 1986). The hatchery winter-run steelhead have a mixed ancestry, derived from populations in Puget Sound (Chambers Creek, Washington) and tributaries of the lower Columbia River (Cowlitz and Elochoman rivers, Washington). Current smolt releases are progeny of hatchery-origin adults returning to Kalama Falls Hatchery (KFH). Source populations for the hatchery summer-run steelhead were from tributaries of the lower (Washougal River, Washington) and mid-Columbia River (Klickitat River, Washington), and current smolt releases are progeny of hatchery-origin adults returning to Skamania Hatchery, Washington, in the Washougal River basin. No wild fish have been used in either hatchery broodstock for approximately 30 years.

Fish sampling

Returning steelhead were intercepted along their upstream migration and sampled at KFH, 70 m above sea level, 135 km from the Pacific Ocean, and 17 km from the confluence with the Columbia River. A modified natural waterfall adjacent to KFH acts as a partial passage barrier, directing fish into a trap. The sampling location is downstream of nearly all natural spawning and downstream of nearly all summer-run steelhead overwintering habitat. Current steelhead management practices permit only wild steelhead to be intentionally passed upstream of KFH.

Steelhead arrive at the Kalama River during all months of the year (Fig. 2). Most hatchery winter-run steelhead arrive at the KFH ladder trap in late December and early January, whereas most wild winter-run steelhead arrive there between March and May. Peak returns of wild and hatchery summer-run steelhead occur in July and August.

Fig. 1. Map of the Kalama River basin in southwestern Washington showing where somatic lipid data and stable isotope samples were collected from returning winter-run and summer-run steelhead.
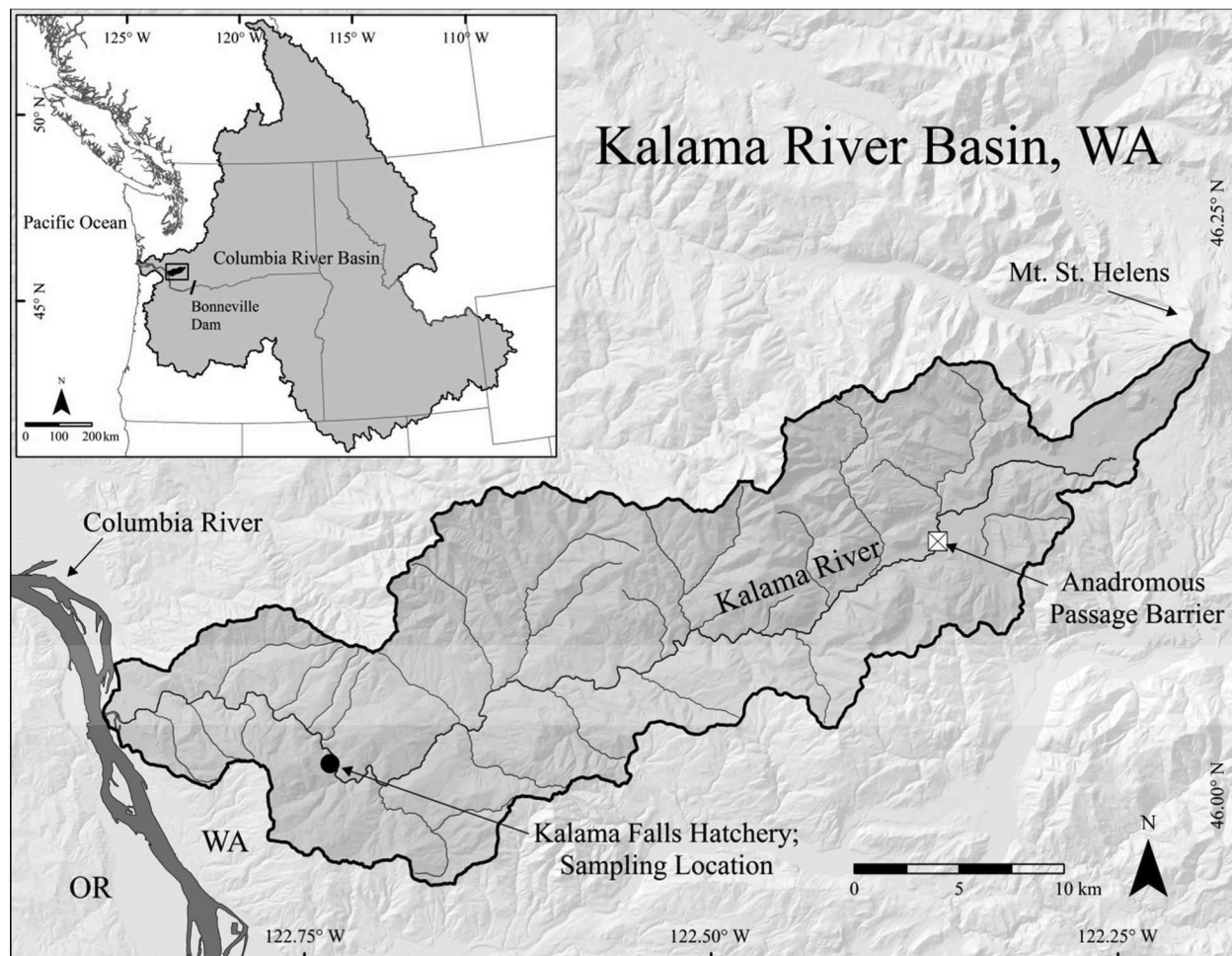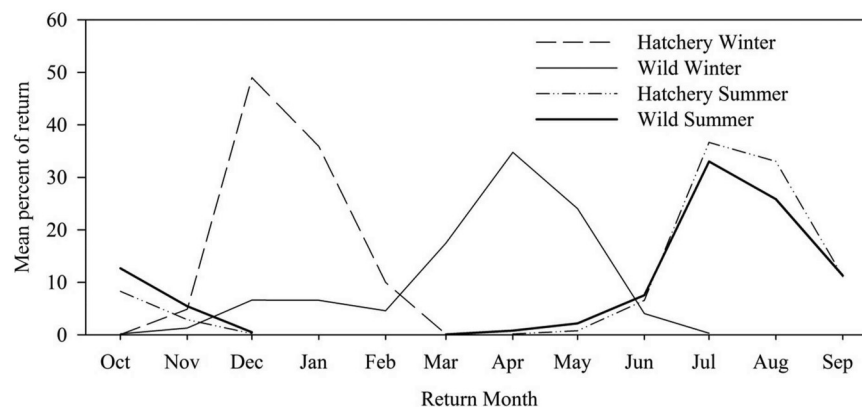

