North American Journal of Fisheries Management 30:1–11, 2010

DOI: 10.1577/M09-048.1

[Article]

$\delta^{13}C$ and $\delta^{15}N$ Signatures in Muscle and Fin Tissues: Nonlethal Sampling Methods for Stable Isotope Analysis of Salmonids

JUSTIN R. HANISCH,* WILLIAM M. TONN, AND CYNTHIA A. PASZKOWSKI
Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

GARRY J. SCRIMGEOUR
Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada; and Parks Canada Agency, Western and Northern Service Centre, Number 1550, 635 8th Avenue SW, Calgary, Alberta T2P 3M3, Canada

Abstract.—Stable isotope analysis has emerged as an important tool in aquatic ecology. For fish, dorsal muscle from sacrificed individuals has traditionally been used in stable isotope studies; however, there are many instances when lethal sampling is undesirable. We evaluated the feasibility of using adipose and caudal fin clips as alternatives to muscle in stable isotope studies for five species of salmonids. Because fish size and water temperature can affect stable isotope ratios, we also determined whether fish length and sampling date affected the difference in isotope signatures between fins and muscle. Biopsied muscle plugs and fin clips were collected from rainbow trout *Oncorhynchus mykiss*, brook trout *Salvelinus fontinalis*, and lake trout *S. namaycush* as well as lake whitefish *Coregonus clupeaformis* and pygmy whitefish *Prosopium coulterii* and analyzed for stable isotopes of carbon and nitrogen. The isotope signatures of both adipose and caudal fins were significantly correlated ($0.33 < R^2 < 0.97$) with those of dorsal muscle from the five salmonid species studied, and in some cases the signatures of fins did not differ from those of muscle. Fish length and sampling date occasionally had a small effect ($0.042 < R^2 < 0.49$) on the relationship between the isotope signatures of fin and muscle. Although muscle biopsy provides a viable, nonlethal method of collecting muscle tissue from suitably sized fish, the strong relationships between the isotope signatures of fin and muscle demonstrate that fin clips should be considered good surrogates for muscle in stable isotope studies of salmonids.

Studies of trophic ecology have traditionally relied on direct field observations and gut content analysis to infer food web relationships (Paine 1980). However, direct observation of aquatic organisms is often difficult, and gut contents provide only a snapshot of what an organism has recently consumed. During the past 20 years, stable isotope analysis (SIA) has become an important tool in ecological research because it can reveal which portions of an organism's diet are assimilated in tissue growth and maintenance (Fry 2006; Grey 2006). Aquatic ecologists have thus used stable carbon and nitrogen isotopes in a variety of applications, including describing food-web relationships (Vander Zanden and Rasmussen 1999; Beaudoin et al. 2001) and elucidating fish movement patterns (Cunjak et al. 2005; Morinville and Rasmussen 2006).

Fishes are often top predators in freshwater food webs (Carpenter et al. 2001; Wissinger et al. 2006) and thus have been the focus of many stable isotope studies. Dorsal white muscle from sacrificed individuals is the most commonly analyzed fish tissue in stable isotope studies because it is available in large quantities, is easy to homogenize, has an intermediate turnover rate, and shows less isotopic variability than other fish tissues (Pinnegar and Polunin 1999; Perga and Gerdeaux 2005). There are many instances, however, when lethal fish sampling is impossible, undesirable, or illegal (Sierszen et al. 2003; COSEWIC 2007). Because salmonids have particularly diverse life history strategies (Klemetsen et al. 2003), stable isotope analysis is especially well suited to studying their ecology. Many salmonid populations are imperiled (Jelks et al. 2008), however, and nonlethal sampling would likely be the only option in isotope studies of these populations. Thus, it is important to establish nonlethal sampling methods to obtain fish tissues for analysis.

Fin-clipping is a widely used methodology in fisheries research, and some studies have considered fins as alternate sources of tissue for stable isotope analysis (Rounick and Hicks 1985; Shannon et al. 2001; Kelly et al. 2006). Only recently have studies begun to evaluate fins as a surrogate for muscle in salmonids. Because white muscle and adipose fin isotopic signatures did not differ in an exploratory study of brown trout *Salmo trutta*, McCarthy and Waldron (2000) recommended further studies on the correlation between muscle and fin in salmonids.

* Corresponding author: hanisch@ualberta.ca

Received April 3, 2009; accepted September 4, 2009
Published online December 21, 2009

Despite this recommendation, few additional studies have been published to date. Statistical differences in $\delta^{13}C$ and $\delta^{15}N$ were found between adipose fin and white muscle of Atlantic salmon *Salmo salar* (Dempson and Power 2004); however, because absolute differences between tissues were only 0.5‰, the authors recommended using adipose fin clips for stable isotope analyses of this species. Additionally, isotopic signatures of caudal fins and white muscle were strongly correlated in both Atlantic salmon and brook trout *Salvelinus fontinalis* from rivers in Atlantic Canada (Jardine et al. 2005). Sanderson et al. (2009) recently found strong relatonships between isotope signatures of muscle and fin of juvenile rainbow trout *Oncorhynchus mykiss* and Chinook salmon *O. tshawytscha*. Conspicuously absent, however, is information on fin–muscle relationships for adult rainbow trout and steelhead, salmonids of special conservation concern (Jelks et al. 2008) and widespread economic importance (Halverson 2008).

In the present study, we compared isotopic signatures of adipose fin, caudal fin, and dorsal white muscle from rainbow trout, brook trout, and lake trout *Salvelinus namaycush*, and lake whitefish *Coregonus clupeaformis* and pygmy whitefish *Prosopium coulterii* from five Alberta lakes. Our specific objectives were to (1) document the relationships between $\delta^{13}C$ and $\delta^{15}N$ signatures of fin tissues with those of dorsal white muscle in five species of salmonids and (2) determine whether the differences between the signatures of fin and muscle vary with fish size (for all five species) and time of year (for rainbow and brook trouts only) because fish size (Harvey et al. 2002) and water temperature (Barnes et al. 2007) can influence stable isotope ratios in fish.

Methods

Study area.—Rainbow and brook trouts were sampled from three lakes and one lake, respectively, in the vicinity of Rocky Mountain House, Alberta (52°22′48.27″N, 114°56′07.71″W). These mesotrophic lakes are small (21.8 ± 10.4 ha [mean ± SD]) and have moderate maximum depths (10.2 ± 3.0 m) with abundant submergent and emergent vegetation. They are among several in the region stocked with rainbow, brook, or brown trout (or a combination thereof) and are inhabited naturally by combinations of dace species (pearl dace *Margariscus margarita*, northern redbelly dace *Phoxinus eos*, and finescale dace *P. neogaeus*), Iowa darter *Etheostoma exile*, fathead minnow *Pimephales promelas*, and brook stickleback *Culaea inconstans* (Hanisch 2009).