Fig. 2. Return timing of winter-run and summer-run steelhead to the Kalama River, Washington, between November 2010 and December 2013 (3 return years), which includes return timing data during this study. Plots show origin, wild and hatchery, for each run type and are mean percentages of trap captures at Kalama Falls Hatchery by month across the 3 return years.

Data were collected from hatchery winter, wild winter, hatchery summer, and wild summer steelhead (four groups) between 8 November 2012 and 20 November 2013 except between 13 July and 6 August 2013 when the SL sampling equipment malfunctioned. The temporal extent of sampling captured nearly the entire return for each group. Data were collected from every other

steelhead from each group and included fish origin (hatchery or wild), fork length (to the nearest 0.5 cm), sex, and run type (winter or summer). Two sets of scales were removed from each fish from the region above the lateral line and behind the dorsal fin. One set was used to determine age and iteroparity (three scales were removed from each side of the fish for a total of six scales per fish),

160

Can. J. Fish. Aquat. Sci. Vol. 74, 2017

and the second set (three scales were removed from one side of the fish) was dried and processed for stable isotope analysis following procedures described by Quinn et al. (2012).

Fish origin was determined by the absence (hatchery) or presence (wild) of an adipose fin. Standard Washington Department of Fish and Wildlife quality assurance – quality control procedures indicate that a very high percentage of hatchery fish returning for this study had the adipose fin removed correctly (range, 99.4%–100.0%; Washington Department of Fish and Wildlife, unpublished data). If clipped correctly, the adipose fin rarely regenerates (Thompson and Blankenship 1997). Inevitably, some small fraction of fish may have regenerated fins, and some wild fish might be injured in a manner that would result in a missing or malformed adipose fin. However, these sources of error would tend to reduce apparent differences between wild and hatchery fish so any observed patterns are likely conservative.

Sex was determined by secondary sexual characteristics and expression of eggs or milt if present. However, sex determination of summer steelhead can be difficult, especially for fish returning very early in the run. Error in assignment was evaluated by tagging fish and holding them at the hatchery until they were spawned. Over 2 years (2012 and 2013), the sex was correctly identified at capture for 94.8% (92 out of 97) of the fish, so we are confident that this is not a significant source of bias or error.

Run type was classified before collecting SL data and was primarily determined by date of capture, physical appearance, and stage of maturation, as described by Leider et al. (1984). These characteristics are redescribed here. Trap captures of the two run types overlap in spring (typically April–June) and late fall to early winter (typically November–January). In the spring, winter-run steelhead are fully mature with dark spawning coloration and marked sexual dimorphism, whereas summer-run steelhead are sexually immature and exhibit bright, chrome coloration. In the late fall and early winter, the situation is reversed, although the summer-run steelhead are typically not as advanced in the sexual maturation process as the winter-run steelhead are in the spring. The presence–absence of freshwater parasitic copepods on the gills is used as an additional characteristic to differentiate run types during the overlap periods. These parasites are often present on summer steelhead in the fall and winter and on winter steelhead in the spring and indicate that the fish has occupied freshwater habitats for an extended period. These phenotypic characteristics have been used to assign run types of Kalama River steelhead since the 1970s and to describe reproductive isolation (Leider et al. 1984) and life history differences (Leider et al. 1986) among the run types.

SL of the dorsal tissue was estimated with a handheld microwave energy meter (Distell Fish Fatmeter, model No. FM 692, Distell Inc., West Lothian, Scotland). Of the forms of energy storage, lipids are primarily used for migration and egg production, and proteins are primarily used for secondary sexual characteristics and metabolism during spawning (Hendry and Berg 1999). This meter estimated water content and converted this value to percent lipids using the strong inverse relationship between the two substances in fish tissue (Hendry and Beall 2004; Crossin and Hinch 2005). Previous work has indicated that these meters provide an accurate, rapid assessment of energy content from live sockeye salmon (Crossin and Hinch 2005). Values can range between 0.0 and 100.0 proportional to the tissue lipid content. The "Trout-1" setting of the energy meter used in this study was calibrated to *Oncorhynchus mykiss* by the manufacturer. The accuracy of the device varies depending on the magnitude of lipid content. At the levels measured for this study, the manufacturer states an accuracy of ±0.5%. Following the manufacturer's instructions, the calibration of the meter was verified before each sampling day. Each steelhead was sampled by collecting eight readings (four on each side) from the dorsal muscle tissue above the lateral line, consistent with the manufacturer's instructions and with the

methods used in similar research (Crossin and Hinch 2005). SL of each steelhead was characterized as the mean of these eight readings. Within individuals, the eight measures of SL had a coefficient of variation of 21.6%.

Stable isotope values were calculated from scale samples (Quinn et al. 2012; Satterfield and Finney 2002). Samples were spread evenly among the four groups to reflect the run timing of each group. Scale tissue was weighed to the nearest 0.001 mg for analysis. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were analyzed at the University of Washington IsoLab with a continuous flow Thermo-Finnigan 253 mass spectrometer. Isotopes were reported as δ values representing a deviation in parts per thousand (‰) from a standard; $\delta^{13}C$ or $\delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Standard material was Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. Measurement precision was estimated to be 0.09‰ for $\delta^{13}C$ and 0.08‰ for $\delta^{15}N$.

Data analysis

Linear models with Gaussian error structure (identity link) were used to test our hypotheses related to SL and stable isotopes of steelhead. Beta distribution models were explored, but they provided no better fit than the Gaussian models and the model outputs did not allow us to calculate a magnitude of association between SL and the explanatory variables (explained below). We evaluated linear model assumptions and problems associated with influential observations using the global model (i.e., all variables included) for each response variable (SL, $\delta^{13}C$, and $\delta^{15}N$). Normal distribution of residuals was tested with the Shapiro–Wilk test ($\alpha = 0.05$), and homoscedasticity of residuals was graphically evaluated. Influential outliers were identified using Cook's distance (influential observation, $D > 8/(n - 2p)$, where D is Cook's distance, n is sample size, and p is number of model parameters) and standardized leverages (influential observation, $h/(1 - h) > 2p/(n - 2p)$, where h is leverage, n is sample size, and p is number of model parameters).

Proportion of SL was logit-transformed to meet model assumptions of normality and homoscedasticity. The transformation greatly improved residual variance structure and distribution compared with the untransformed response, but the residuals still were not normally distributed (Shapiro–Wilk; $P = 0.01$). In addition, several observations had high influence on the estimated parameters or fitted values. After removing all residual outliers (|standardized residuals| > 3.0) and influential observations, the data met model assumptions (e.g., Shapiro–Wilk; $P = 0.08$). To determine whether including or excluding the offending observations would change the inferences from our analytical approach (range in partial η^2 values for each explanatory variable; see below for details), data sets with and without the offending observations were compared. Including or excluding these observations did not change the results, so all observations were retained. For the stable isotope models, diagnostics were run with the nontransformed response variables and the models met the assumptions; the residuals were normally distributed (Shapiro–Wilk; $\delta^{13}C$, $P = 0.07$; $\delta^{15}N$, $P = 0.75$) and had equal variances, and there were no issues with influential outliers.

To test our hypotheses related to SL of returning steelhead, we used a two-step analytical approach to determine the relative magnitude of association between SL of returning steelhead and a series of explanatory variables including run type (winter or summer), origin (hatchery or wild), sex (female or male), and return date (a continuous variable). Data were limited to first-time spawners to remove additional complexity associated with energy acquisition and consumption by steelhead that have spawned multiple times. Return date was normalized to days from the first return date for each group, termed relative return date (RRD), to improve comparisons among steelhead groups. In the first step of the analysis, we used information theory to identify the best approximating models among all possible model subsets. The global

Table 1. Summary statistics of somatic lipids and the date of return to Kalama Falls Hatchery for steelhead sampled for this study.

| Origin and run type | Sex | Proportion of somatic lipids | | | | | | Return date | | |
		n	Mean	SD	Min.	Median	Max.	Min.	Median	Max.
Hatchery winter	F	49	0.015	0.007	0.008	0.013	0.042			
	M	47	0.022	0.010	0.009	0.020	0.048			
	All	96	0.018	0.009	0.008	0.015	0.048	19 Nov. 2012	16 Jan. 2013	14 Feb. 2013
Wild winter	F	176	0.017	0.009	0.008	0.013	0.050			
	M	157	0.022	0.011	0.009	0.021	0.080			
	All	333	0.019	0.011	0.008	0.015	0.080	8 Nov. 2012	4 April 2013	3 July 2013
Hatchery summer	F	106	0.036	0.012	0.009	0.036	0.078			
	M	123	0.030	0.012	0.007	0.028	0.068			
	All	229	0.033	0.012	0.007	0.031	0.078	3 June 2013	5 Aug. 2013	20 Nov. 2013
Wild summer	F	64	0.042	0.023	0.011	0.040	0.123			
	M	42	0.037	0.016	0.014	0.036	0.095			
	All	106	0.040	0.020	0.011	0.037	0.123	7 June 2013	19 July 2013	20 Nov. 2013

Note: Four groups of steelhead were sampled defined by their rearing origin (hatchery or river (i.e., wild)) and season of return (winter or summer). n, sample size; SD, standard deviation; Min., minimum value observed; Max., maximum value observed. Return date is summarized at the group level.

or saturated model included the four main effects identified above and all two-way interactions (10 variables and 113 models in total). The models were ranked using Akaike's information criterion corrected for small sample sizes (AIC$_c$; Hurvich and Tsai 1989). The difference between the AIC$_c$ of a candidate model and the one with the lowest AIC$_c$ (ΔAIC$_c$) was used to identify the models that had strong support for being the best approximating model (ΔAIC$_c$ < 3; Burnham and Anderson 2002). In the second step, the magnitude of association between SL and each variable in the strongly supported models (ΔAIC$_c$ < 3) was quantified using partial η^2 (effect size). Partial η^2 was calculated from type III sum of squares (SS$_{between}$ / (SS$_{between}$ + SS$_{residual}$); Cohen 1973). The range of partial η^2 was used to quantify the relative magnitude of association between SL and individual variables and was used to test our hypotheses. We chose this approach to identify variable importance as opposed to summing Akaike weights, because the latter approach does not provide information on the magnitude of association between the explanatory variables and SL, a metric we were interested in for this study.