Lake trout and lake and pygmy whitefishes were collected from the upper and middle basins of Waterton Lake, Alberta (49°03′16.52″N, 113°54′42.78″W). Waterton Lake is a cold, deep oligotrophic lake located in Waterton Lakes and Glacier National parks, Alberta and Montana (Brinkmann 2007). Waterton Lake contains a coldwater fish assemblage, including whitefishes *Coregonus* spp. and *Prosopium* spp., bull trout *Salvelinus confluentus* and lake trout (Cuerrier and Schultz 1957), and "glacial relict" species such as the deepwater sculpin *Myoxocephalus thompsonii* (Kontula and Väinölä 2003).

Fish collection and tissue sampling.—Trout were angled from the four Rocky Mountain House lakes with barbless hook and line May–September 2007. Angling is a low-mortality sampling method (Schisler and Bergersen 1996) and has been shown to have no size bias among catchable (≥120-mm) rainbow trout (Hetrick and Bromaghin 2006). Several recreational anglers also offered their freshly caught fish for sampling. Lake trout and lake and pygmy whitefishes were caught with gill nets 15–21 October 2007. Although our primary focus was on rainbow trout, we also analyzed samples from brook and lake trouts and lake and pygmy whitefishes to broaden the study to include several salmonid species; these four were used because they were readily available in sufficient numbers from our study lakes.

Angled trout were held in a solution of clove oil (100 mg/L) and water for approximately 1 min until the fish lost equilibrium, no longer responded to touch, and exhibited slowed but regular breathing (stage 4 anesthesia; Keene et al. 1998). A 2–3-mm dorsal incision was made posterior to and slightly below the dorsal fin with a scalpel to pierce the skin. A 14-gauge Tru-Cut soft tissue biopsy needle was then inserted through the incision, parallel to the skin, into the dorsal muscle (McAndrew 1981). Fish under 245 mm were generally not biopsied as we deemed the procedure too invasive for smaller individuals; this also precluded sampling newly stocked trout. No topical antiseptic was used as use of antiseptic has either no effect on healing time in fish (Wagner et al. 1999) or may actually hinder healing (Stoskopf 1993). Because the incision was small and no epidermal tissue was removed, the postbiopsy wound was very small (<5 mm). Removal of muscle samples using this biopsy method was rapid (<10 s). We used a sharp pair of scissors to remove adipose and caudal fin clips (1–3-cm^2 clips from the lower lobe of the tail) from each fish; however, collection of caudal fin clips did not begin until 6 August 2007. Individual tissue samples were transferred to Eppendorf tubes and immediately placed on ice. After biopsy, trout were held in a flow-through recovery chamber suspended from floats in the

lake. Once a trout had recovered from anesthesia (stage 5; Keene et al. 1998), typically 3–5 min after sampling, it was released. The biopsy needle, scalpel, and scissors were sterilized in a 95% solution of ethanol between procedures.

Muscle tissue, adipose fins, and caudal fins from lake whitefish, lake trout, and pygmy whitefish were excised and frozen for future analysis. Adipose fin clips from pygmy whitefish did not provide enough material for stable isotope analysis, so only caudal fin clips were analyzed for this species.

Laboratory methods.—Fin clips were rinsed in distilled water prior to analysis and all tissue samples were freeze-dried for 24 h. Muscle samples were homogenized into a fine powder and fin clips were cut into small pieces with scissors. Dried samples were weighed (1.0 ± 0.10 mg) into tin capsules and submitted to the University of Saskatchewan Department of Soil Sciences for stable carbon and nitrogen isotope analysis. Samples were processed with an ANCA G/S/L elemental analyzer coupled to a Tracer/20 mass spectrometer (Europa Scientific, Crewe, UK); measurement error (95% confidence interval [CI]) was ± 0.20‰ for both $\delta^{15}N$ and $\delta^{13}C$ (M. Stocki, University of Saskatchewan, personal communication). Results are presented in δ notation (where $\delta^{15}N$ or $\delta^{13}C = [\{R_{sample}/R_{standard} - 1\}] \times 1{,}000$, with $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$). The international reference standards are PeeDee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The internal reference is egg albumen. The SD of reference materials ($n = 91$) was 0.09‰ for $\delta^{13}C$ and 0.05‰ for $\delta^{15}N$. Twelve samples were also analyzed in duplicate to test the efficiency of our homogenization procedure.

Carbon-to-nitrogen elemental ratios (C:N) are indicative of a sample's lipid content (Post et al. 2007), and, because lipids are depleted in ^{13}C, many researchers elect to remove them chemically (e.g., Beaudoin et al. 2001) or correct for them mathematically (Post et al. 2007). In samples with low C:N ratios (<3.5 in aquatic animals), and thus low lipid content, lipid removal has little effect on $\delta^{13}C$ signatures (Post et al. 2007). Therefore, we did not remove or correct for lipids, as mean C:N ratios for the three tissues in this study were typically less than or not different from 3.5 (Hanisch 2009).

Statistical analyses.—Paired *t*-tests were used to assess differences between fins and muscle in $\delta^{15}N$ and $\delta^{13}C$ and between C:N ratios of fins and muscle. Relationships between fin and muscle isotope signatures were examined by linear regression analysis (Zar 1999), which was also used to determine if differences in isotope signatures between fin and muscle pairs were affected by fish length and sampling date. Additionally, linear regression analysis was used to generate models to convert isotope signatures of fin to those of muscle. When relationships between muscle and fin were statistically significant, we used 95% CIs of the slope to determine if slopes differed significantly from 1.0. If fish length or sampling date had a significant effect on the difference between fin and muscle signatures, analysis of covariance (ANCOVA; Zar 1999) was used to test for an interaction. Sample size estimations (Cohen 1969; Zar 1999) were performed to determine how many individuals were needed to establish regression equations to convert fin signatures to those of muscle. Analysis of stem and leaf plots identified the $\delta^{13}C$ signature and C:N ratio of one lake trout dorsal muscle sample as outliers. These two values were removed for all $\delta^{13}C$ and C:N analyses of lake trout. All data analyses were conducted in SPSS for Mac, version 16.0.1 (SPSS, Inc., Chicago, Illinois). Tests were considered statistically significant when P was less than 0.05.

Results

Tissue Biopsy

Two hundred forty-three trout were biopsied. For ca. 80% of fish, only one biopsy (yielding 3–4 mg of dried muscle tissue) was needed for SIA, and no fish required more than two biopsies. Short-term sampling mortality was 2.5% in biopsied fishes (6 of 243). Three of these fish died due to blood loss from deep hooking, whereas the remaining three did not recover from anesthesia.

Tissue Comparison

There was no difference between 12 duplicate subsamples for either $\delta^{15}N$ (paired *t*-test: $t_{11} = 0.066$, $p = 0.948$) or $\delta^{13}C$ (paired *t*-test: $t_{11} = 0.200$, $P = 0.845$) submitted to test the efficiency of our homogenization procedure. Differences in C:N ratios sometimes occurred between dorsal muscle and fin tissues, but when these differences occurred, they were small: rainbow trout muscle versus caudal fin (–0.21 ± 0.14 [mean difference ± SD]; $P < 0.01$), brook trout muscle versus caudal fin (0.34 ± 0.31; $P < 0.01$), lake whitefish muscle versus adipose fin (–0.10 ± 0.31; $P < 0.01$), lake trout muscle versus adipose fin (0.15 ± 0.18; $P < 0.01$), and pygmy whitefish muscle versus caudal fin (–0.58 ± 0.36; $P < 0.01$).

For rainbow trout, $\delta^{15}N$ signatures of neither adipose nor caudal fins were different from those of muscle (Table 1), and $\delta^{13}C$ signatures did not differ between caudal fin and muscle, although adipose fins were slightly enriched in ^{13}C relative to muscle tissue (Table

TABLE 1.—Mean ± SD $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of muscle and fin (adipose and caudal) tissue for five salmonid species. "Difference" is the mean (± SD) difference of each fin type from muscle for each isotope–tissue pair. Also shown are the results from paired *t*-tests of significance examining fin–muscle differences ($P < 0.05$*, $P < 0.01$**, $P < 0.0001$***). Because one outlier was removed, $n = 15$ for all lake trout $\delta^{13}C$ comparisons.

		Carbon		Nitrogen	
Species	Tissue (*n*)	$\delta^{13}C$	Difference	$\delta^{15}N$	Difference
Rainbow trout	Muscle (143)	−24.10 ± 4.17		7.73 ± 0.71	
	Adipose fin (143)	−23.43 ± 4.21	+0.67 ± 0.76***	7.72 ± 0.70	−0.012 ± 0.41
	Caudal fin (55)	−22.70 ± 3.97	+0.090 ± 1.00	7.89 ± 0.77	+0.14 ± 0.63
Brook trout	Muscle (16)	−27.04 ± 1.37		8.54 ± 0.43	
	Adipose fin (16)	−25.22 ± 1.39	+1.82 ± 0.44***	8.52 ± 0.51	−0.019 ± 0.24
	Caudal fin (5)	−25.31 ± 1.61	+1.63 ± 0.58**	9.35 ± 0.62	+0.74 ± 0.21***
Lake trout	Muscle (15,16)	−27.16 ± 0.50		10.14 ± 1.35	
	Adipose fin (15,16)	−26.02 ± 0.55	+1.13 ± 0.49***	9.51 ± 1.34	−0.63 ± 0.45***
	Caudal fin (15,16)	−25.99 ± 0.51	+1.17 ± 0.61***	10.28 ± 1.59	+0.14 ± 0.54
Lake whitefish	Muscle (17)	−27.64 ± 1.12		8.16 ± 0.93	
	Adipose fin (16)	−27.42 ± 1.08	+0.22 ± 0.37*	7.93 ± 0.94	−0.23 ± 0.52
	Caudal fin (17)	−26.62 ± 1.23	+1.11 ± 0.42***	8.17 ± 1.37	+0.22 ± 0.49
Pygmy whitefish	Muscle (26)	−29.13 ± 0.82		10.15 ± 0.69	
	Caudal fin (26)	−28.03 ± 1.06	+1.10 ± 0.67***	8.95 ± 0.76	−1.21 ± 0.40***

1). $\delta^{15}N$ signatures of brook trout adipose fins did not differ from those of muscle, while $\delta^{15}N$ signatures of caudal fins and $\delta^{13}C$ signatures of adipose and caudal fins were significantly higher than muscle signatures (Table 1). For lake trout, $\delta^{15}N$ signatures of adipose, but not caudal, fins were significantly lower than those of muscle (Table 1). In contrast, $\delta^{13}C$ signatures of adipose and caudal fins were both higher than muscle (Table 1). $\delta^{15}N$ signatures of adipose and caudal fins for lake whitefish did not differ from those of muscle, but $\delta^{13}C$ signatures of both fins were higher than muscle (Table 1). For pygmy whitefish, $\delta^{15}N$ signatures of caudal fins were lower than those of muscle (Table 1), while $\delta^{13}C$ signatures of caudal fins were higher than muscle (Table 1). Fins from all five species were strongly correlated with muscle $\delta^{15}N$ and $\delta^{13}C$ signatures except for $\delta^{13}C$ signatures of lake trout caudal fins (Figure 1). Ninety-five-percent CIs of all significant regression line slopes (Figure 1) included 1.0 except for the relationship between $\delta^{15}N$ signatures of rainbow trout muscle and adipose fin (95% CI of slope = 0.710–0.904).

Effects of Sampling Date and Body Length

Sampling date and fish body length occasionally had statistically significant effects on the difference in isotope signatures between fin and muscle; however, these effects were generally small (Figure 2). For rainbow trout, the difference in $\delta^{15}N$ between adipose fin and muscle signatures was positively related to sampling date ($R^2 = 0.055$) but negatively related to trout length ($R^2 = 0.095$; Figure 2). In contrast, the difference between caudal fin and muscle was unrelated to sampling date but positively correlated with trout length ($R^2 = 0.25$; Figure 2). The interaction term between the two fin types was significant for trout length (ANCOVA: $F_{1,\ 194} = 38.67$, $P < 0.001$). Neither rainbow trout length nor sampling date had a significant effect on the difference in $\delta^{13}C$ between adipose fin and muscle (Figure 2), but the difference with muscle was positively related to fish length ($R^2 = 0.14$) and negatively related to sampling date ($R^2 = 0.042$) for caudal fin (Figure 2).

For lake trout, the difference in $\delta^{15}N$ between adipose fin and muscle and the difference in $\delta^{13}C$ between caudal fin and muscle were positively related to fish length ($R^2 = 0.37$ and $R^2 = 0.49$, respectively; Figure 2). Differences in $\delta^{15}N$ between either fin type and muscle were unrelated to length in lake whitefish, but differences in $\delta^{13}C$ between caudal fin and muscle were positively related to fish length ($R^2 = 0.37$; Figure 2). For pygmy whitefish, differences in $\delta^{15}N$ and $\delta^{13}C$ signatures between caudal fin and muscle were unrelated to fish length (data not shown; $P > 0.05$), and differences in $\delta^{15}N$ and $\delta^{13}C$ between both fins and muscle were unrelated to brook trout length or sampling date (data not shown; $P > 0.05$).