To test our hypotheses regarding steelhead ocean feeding ecology, we used the same two-step analytical approach for each response (δ^{13}C and δ^{15}N) but limited the explanatory variables to run, origin, and run × origin (three variables and five models in total). Data were limited to fish that entered the ocean in 2011 and occupied the marine environment for two full summers (i.e., ocean age 2) to remove effects of interannual variation in ocean growing conditions (Atcheson et al. 2012b; Quinn et al. 2012) and to first-time spawners. Statistical analyses were performed with R software 2.15.2 (R Core Team 2012). Model selection was performed with the glmulti package (Calcagno and de Mazancourt 2010).

Results

Factors affecting somatic lipid content

SL data were collected from 96 hatchery winter, 333 wild winter, 229 hatchery summer, and 106 wild summer steelhead (Table 1). Winter steelhead were sampled between 8 November 2012 and 3 July 2013; the median return date of hatchery steelhead was earlier (median = 16 January 2013) than wild steelhead (median = 4 April 2013). Summer steelhead were sampled between 3 June and 20 November 2013 with similar return timing for hatchery (median = 5 August 2013) and wild fish (median = 29 July 2013).

SL in returning steelhead ranged 18-fold (range = 0.007–0.123) among individuals over the study period (Table 1). Seven out of all possible model subsets had strong support (ΔAIC$_c$ < 3), whereas the null model had no support (ΔAIC$_c$ = 886.36; Table 2). Model-

averaged parameter estimates are presented in the online Supplementary Material[1] (Table S1). Each of the 10 variables was included in at least one of the top models, and six variables (run, RRD, origin, sex, run × origin, and run × sex) were included in all top models. Relative contribution of each variable, reflected by the range of partial η^2 values, was greatest for run (0.304–0.396) and RRD (0.216–0.315) as main effects and run × origin (0.084–0.116) and run × sex interactions (0.068–0.090; Fig. 3), indicating that these variables best explained the observed variation in SL.

SL was nearly twice as high for summer steelhead (0.035 ± 0.002 (mean ± 95% CI), n = 335) as it was for winter steelhead (0.019 ± 0.001, n = 429; Fig. 4A), but among all steelhead, SL decreased as a function of arrival date, converging on ~0.01 by the latest arrival dates for each run (Fig. 4B). Early-arriving (cumulative percentage of returns < 10%) winter steelhead had more stored energy than late-arriving (cumulative percentage of returns > 90%) summer steelhead (winter = 0.033 ± 0.005, n = 44; summer = 0.019 ± 0.003, n = 51).

Differences in SL between origin types and sexes were only apparent within runs. SL was 21% higher in wild summer steelhead (0.040 ± 0.004, n = 106) than in hatchery summer steelhead (0.033 ± 0.002, n = 229; Fig. 4C) and 19% higher in summer females (0.038 ± 0.003, n = 170) than in summer males (0.032 ± 0.002, n = 165; Fig. 4D). Winter steelhead did not differ in SL between wild (0.019 ± 0.001, n = 333) and hatchery origin (0.018 ± 0.002, n = 96; Fig. 4C), but SL was 27% lower in winter females (0.016 ± 0.001, n = 225) than in winter males (0.022 ± 0.002, n = 204; Fig. 4D).

Stable isotope analysis

Stable isotope values were calculated for 47 hatchery winter, 63 wild winter, 55 hatchery summer, and 52 wild summer steelhead (Table 3). Values ranged between –18.46‰ and –16.46‰ for δ^{13}C and between 9.75‰ and 12.75‰ for δ^{15}N and were similar among groups. The null model was the top model (ΔAIC$_c$ = 0.00; δ^{13}C) or a strongly supported model (ΔAIC$_c$ = 0.12; δ^{15}N) among the candidate models for each response, suggesting that none of the variables examined (run type, origin, or run × origin) provided additional information to explain the observed variability in stable isotopes.

Discussion

Despite being a single-year study, our results indicated that multiple interacting variables are connected to the SL of steelhead returning to the Kalama River and provided a meaningful understanding of factors associated with the energetics of steelhead and

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Table 2. Model variables and model selection metrics for the top somatic lipid models ($\Delta AIC_c < 3$) out of all possible model subsets, and the null model.

Variable	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Null model
Run	×	×	×	×	×	×	×	
RRD	×	×	×	×	×	×	×	
Origin	×	×	×	×	×	×	×	
Sex	×	×	×	×	×	×	×	
Run × Origin	×	×	×	×	×	×	×	
Run × Sex	×	×	×	×	×	×	×	
RRD × Origin	×	×		×	×	×	×	
RRD × Run		×	×			×	×	
Origin × Sex					×		×	
RRD × Sex				×		×		
k	9	10	9	10	10	11	11	2
LogL	−217.41	−216.49	−218.27	−217.37	−217.38	−216.42	−216.47	−667.71
AIC_c	453.06	453.28	454.77	455.04	455.06	455.20	455.29	1339.43
ΔAIC_c	0.00	0.21	1.71	1.97	1.99	2.13	2.23	886.36
df	756	755	756	755	755	754	754	763

Note: The response variable was the logit of the proportion of somatic lipids. The symbol "×" signifies variable presence in the model; k, number of estimated parameters; LogL, log-likelihood; AIC_c, Akaike's information criterion corrected for small sample sizes; ΔAIC_c, AIC_c difference between model i and the top model (i.e., Model 1); RRD, the relative return date or the number of days from the first return date for each steelhead group.