$\delta^{15}N$ and $\delta^{13}C$ Regression Conversion Models

The majority (17 of 18) of regression models that can be used to convert $\delta^{15}N$ and $\delta^{13}C$ signatures of adipose and caudal fins to those of muscle revealed significant ($P < 0.05$) relationships (Table 2). These models typically explained between 68% and 97% of variance in isotopic signatures of dorsal white muscle (Figure 1). In 10 of the 17 significant conversion models, 95% CIs of slopes included 1.0, and of the seven slopes that did not include 1.0, the upper limits of most closely

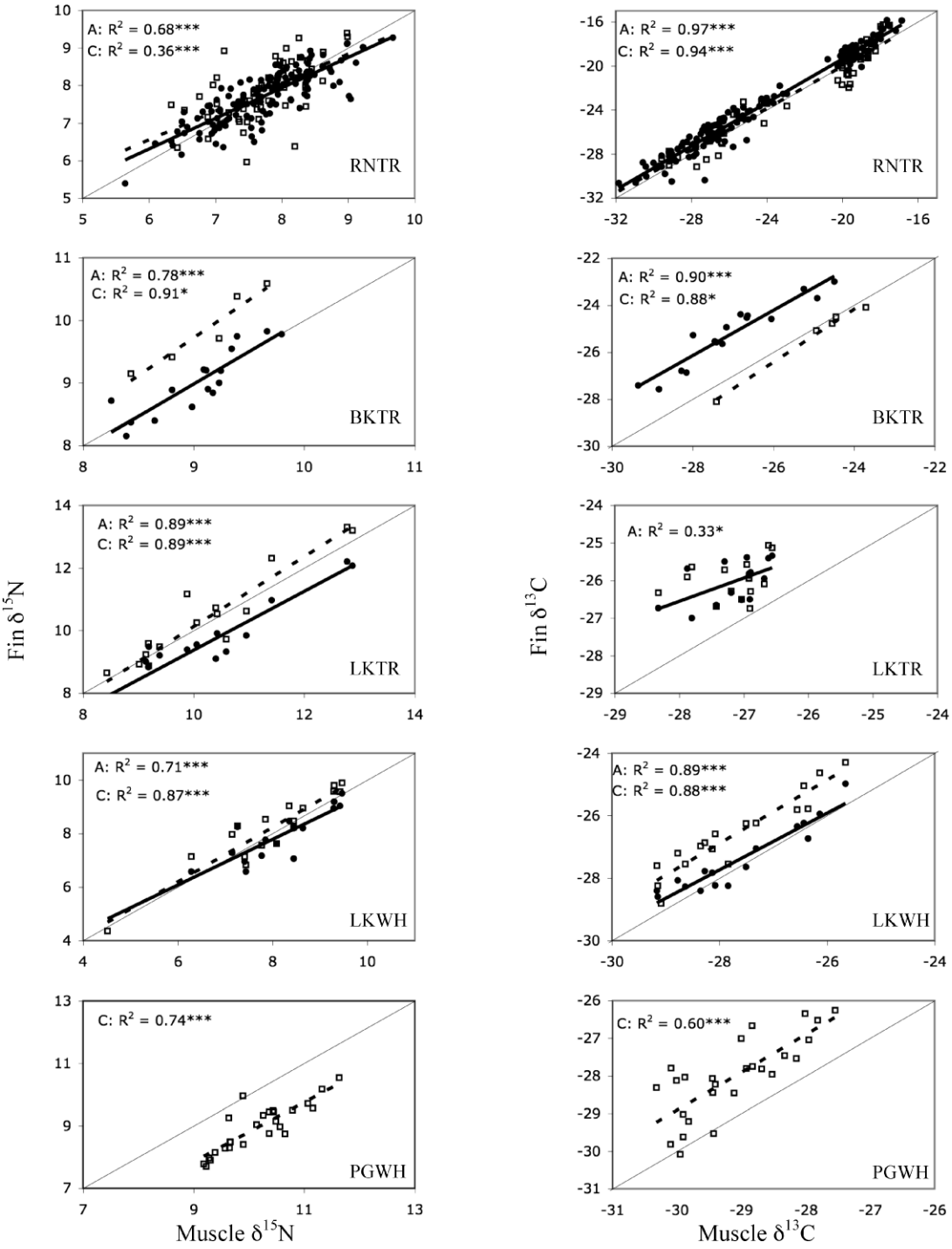

FIGURE 1.—Relationships between $\delta^{15}N$ (left panels) and $\delta^{13}C$ (right panels) isotope signatures of dorsal muscle and adipose (A; solid circles and solid lines) and caudal fins (C; open squares and dashed lines) of rainbow trout (RNTR), brook trout (BKTR), lake trout (LKTR), lake whitefish (LKWH), and pygmy whitefish (PGWH). R^2-values are presented for significant regressions ($P < 0.05$*; $P < 0.001$***). The fine dashed lines indicate 1:1 relationships.

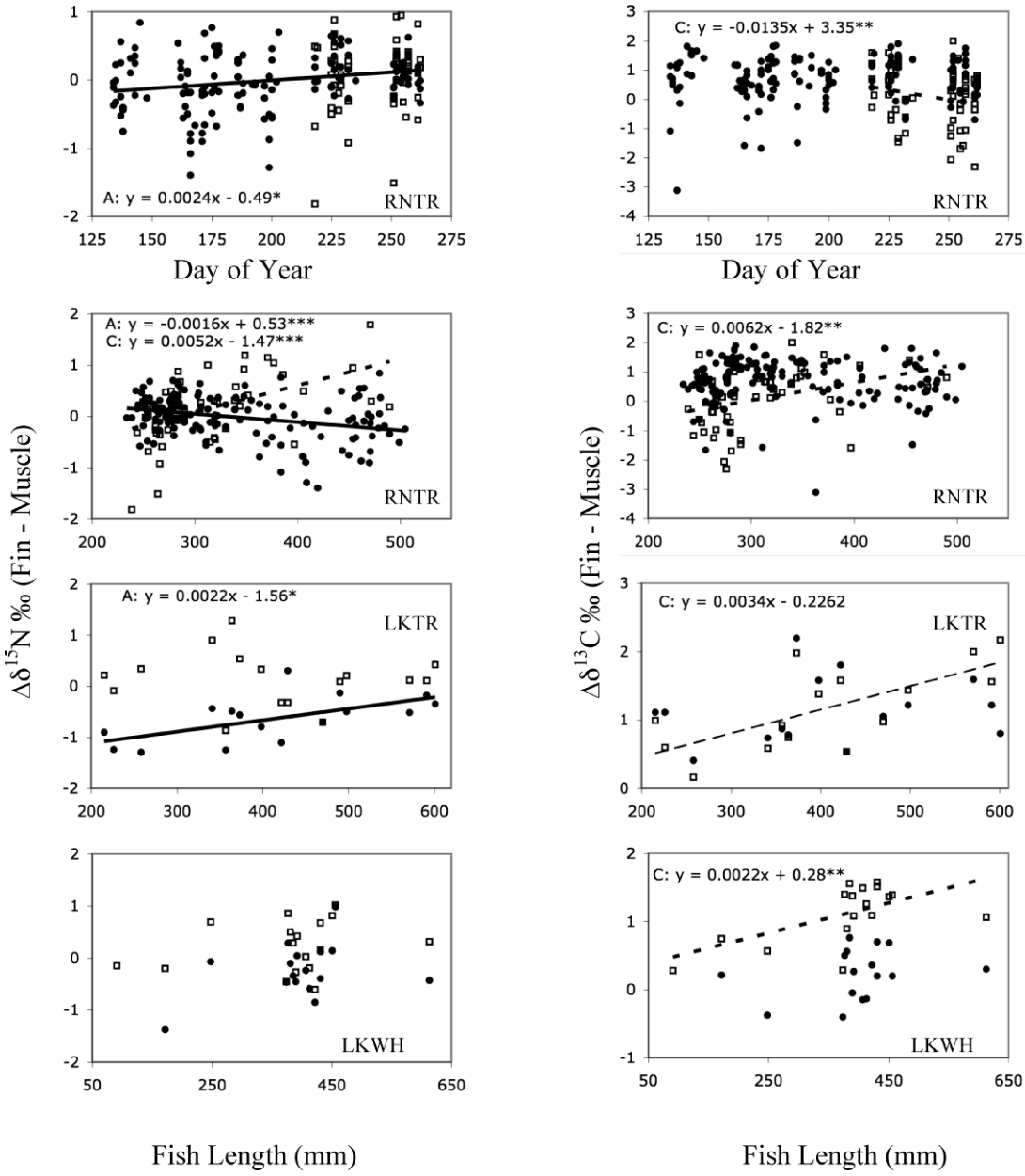

FIGURE 2.—Regressions of the differences in the $\delta^{15}N$ (left panels) and $\delta^{13}C$ (right panels) signatures between adipose (A; solid circles and solid lines) and caudal fins (C; open squares and dashed lines) and muscle on day of the year and fish length for rainbow trout (RNTR), lake trout (LKTR), and lake whitefish (LKWH). Regression lines and equations are presented for significant regressions ($P < 0.05$*; $P < 0.01$**; $P < 0.001$***); the R^2-values are presented in the text.

approached 1.0 (Table 2). A priori sample size estimations based on preliminary data from 2006 (J. R. Hanisch, unpublished) indicated that between 5 and 20 samples would be needed to establish regression conversion models at power of 0.9 with correlation coefficients between 0.99 and 0.66.

Discussion

Tissue Comparison

Our muscle biopsy method was accompanied with low mortality for fish greater than 245 mm (Hanisch 2009); thus, it should be considered as a nonlethal

TABLE 2.—Linear regression equations and 95% confidence intervals (CIs) of slopes to convert stable isotope signatures of adipose (A) and caudal (C) fins to signatures of dorsal muscle (M) for five salmonid species. ($P < 0.05$*, $P < 0.01$**, $P < 0.0001$***). R^2 = values are presented in Figure 1.

Species	Stable isotope	Adipose fin–muscle	95% CI of slope	Caudal fin–muscle	95% CI of slope
Rainbow trout	Nitrogen	M = 0.8412(A) + 1.2387***	0.746–0.937	M = 0.4748(C) + 4.0063***	0.301–0.649
	Carbon	M = 0.9753(A) − 1.2507***	0.946–1.005	M = 0.9798(C) − 0.5486***	0.910–1.049
Brook trout	Nitrogen	M = 0.7504(A) + 2.147***	0.519–0.982	M = 0.7531(C) + 1.5648*	0.320–1.186
	Carbon	M = 0.9356(A) − 3.4445***	0.758–1.113	M = 0.9280(C) − 3.4649*	0.248–1.571
Lake trout	Nitrogen	M = 0.9479(A) + 1.1295***	0.758–1.138	M = 0.8002(C) + 1.914***	0.642–0.959
	Carbon	M = 0.5253(A) − 13.489*	0.072–0.979	Nonsignificant relationship	
Lake whitefish	Nitrogen	M = 0.8324(A) + 1.5615***	0.528–1.137	M = 0.8574(C) + 0.9367***	0.677–1.038
	Carbon	M = 0.9803(A) − 0.7678***	0.785–1.176	M = 0.8677(C) − 4.6371***	0.693–1.042
Pygmy whitefish	Nitrogen	Not analyzed		M = 0.7742(C) + 3.228***	0.578–0.970
	Carbon	Not analyzed		M = 0.5948(C) − 12.456***	0.389–0.801

alternative to sacrificing fish to obtain muscle for stable isotope analysis. Several ecotoxicology studies also found biopsied muscle plugs to be an effective, generally nonlethal alternative to terminal sampling (e.g., Baker et al. 2004); however, particularly because the procedure involves anesthesia, tissue biopsy can sometimes result in mortality (McAndrew 1981). The results of our research demonstrate that salmonid fins are good surrogates for muscle in stable isotope studies. Significantly, we found $\delta^{15}N$ and $\delta^{13}C$ signatures of adipose fins of rainbow, brook, and lake trouts, and lake and pygmy whitefishes to be strongly correlated with signatures of dorsal white muscle, and only $\delta^{13}C$ signatures for caudal fins of lake trout were not strongly correlated with muscle. As in previous studies, differences between fin and muscle, when they occurred, were generally small (Dempson and Power 2004; Jardine et al. 2005; Sanderson et al. 2009). Thus, a total reliance on muscle tissue for fish SIA is not required for these salmonids.

It is not clear why fin tissues of these salmonid species were consistently enriched in ^{13}C relative to muscle. Lipids are among the macromolecules most depleted in ^{13}C (DeNiro and Epstein 1978); thus, an adipose fin might be expected to be depleted in ^{13}C relative to muscle. Contrary to its name, however, the salmonid adipose fin contains very little fat (Weisel 1968), and C:N ratios of adipose fins we analyzed were low and similar to dorsal muscle (Hanisch 2009), suggesting a low lipid content (Post et al. 2007). Low C:N ratios confirmed qualitative observations during homogenization that adipose fins were composed predominately of skin and muscle, and it is possible that the presence of skin caused the observed ^{13}C enrichment. At least one study has reported higher $\delta^{13}C$ values in skin relative to muscle in an ectothermic animal (loggerhead sea turtle *Caretta caretta*; Revelles et al. 2007), and Sotiropoulos et al. (2004) found that whole juvenile minnows (including skin) were enriched in ^{13}C compared with muscle-only samples. The presence of bone from fin rays, which is generally enriched in ^{13}C relative to muscle (Syväranta et al. 2008), may explain the higher $\delta^{13}C$ values of caudal fins. However, C:N ratios of caudal fins, like those of adipose fins, generally did not differ from muscle. When differences in C:N ratios did occur, they were often small, indicating that C:N ratios alone cannot explain the consistent enrichment of fins in ^{13}C relative to muscle.