Fig. 3. Effect sizes of variables used to explain the variation in somatic lipid content of steelhead returning to the Kalama River, Washington. Values are the range of partial η^2 across the seven best models ($\Delta AIC_c < 3$) identified through model selection. m is the number of models the variable was present, run refers to the season of return (summer or winter), origin refers to the environment in which the steelhead reared (hatchery or river (i.e., wild)), and RRD is the relative return date within each group.
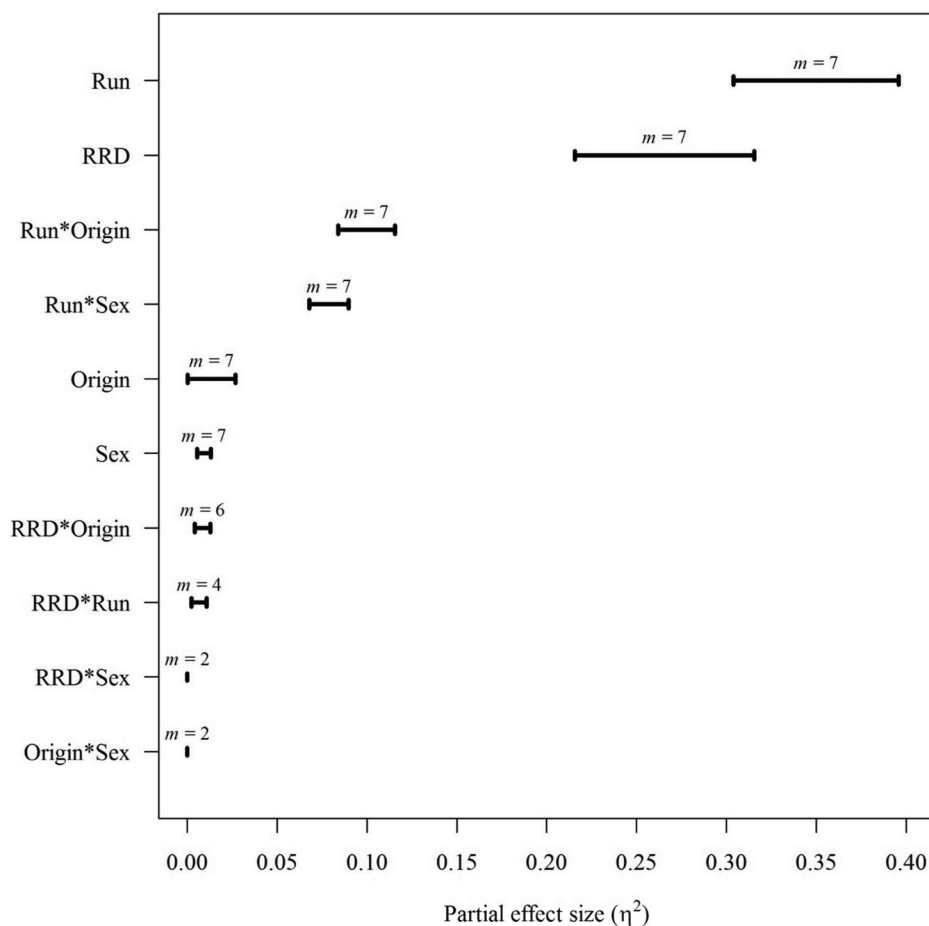
other anadromous fishes. Variables with the largest magnitude included run type, RRD, and interactions between run type by origin and run type by sex. Analysis of estimated SL levels revealed the expected higher energy content in summer (stream-maturing) compared with winter (ocean-maturing) steelhead. This fundamental difference between the runs reflects the need to store sufficient energy prior to leaving the ocean for both the migration to the spawning grounds and prespawning freshwater residency

Fig. 4. Contribution of run type (A), relative return date (B), interaction between run type and origin (hatchery or wild, C), and interaction between run type and sex (male or female, D) to the variation in somatic lipid content for steelhead returning to the Kalama River, Washington. The untransformed (i.e., raw) proportion of somatic lipid content is shown on the *y* axis for each plot. Plots A, C, and D show the mean ± 95% confidence intervals for each group using the raw somatic lipid data. In plot B, day 0 of the relative return date is the first day of return for each group.
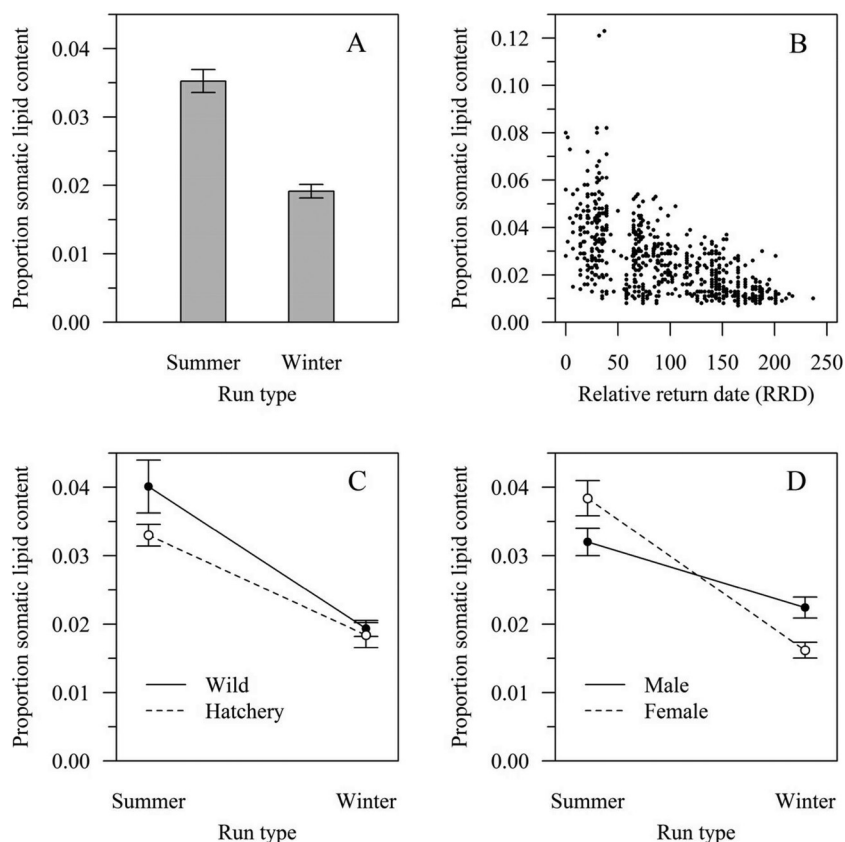

Table 3. Summary statistics of Kalama River steelhead carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope values calculated from scale samples of steelhead that had spent two full summers in the marine environment.

Steelhead group	n	δ^{13}C (‰)					δ^{15}N (‰)				
		Mean	SD	Min.	Median	Max.	Mean	SD	Min.	Median	Max.
Hatchery winter	47	−17.32	0.50	−18.46	−17.15	−16.56	11.18	0.54	9.98	11.12	12.32
Wild winter	63	−17.29	0.34	−18.09	−17.24	−16.51	11.09	0.64	9.75	11.06	12.42
Hatchery summer	55	−17.20	0.27	−17.83	−17.21	−16.46	11.02	0.48	10.00	10.99	12.05
Wild summer	52	−17.27	0.32	−17.90	−17.28	−16.60	11.01	0.55	9.87	10.92	12.75

Note: n, sample size; SD, standard deviation; Min., minimum calculated value; Max., maximum calculated value.

(Smith 1969). Throughout the range of steelhead, summer runs generally migrate farther inland than winter runs, and therefore those fish need more stored energy for both migration and metabolic demands. Our study site on the Kalama River presumably minimized the confounding of these variables because the two ecotypes migrate virtually identical distances while differing in their duration of freshwater residency.

The nonlethal, field-based sampling of the Distell Fatmeter minimizes impact to the fish compared with more invasive measures of body composition (Hartman and Brandt 1995; Schreckenbach et al. 2001). However, our results rely on the assumption that the instrument provided a reliable index of steelhead energetic status. This assumption was not tested for this study and needs to be evaluated in the future. Despite this shortcoming, the assumption is supported by several lines of evidence. First, lipid content estimated by the Distell Fatmeter relies on the inverse relationship between water and lipid, a relationship supported by numerous studies (Schreckenbach et al. 2001; Crossin and Hinch 2005). Fatmeter scans of the dorsal muscle tissue are assumed to be an index

of total SL, an assumption that relies on a proportional relationship of lipids in the dorsal muscle versus other somatic energy storage occurring in the viscera or skin. Although this index has generally been supported in adult salmon (Colt and Shearer 2001; Crossin and Hinch 2005; Penney and Moffitt 2014a), the relationship appears to be curvilinear, and when somatic lipid content is low (<2%), energy meter readings from the dorsal muscle are less sensitive to changes in total body lipids (Colt and Shearer 2001; Crossin and Hinch 2005). As a result, our study may have overestimated the lowest levels of SL in both summer and winter steelhead observed in the latter portion of their respective entry timing. In addition, both lipid and protein are important energy sources during migration and spawning of anadromous fishes (Hendry and Berg 1999; Penney and Moffitt 2014a). However, lipids are depleted more rapidly than protein during migration and spawning (Penney and Moffitt 2014a), indicating that lipids are an important indicator of changing energetic status once steelhead enter fresh water.

Steelhead in this study that arrived later at the sampling location had much lower SL than those that arrived earlier. This observation is consistent with hypothesis 4 and indicates that SL are depleted as steelhead approach spawning time, as has been observed for Pacific salmon (Hendry and Berg 1999; Crossin et al. 2004; Hearsey and Kinziger 2015) and steelhead elsewhere (Penney and Moffitt 2014a). Wild Kalama River steelhead representing each run arrived over periods that spanned nine months, with overlap between runs (Leider et al. 1984, 1986). The spawning period is less protracted than the arrival period; thus, the duration of prespawning residency varies greatly among individuals. Our results showed that within each run type, early arrivals entered with more stored energy than later arrivals of that run, and early-arriving winter steelhead had more stored energy than late-arriving summer steelhead, so the primary factor associated with energy storage was timing within the run and perhaps also the duration of prespawning residency.

Phenological traits are heritable in salmonid fishes (Carlson and Seamons 2008) and vary among populations (Ricker 1972; Brannon 1987). These traits respond quickly to artificial selection (Siitonen and Gall 1989) and evolve quickly in transplanted populations (Quinn et al. 2000). The highly protracted migration timing observed in the wild steelhead in the Kalama River is interpreted to reflect the absence of stabilizing selection on a narrow optimal migration period, unlike many other populations or species. Steelhead in coastal areas typically migrate during the winter and spring; however, populations often display more diverse run timing in coastal watersheds that contain an appreciable amount of spawning habitat above seasonal passage barriers such as waterfalls or cascades that are impassable during high discharge periods (Withler 1966). Prior work in the Kalama River showed that only steelhead returning during low discharge periods (i.e., summer) were able to ascend the falls adjacent to KFH (Bradford et al. 1996). This suggests that before the construction of a fishway at KFH, the spawning habitat above the falls was only accessible to summer migrants and nearly all winter migrants spawned below the falls in the Kalama River. Salmonids not only show genetic control over timing of migration and spawning, but they also show "adaptation by date" within breeding populations in features such as the investment of energy in soma versus gonads and rates of senescence (Hendry et al. 1999; Hendry and Day 2005). Thus, the heritability of spawning date and the differences in selection over the breeding season have resulted in important adaptations within populations. Just as seasonal runs of steelhead differ in patterns of energy storage, even when held under similar conditions (Smith 1969), individuals within runs differ in the timing of return and also the storage of energy prior to leaving the ocean. Such energy is needed to ensure that they have sufficient energy for migration, prespawning metabolic demands while in freshwater, reproduction, and, if possible, postspawning reconditioning (Jonsson et al. 1991, 1997). Similarly, the spring run of Chinook salmon in the Klamath River also enters with higher fat content than the fall run, even though they migrate to the same hatchery (Hearsey and Kinziger 2015).