The lack of strong correlations of $\delta^{13}C$ between caudal fin and muscle for lake trout was surprising and contrary to other patterns seen in our study and to previously published results for salmonids. In the absence of a good mechanistic explanation, we cannot rule out sample contamination, and because a lack of correlation between fin and muscle appears uncommon, it should not be expected frequently in other species or sites.

Effects of Body Length and Sampling Date

If the isotope signatures of prey items change from spring through fall, the primary period of growth in northern temperate fishes, tissues of predatory fish will reflect these changes (Perga and Gerdeaux 2005) after a lag caused by tissue turnover. However, because different fish tissues often differ in turnover rates (Pinnegar and Polunin 1999) and because fish growth can lead to differences in isotope signatures between tissues (e.g., Miller 2006), a tissue exhibiting a slower turnover or growth rate would "lag behind" another tissue in expressing the changing stable isotope signal of prey. Consistent with these scenarios, date of capture sometimes had a significant effect on the difference between the $\delta^{15}N$ signatures of adipose fin and muscle and between the $\delta^{13}C$ signatures of caudal fin and muscle. Nevertheless, the R^2-values were small (<0.1), suggesting that temporal patterns should not confound interpretation of SIA data from fins. Furthermore, Suzuki et al. (2005) recommended using fins as surrogates for muscle in stable isotope studies because

fin and muscle of juvenile Japanese temperate bass *Lateolabrax japonicus* had similar turnover rates.

Fish length occasionally affected the difference between isotope signatures of fin and muscle. Changes in turnover rates during ontogenetic diet shifts may explain this size effect (Jardine et al. 2005). It is also possible that the relative composition of fins changes with fish length, as reflected by C:N ratios. The C:N ratios of dorsal muscle in this study were not affected by length of rainbow trout, but ratios did decrease with length in both adipose and caudal fins (Hanisch 2009). The C:N ratios of lake whitefish adipose fins, pygmy whitefish dorsal muscle, and lake trout caudal fins also decreased with fish length (Hanisch 2009). Qualitatively, fins from larger fish appeared to have more muscle tissue than fins from smaller fish. Thus, it appears that the relative compositions (e.g., proportions of muscle, connective tissue, skin, etc.) of fins may be different in fish of different size. While suggestive, C:N ratios are only a coarse indicator of tissue composition, and a more detailed analysis of how fins change as fish grow would be required to understand mechanisms underlying these patterns.

Fins as Surrogates for Muscle

Because of similarities in isotopic signatures between fin and muscle, fin clips could be used as a direct surrogate for muscle without applying a correction factor. When used in mixing models, however, the absolute difference between the two tissues has potential to be magnified as an increased error rate. Post et al. (2007) presented figures to estimate the error introduced into two-source mixing models if samples are not corrected for lipid content. Because percent lipid content can be converted to a difference in $\delta^{13}C$, ($\Delta\delta^{13}C$), Post et al.'s figures can be used effectively to predict the potential error introduced into a two-source mixing model by the absolute $\Delta\delta^{13}C$ between fin and muscle. According to Post et al. (2007), 1‰ $\Delta\delta^{13}C$ could introduce an error as small as 5–10% when working with a mixing model having 10–12‰ between end members or an error as large as 25–50% in a mixing model having a difference of only 2‰ between end members. A common use of mixing models in aquatic systems is to determine the importance of littoral and pelagic primary production in an organism's diet, where the difference in $\delta^{13}C$ between end members (e.g., pelagic and littoral algae) is typically 7–8‰ (France 1995). In such a model, a 1‰ $\Delta\delta^{13}C$ would introduce a 10–15% error. This error is not large but could be ecologically significant, suggesting that a difference of 1‰ between fin and muscle may represent an upper limit for using $\delta^{13}C$ values of fins as a surrogate for muscle without correction. While fish length did not have a large effect on the difference between fin and muscle in our study, the effects of length appear to be tissue dependent, and researchers should quantify this difference in their own research. Accounting for a large effect of fish length, if it occurs, could potentially improve fin-to-muscle conversion equations or reduce the error introduced into mixing models when using fin as a surrogate for muscle. While stable isotope data from fins are not necessarily less ecologically meaningful than data from muscle, fin signatures should be similar to muscle if direct comparisons with other published studies are made.

When the difference between fin and muscle is greater than 1‰ but the fin–muscle isotope relationships are significant, we recommend using linear regression to estimate muscle signatures from fin signatures. When the relationship between the fin and muscle signatures is very strong, as was usually the case in our study, samples from only a few fish are needed to derive this equation. For example, only 10 fish would be needed to establish a conversion equation at a power of 0.90 and a significance level of $\alpha = 0.05$ when samples have a correlation of 0.85 (Cohen 1969; Zar 1999). As discussed previously, small differences between fin and muscle may be magnified in quantitative modeling. Thus, caution should be used when applying conversion equations with low R^2-values or slopes that differ largely from 1.0.

Strong correlations between stable isotope signatures of salmonid fin and muscle and, in some cases, the lack of statistical differences between the two tissue types make fin clips an attractive, nonlethal alternative for dorsal muscle in the five salmonid species included in our study. Sampling fins is easier and less time-consuming than muscle biopsy or sacrificing fish. Sampling fins also reduces the amount of time fish are held out of the water or under anesthesia and thus causes less stress. With one exception ($\delta^{13}C$ signatures of lake trout caudal fins), the $\delta^{13}C$ and $\delta^{15}N$ signatures of fin tissue were either not different from muscle or were strongly correlated with muscle. Because fish length and sampling date sometimes affected the difference between fin and muscle isotope signatures, fish representative of the expected length distribution and sampling seasons should be included if fin to muscle conversion equations are created. However, because the effects of sampling date and fish length seem to be minimal (Sanderson et al. 2009; this study), accounting for their effects may only slightly improve the relationships between fin and muscle.

Other portions of fish have been evaluated as alternatives for muscle in stable isotope studies, including mucus (which has a faster turnover time

than fin or muscle; Church et al. 2009) and scales (which have a slower turnover time; Sinnatamby et al. 2008). Each method of nonlethal sampling may have unintended consequences; for example, removing an adipose fin may impair a salmonid's ability to swim in turbulent water (e.g., Reimchen and Temple 2004) or may suggest to an angler that a fish is of hatchery origin, while removing protective mucus may expose a fish to infection. Additionally, an adipose fin will not regenerate after clipping, while caudal fins clips regenerate within several weeks (S. Herman, Alberta Sustainable Resources Department, personal communication). Mucus, fin, and scale could possibly be analyzed jointly to elucidate, respectively, short, intermediate, and long-term feeding habits or migration patterns when the relationships of these tissues to muscle is known. Researchers should thus evaluate the purpose and temporal scale of their stable isotope study when deciding which nonlethal tissue to sample and how large a difference between tissues is acceptable without correction.

Our research is the first to document fin–muscle isotope relationships in lake trout, and lake and pygmy whitefishes. Fins of other *Coregonus*, *Prosopium*, *Oncorhynchus*, and *Salvelinus* species—each of which has some forms that are of conservation concern at the national, state, or provincial level in North America (NatureServe 2009)—would likely be good surrogates for muscle. In addition, initial evidence suggests that small-bodied fish species, typically sacrificed to obtain muscle tissue, also exhibit strong relationships between fin and muscle tissues (e.g., Kelly et al. 2006).

It should be incumbent upon ecologists to avoid lethal sampling when possible. Removing individuals could alter the structure of a population under study, and some researchers have limited their sample sizes for precisely this reason (e.g., Sierszen et al. 2003). Animal welfare, including fish welfare, has attained prominence in popular and scientific literature (Huntingford et al. 2006), and the search for less-invasive alternatives to destructive sampling is gaining importance. Ecologists often work in populated areas and are frequently approached by interested members of the public. Lethal sampling in these cases has the potential to engender negative public opinion. This study demonstrates that lethal sampling of salmonids to obtain tissue in stable isotope studies is not necessary. Future research should focus on the mechanisms controlling isotopic turnover in different fish tissue types, document turnover rates in fish fins (e.g., Suzuki et al. 2005), and continue to evaluate nonlethal sampling alternatives for other taxa and other applications (e.g., mercury analysis; Baker et al. 2004).

Acknowledgments

We thank Alex Wolfe and Brian Parker for guidance during the project, and Heather Currie, Darryl Edwards, Eric Hegerat, Alissa Moenting, Leslie Nasmith, Candra Schank, and Homun Yee for assistance in the field and laboratory. Lars Brinkman and Joe Rasmussen (University of Lethbridge), and Rob Watt, Queenie Gray, Sean LeMoine, and Tyler Tilson (Waterton Lakes National Park) assisted with collections from Waterton Lake. We also thank Steve Herman, Rocky Konynenbelt, and Rod Burns (Alberta Sustainable Resource Development), and Corey Rasmussen (Alberta Conservation Association) for logistical support. Myles Stocki deserves special thanks for quickly processing large numbers of SIA samples. Thanks also to three anonymous reviewers for their helpful comments. This research was funded by grants from the Alberta Conservation Association, Alberta Ingenuity Fund, Canadian Circumpolar Institute, and Alberta Sport, Recreation, Parks and Wildlife Foundation; a D. Alan Birdsall Memorial Scholarship to J.R.H.; and Parks Canada funds to G.J.S.

References

Baker, R. F., P. J. Blanchfield, M. J. Paterson, R. J. Flett, and L. Wesson. 2004. Evaluation of nonlethal methods for the analysis of mercury in fish tissue. Transactions of the American Fisheries Society 133:568–576.

Barnes, C., C. J. Sweeting, S. Jennings, J. T. Barry, and N. V. C. Polunin. 2007. Effect of temperature and ration size on carbon and nitrogen stable isotope fractionation. Functional Ecology 21:356–362.

Beaudoin, C. P., E. E. Prepas, W. M. Tonn, L. I. Wassenaar, and B. G. Kotak. 2001. A stable carbon and nitrogen isotope study of lake food webs in Canada's Boreal Plain. Freshwater Biology 46:465–477.

Brinkmann, L. 2007. Mercury biomagnification in the upper South Saskatchewan River basin. Master's thesis. University of Lethbridge, Lethbridge, Alberta.

Carpenter, S. R., J. J. Cole, J. R. Hodgson, J. F. Kitchell, M. L. Pace, D. Bade, K. L. Cottingham, T. E. Essington, J. N. Houser, and D. E. Schindler. 2001. Trophic cascades, nutrients, and lake productivity: whole-lake experiments. Ecological Monographs 71:163–186.

Church, M. R., J. L. Ebersole, K. M. Rensmeyer, R. B. Couture, F. T. Barrows, and D. L. G. Noakes. 2009. Mucus: a new tissue fraction for rapid determination of fish diet switching using stable isotope analysis. Canadian Journal of Fisheries and Aquatic Sciences 66:1–5.

Cohen, J. 1969. Statistical power analysis for the behavioral sciences. Academic Press, New York.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2007. COSEWIC Annual Report. Available: www.sararegistry.gc.ca/. (September 2008.)

Cuerrier, J. P., and F. H. Schultz. 1957. Studies of lake trout and common whitefish in Waterton Lakes, Waterton

Lakes National Park, Alberta. Canadian Wildlife Service, Series 3, Number 5, Ottawa.

Cunjak, R. A., J. M. Roussel, M. A. Gray, J. P. Dietrich, D. F. Cartwright, K. R. Munkittrick, and T. D. Jardine. 2005. Using stable isotope analysis with telemetry or mark–recapture data to identify fish movement and foraging. Oecologia 144:636–646.

Dempson, J. B., and M. Power. 2004. Use of stable isotopes to distinguish farmed from wild Atlantic salmon, *Salmo salar*. Ecology of Freshwater Fish 13:176–184.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of stable carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

France, R. L. 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnology and Oceanography 40:1310–1313.

Fry, B. 2006. Stable isotope ecology. Springer, New York.

Grey, J. 2006. The use of stable isotope analysis in freshwater ecology: current awareness. Polish Journal of Ecology 54:563–584.

Halverson, M. A. 2008. Stocking trends: a quantitative review of governmental fish stocking in the United States, 1931 to 2004. Fisheries 33:69–75.

Hanisch, J. R. 2009. $\delta^{13}C$ and $\delta^{15}N$ signatures in salmonid muscle and fin tissues: nonlethal sampling methods for stable isotope analysis of fish. Master's thesis. University of Alberta, Edmonton.

Harvey, C. J., P. C. Hanson, T. E. Essington, P. B. Brown, and J. F. Kitchell. 2002. Using bioenergetics models to predict stable isotope ratios in fishes. Canadian Journal of Fisheries and Aquatic Sciences 59:115–124.