For each seasonal run of steelhead, the wild fish tended to have higher SL than the hatchery fish, but the difference was greater for the summer than winter steelhead. The wild and hatchery winter steelhead entered with low SL, and so there was less scope for a difference related to their origin, whereas summer-run steelhead ranged more widely in SL, and wild steelhead differed more from the hatchery steelhead. These differences between wild and hatchery steelhead were of lesser magnitude than the differences between seasonal runs but were nevertheless consistent with hypothesis 2 that hatchery steelhead would have less SL than wild steelhead. In the hatchery environment, steelhead are regularly checked for ripeness (e.g., every week), and once females are judged to be ripe, they are killed and the eggs removed and fertilized by milt from one or more males that were ripe on that date.

Therefore, the hatchery operations eliminate the natural selection that would favor males with sufficient energy to spawn with multiple females over a long period of time and females with enough energy to survive and reproduce again. Genetic parentage studies have revealed the importance of subsequent breeding events in the lifetime reproductive success in steelhead (Seamons and Quinn 2010), so this change in selection regime may be important.

The Kalama River hatchery steelhead were not derived from the native populations, so SL may reflect ancestral patterns as well as current selection regimes. The hatchery winter run was derived from two populations that are closer to the marine environment and lower in elevation (Elochoman River and Chambers Creek) and one population (Cowlitz River) that traveled a similar distance as the wild Kalama population. If ancestral origin (i.e., migration distance) was the major factor controlling energy storage, we might expect lower SL in the winter hatchery population than the wild winter population, but we detected no such difference. The summer run was derived from the Washougal and Klickitat river populations that travel farther than the wild Kalama River population. If ancestral origin influenced energy storage of summer steelhead, we might expect hatchery summer steelhead should have more stored energy than the wild summer Kalama steelhead, but this also was not observed. Thus, indirect selection over multiple generations within the hatchery remains a viable hypothesis for the reduced levels of stored energy compared with the wild fish.

Consistent with our predictions, differences in SL between males and females depended on run type; female winter steelhead had less SL than did males, but female summer steelhead had more SL than males. This reversal may be related to the energetic requirements and the sequence of energy allocation to gonadal development between sexes in the two runs. In salmonids, gonads are a much greater proportion of the mass in mature females than in males (e.g., 20% versus 2% in sockeye salmon (Hendry and Berg 1999), 21% versus 4% in chum salmon (*Oncorhynchus keta*) and 19% versus 5% in pink salmon (*Oncorhynchus gorbuscha*) (Gende et al. 2004), 21.5% versus 2.8% in anadromous brown trout (*Salmo trutta*) (Jonsson and Jonsson 1997), and 28.6% versus 3.2% in Atlantic salmon (Jonsson et al. 1997)). Moreover, the eggs are richer in energy than male gonads (e.g., 7.8 versus 3.9 kJ·g^{-1} wet mass prior to spawning in sockeye salmon (Hendry and Berg 1999), 7.8 versus 5.7 kJ·g^{-1} in anadromous brown trout (Jonsson and Jonsson 1997), and 8.4 versus 3.8 kJ·g^{-1} in Atlantic salmon (Jonsson et al. 1997)) and therefore require a disproportionate overall investment. The female summer steelhead would have arrived with less-developed gonads than the female winter steelhead, and so the summer females would be expected to have more SL in their muscle than males, whereas the reverse might be true in winter steelhead. In the Cheakamus River, British Columbia, steelhead that arrived earlier spent more time in the spawning grounds, and males tended to stay longer than females (Korman et al. 2007); both of these patterns would be consistent with higher energetic investment in early-arriving fish and in male winter steelhead, as we observed. Notwithstanding the larger energetic investment in gonads by females, males are less likely to survive spawning (Keefer et al. 2008), consistent with the general pattern in iteroparous salmonids (Fleming 1998), suggesting that reproductive activity is very taxing. The attributes of individual fish that contribute to the storage and mobilization of energy and the resumption of feeding and eventual survival in iteroparous salmonids are an important topic that has received little attention until recently (Penney and Moffitt 2014b).