Hetrick, N. J., and J. F. Bromaghin. 2006. Sampling bias of hook-and-line gear used to capture rainbow trout in Gertrude Creek, Alaska. North American Journal of Fisheries Management 26:13–23.

Huntingford, F. A., C. Adams, V. A. Braithwaite, S. Kadri, T. G. Pottinger, P. Sandøe, and J. F. Turnbull. 2006. Current issues in fish welfare. Journal of Fish Biology 68:332–372.

Jardine, T. D., M. A. Gray, S. M. McWilliam, and R. A. Cunjak. 2005. Stable isotope variability of tissues of temperate stream fishes. Transactions of the American Fisheries Society 134:1103–1110.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Díaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Tayler, and M. L. Warren Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372–407.

Keene, J. L., D. L. G. Noakes, R. D. Moccia, and C. G. Soto. 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research 29:89–101.

Kelly, M. H., W. G. Hagar, T. D. Jardine, and R. A. Cunjak. 2006. Nonlethal sampling of sunfish and slimy sculpin for stable isotope analysis: how scale and fin tissue compare with muscle tissue. North American Journal of Fisheries Management 26:921–925.

Klemetsen, A., P. Amundsen, J. B. Dempson, B. Jonsson, N. Jonsson, M. F. O'Connel, and E. Mortensen. 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecology of Freshwater Fish 12:1–59.

Kontula, T., and R. Väinölä. 2003. Relationships of Palearctic and Nearctic 'glacial relict' *Myoxocephalus* sculpins from mitochondrial DNA data. Molecular Ecology 12:3179–3184.

McAndrew, B. J. 1981. Muscle biopsy technique for fish stock management. Veterinary Record 108:516–516.

McCarthy, I. D., and S. Waldron. 2000. Identifying migratory *Salmo trutta* using carbon and nitrogen stable isotope ratios. Rapid Communications in Mass Spectrometry 14:1325–1331.

Miller, T. W. 2006. Tissue-specific response of $\delta^{15}N$ in adult Pacific herring (*Clupea pallasi*) following an isotopic shift in diet. Environmental Biology of Fishes 76:177–189.

Morinville, G., and J. B. Rasmussen. 2006. Marine feeding patterns of anadromous brook trout (*Salvelinus fontinalis*) inhabiting an estuarine river fjord. Canadian Journal of Fisheries and Aquatic Sciences 63:2011–2027.

NatureServe. 2009. NatureServe Explorer: an online encyclopedia of life, version 7.1. NatureServe, Arlington, Virginia. Available: http://www.natureserve.org/explorer. (March 2009.)

Paine, R. T. 1980. Food webs: linkage, interaction strength, and community infrastructure. Journal of Animal Ecology 49:667–685.

Perga, M. E., and D. Gerdeaux. 2005. Are fish what they eat all year round? Oecologia 144:598–606.

Pinnegar, J. K., and N. V. C. Polunin. 1999. Differential fractionation of ^{13}C and ^{15}N among fish tissues: implications for the study of trophic interactions. Functional Ecology 13:225–231.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montaña. 2007. Getting to the fat of the matter: models, methods, and assumptions for dealing with lipids in stable isotope analysis. Oecologia 152:179–189.

Reimchen, T. E., and N. F. Temple. 2004. Hydrodynamic and phylogenetic aspects of the adipose fin in fishes. Canadian Journal of Zoology 82:910–916.

Revelles, M., L. Cardona, A. Aguilar, A. Borrell, G. Fernández, and M. San Félix. 2007. Stable C and N isotope concentration in several tissues of the loggerhead sea turtle *Caretta caretta* from the western Mediterranean and dietary implications. Scientia Marina 71:87–93.

Rounick, J. S., and B. J. Hicks. 1985. The stable carbon isotope ratios of fish and their invertebrate prey in four New Zealand rivers. Freshwater Biology 15:207–214.

Sanderson, B. L., C. D. Tran, H. J. Coe, V. Pelekis, E. A. Steel, and W. L. Reichert. 2009. Nonlethal sampling of fish caudal fins yields valuable stable isotope data for threatened and endangered fishes. Transactions of the American Fisheries Society 138:1166–1177.

Schisler, G. J., and E. P. Bergersen. 1996. Postrelease hooking mortality of rainbow trout caught on scented artificial baits. North American Journal of Fisheries Management 16:570–578.

Shannon, J. P., D. W. Blinn, G. A. Haden, E. P. Benenati, and K. P. Wilson. 2001. Food web implications of $\delta^{13}C$ and $\delta^{15}N$ variability over 370 km of the regulated Colorado

River, USA. Isotopes in Environmental and Health Studies 37:179–191.

Sierszen, M. E., M. E. McDonald, and D. A. Jensen. 2003. Benthos as the basis for arctic lake food webs. Aquatic Ecology 36:437–445.

Sinnatamby, R. N., J. B. Dempson, and M. Power. 2008. A comparison of muscle- and scale-derived $\delta^{13}C$ and $\delta^{15}N$ across three life history stages of Atlantic salmon, *Salmo salar*. Rapid Communications in Mass Spectrometry 22:2773–2778.

Sotiropoulos, M. A., W. M. Tonn, and L. I. Wassenaar. 2004. Effects of lipid extraction in stable carbon and nitrogen isotope analysis of fish tissue: potential consequences for food web studies. Ecology of Freshwater Fish 13:155–160.

Stoskopf, M. K. 1993. Surgery. Pages 91–97 *in* M. K. Stoskopf, editor. Fish medicine. Saunders, Philadelphia.

Suzuki, K. W., A. Kasai, K. Nakayama, and M. Tanaka. 2005. Differential isotopic enrichment and half-life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: implications for analyzing migration. Canadian Journal of Fisheries and Aquatic Sciences 62:671–678.

Syväranta, J., S. Vesala, M. Rask, J. Ruuhijärvi, and R. I. Jones. 2008. Evaluating the utility of stable isotope analysis of archived freshwater sample materials. Hydrobiologia 600:121–130.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80:1395–1404.

Wagner, G. N., E. D. Stevens, and C. Harvey-Clark. 1999. Wound healing in rainbow trout following surgical site preparation with a povidone–iodine antiseptic. Journal of Aquatic Animal Health 11:373–382.

Weisel, G. F. 1968. The salmonoid adipose fin. Copeia 1968:626–627.

Wissinger, S. A., A. R. McIntosh, and H. S. Greig. 2006. Impacts of introduced brown and rainbow trout on benthic invertebrate communities in shallow New Zealand lakes. Freshwater Biology 51:2009–2028.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, New Jersey.