This study was not designed to quantify the cost of reproduction or link the amount of SL with the temporal extent of freshwater residency (i.e., from arrival to migration back to sea) for individual steelhead. We are limited in our interpretation of the data because we do not know the date, energy storage levels, or stage of

maturation when the fish first entered the Columbia River. Consequently, we do not know how long individuals were in fresh water prior to arriving at the Kalama Falls trap (presumably fasting), when individuals spawned, or which individuals survived to spawn again. However, we do know that Kalama River steelhead spawn in late winter and spring (Leider et al. 1984), and our data show that SL decreased as the spawning period was approached. Other work has shown that energy reserves are negatively related to gonadal development (Jonsson et al. 1997; Hearsey and Kinziger 2015), and we expect the same relationship existed for the steelhead in this study. Therefore, the energy depletion pattern we observed may be explained by the duration of prespawning freshwater residency and the energetic requirements of gamete development, and there may be a minimal level of SL necessary for successful reproduction. For example, our measure of SL converged at approximately 1% in later-returning fish of each run type. Other work has found similar lipid content values just prior to spawning: Atlantic salmon ~2% (Jonsson et al. 1997); Atlantic salmon ~1% (Hendry and Beall 2004); sockeye salmon 1.6% (Hendry and Berg 1999); steelhead ~2% (Penney and Moffitt 2014a), but direct comparisons with these studies are not possible as methodologies differed. In our study, the Distell Fatmeter measured the proportion of lipid in the dorsal muscle tissue, which is an index rather than an absolute representation of total body lipids. In summary, we hypothesize that the early-arriving fish spend more time in the river prior to spawning compared with fish arriving later, rather than the alternative that all fish have similar duration of freshwater residency prior to spawning.

In contrast with the marked differences in stored energy between seasonal runs and, to a lesser extent, between wild and hatchery fish, we detected no differences in stable isotopes of C or N, as might have resulted from differences in trophic position or regions of the ocean where the fish had been feeding. Nitrogen isotopes are most informative for trophic position, as $\delta^{15}N$ increases with trophic position (Post 2002). Carbon isotopes vary only slightly with trophic position but are informative for sources of carbon; $\delta^{13}C$ values tend to be increasingly negative in offshore waters (Davenport and Bax 2002; Johnson and Schindler 2009). Marine foraging patterns, inferred from stable isotopes, differ among Pacific salmon species; Chinook and coho salmon tend to feed at higher trophic levels in coastal environments, whereas sockeye, pink, and chum salmon feed at lower trophic levels in more offshore environments (Satterfield and Finney 2002; Johnson and Schindler 2009). Stable isotope values of steelhead in this study were calculated from scale tissue and thus not directly comparable to values reported for Pacific salmon based on muscle tissue. Several studies have shown that $\delta^{15}N$ and $\delta^{13}C$ correlations between scale and muscle tissue have a slope that differs from a value of one (Satterfield and Finney 2002; Quinn et al. 2012). If the scale-tissue values from this study are adjusted to muscle-tissue values using the equations provided in Quinn et al. (2012), the adjusted $\delta^{15}N$ (12.06) and $\delta^{13}C$ (–21.93) for Kalama River steelhead indicate that they foraged at an intermediate trophic level and in a more offshore environment than other Pacific salmon species.

Given the large differences in SL between the seasonal runs to the Kalama River, the similarity in isotope ratios between wild and hatchery steelhead and between the seasonal runs was surprising. There was no indication of differences in trophic level or foraging region, although the Kalama River samples were taken from a single year, and interannual variation could not be explored. Thus, the differences in SL among Kalama River groups likely resulted from differences in energy storage and use rather than differences in marine foraging. The winter hatchery population in the Kalama River was derived from the same lineage as the hatchery run to Forks Creek, though the two hatchery stocks have had different selection regimes since each run was established over multiple generations at their respective hatcheries. Stable isotope ratios of these two winter steelhead groups (Kalama River,

Forks Creek) differed significantly between the two watersheds. The Forks Creek hatchery steelhead (Quinn et al. 2012) had lower values of $\delta^{15}N$ (10.51, SD = 0.59, pooling samples from 2 years) than did the Kalama River steelhead (11.18, SD = 0.54; t = 6.09, df = 105, P < 0.001), and the Forks Creek fish also had higher (less negative) values of $\delta^{13}C$ (–16.73, SD = 0.31) than the Kalama River steelhead (–17.32, SD = 0.50; t = 7.45, P < 0.001). These data indicated that the Kalama River fish were feeding at a somewhat higher trophic level (higher values of $\delta^{15}N$) and farther offshore (more negative values of $\delta^{13}C$) than the Forks Creek fish. However, steelhead in these two data sets went to sea in different years and entered the ocean at different locations, complicating this interpretation. Nevertheless, our results show that stable isotopes can be a useful tool for interpreting the role that marine foraging patterns may play in population-specific differences. In the Forks Creek study, the differences in stable isotopes suggested population- or year-specific marine foraging patterns. In the comparison among Kalama River populations, the lack of difference in stable isotopes provides a basis for evaluating factors other than marine foraging patterns that contribute to energetic condition of the fish. Together, these results suggest that additional study is needed to understand when and how ocean growth of steelhead is influenced by population-specific or interannual environmental variation.

In conclusion, the results revealed complex processes controlling the storage of energy needed for migration, reproduction, and postspawning survival in steelhead and, by extension, other anadromous fishes. After controlling for the distance the fish migrated, the two seasonal runs differed markedly in stored energy upon arrival; earlier-arriving fish had more energy than those arriving later, and wild fish tended to have more energy than hatchery fish. Considering the reduced in-stream lifespan of later-arriving semelparous salmonids (Hendry et al. 1999; Doctor and Quinn 2009), the storage of energy is likely part of a suite of adaptations associated with the timing of freshwater entry, rigors of migration, duration of freshwater residency, and spawning. Artificial selection on timing often occurs in hatcheries, typically favoring early-arriving fish (Flagg et al. 1995; Quinn et al. 2002; McLean et al. 2005), and there may be correlated, inadvertent selection on energy storage as well. If so, this might be part of the complex of traits that cause hatchery-origin salmonids to produce fewer offspring when breeding in rivers compared with sympatric wild fish (Chilcote et al. 1986; Araki et al. 2007; Seamons et al. 2012).

Acknowledgements

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166

Can. J. Fish. Aquat. Sci. Vol. 74, 2017

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