CSIRO PUBLISHING

Marine and Freshwater Research
http://dx.doi.org/10.1071/MF13346

Important sources of variation to be considered when using fin clips as a surrogate for muscle in trophic studies using stable isotopes

David E. Galván[A,D], *Manuela Funes*[B], *Ana L. Liberoff*[A], *Florencia Botto*[C] *and Oscar O. Iribarne*[C]

[A]Centro Nacional Patagónico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Boulevard Brown 2915, (U9120ACV) Puerto Madryn, Chubut, Argentina.
[B]Facultad de Ciencias Naturales (FCN) – Universidad Nacional de la Patagonia, Boulevard Brown 3051, (U9120ACV) Puerto Madryn, Chubut, Argentina.
[C]Laboratorio de Ecología, Instituto de Investigaciones Marinas y Costeras (IIMyC), CONICET – UNMDP – 3er Piso, Funes 3250, (B7600WAG) Mar del Plata, Argentina.
[D]Corresponding author. Email: galvan@cenpat-conicet.gob.ar

Abstract. White muscle is the prevalent tissue for C and N stable isotope analysis in fish, requiring the death of the fish or biopsy procedures that could lead to infections or severe damage. Given that caudal fin-clipping does not seriously affect growth or condition, the present study assessed the suitability of caudal fin tissue as replacement for muscle tissue in trophic studies. Clips of caudal fin were a useful non-lethal surrogate of muscle samples in four studied reef-fish (*Diplodus argenteus*, *Pagrus pagrus*, *Acanthistius patachonicus* and *Pinguipes brasilianus*). Fin clips were easy to collect in quantities adequate for mass spectrometry analyses and had C : N ratios similar to those of white muscle with low lipid content. However, results showed that fin-muscle correction models should be specific and sampling design should be conducted to reduce spatial and temporal variation. Moreover, species-specific correction factors may not be valid for other populations of the same species if the presumed range of δX values differ from the population used to estimate the correction models. Results also showed that the fin-muscle relationship could vary with size. Thus, unless a non-ecological meaningful fin-muscle correlation with body size was previously identified, correction models should be estimated sampling a representative size range and fin samples should be used with caution to study size-related trophodynamics.

Additional keywords: carbon, growth, marine fish, nitrogen, sampling design, trophodynamic.

Received 31 December 2013, accepted 18 September 2014, published online 10 March 2015

Introduction

Identifying and quantifying the magnitude of sources of variation other than strictly feeding-related differences is critical for the correct interpretation of stable isotope data in trophic studies. The use of stable isotope data for carbon (C) and nitrogen (N) in trophic ecology is predicated on the assumption that stable isotope ratios of consumers' tissues are similar to that of the dominant food sources after accounting for discrimination (DeNiro and Epstein 1976). However, tissue-specific turnover rates and fractionation (Pinnegar and Polunin 1999) lead to tissues with varying stable isotopic compositions (Miller 2006) differing in their capability or usefulness to reflect consumers' food sources. In contrast, stable isotopic differences between tissues is a powerful tool for inferring movement patterns and home-range shifts by comparing tissues that integrate the stable isotopic composition of the food items consumed over different periods (e.g. blue cod, *Parapercis colias*, Rodgers and Wing 2008) or assess nutritional status by comparing tissues with different metabolic function (e.g. stream fish: Jardine *et al.* 2005).

A wide variety of fish tissues are used in ecological research, including bones (Estrada *et al.* 2005), gonads (Jardine *et al.* 2005), heart, blood, eyes (Miller 2006), red muscle (Pinnegar and Polunin 1999) and liver (Hesslein *et al.* 1993; Perga and Gerdeaux 2005). However, white muscle has been suggested to be the best tissue for ecological studies because of its low variability in $\delta^{13}C$ and $\delta^{15}N$ values when compared to other tissues in individuals with constant diet (Pinnegar and Polunin 1999; Sweeting *et al.* 2005). In contrast, the common procedure of obtaining samples of dorsal white muscle typically involves the death of the fish. Even though muscle biopsy for large fishes is possible, it could lead to infections or severe damage and thus it is not recommended for the study of endangered species such as sturgeons, *Scaphirhynchus albu* (Andvik *et al.* 2010). Given

that fish welfare is of increasing scientific and public concern (Huntingford *et al.* 2006), killing or damaging any fish for scientific purposes should be avoided.

Therefore, clips of caudal or dorsal fin and samples of mucus have been proposed as possible substitutes for white muscle (e.g. Church *et al.* 2009; Valladares and Planas 2012). Fin-clipping is a desirable non-lethal sampling technique because (1) it requires minimal equipment, handling time, and training (Sanderson *et al.* 2009), (2) fins regenerate fast (clipped fins require 1 month for complete regeneration, German and Miles 2010) and could regenerate even from complete amputation (Wills *et al.* 2008), and (3) caudal fin clipping does not affect growth or condition factors (Dietrich and Cunjak 2006). In addition, structural units of radial fins (lepidotrichia, actinotrichia and tegument) are primarily composed of proteins, mainly collagen, surrounded, in the case of lepidotrichia, by a fundamental mineralised matrix of chondroitin sulphate (Böckelmann and Bechara 2009), assuring low lipid and carbonate content, which helps to reduce sample treatments such as acidification or lipid extraction.

Most studies using fin as replacement for muscle in stable isotope analysis have been conducted on salmonids (McCarthy and Waldron 2000; Jardine *et al.* 2005; Sanderson *et al.* 2009; Hanisch *et al.* 2010) or other related freshwater species (Suzuki *et al.* 2005; Kelly *et al.* 2006; Andvik *et al.* 2010; Jardine *et al.* 2011; Fincel *et al.* 2012; Tronquart *et al.* 2012) and few studies have been conducted on strictly marine fish such as *Hippocampus guttulatus* (Valladares and Planas 2012), *Parapercis colias* and *Notolabrus celidotus* (Willis *et al.* 2013). Overall, results on fins (caudal, Jardine *et al.* 2005, 2011; Kelly *et al.* 2006; Sanderson *et al.* 2009; Hanisch *et al.* 2010; dorsal, Valladares and Planas 2012; Willis *et al.* 2013; pectoral, Andvik *et al.* 2010; Fincel *et al.* 2012), excluding adipose fins, as a substitute of white muscle, could be summarised as: (1) fin tissue correlates closely with muscle for $\delta^{13}C$ and $\delta^{15}N$; but fin tissue has higher $\delta^{13}C$ values than muscle in a predictable amount, whereas the reported differences between fin $\delta^{15}N$ and muscle $\delta^{15}N$ show no clear patterns (Jardine *et al.* 2005, 2011; Tronquart *et al.* 2012); (2) determinations of isotope ratios in fin samples have higher analytical error than in muscle samples and, therefore, a larger sample size is required (Sanderson *et al.* 2009; Jardine *et al.* 2011); (3) some studies pointed out that fin as a substitute of muscle should be used with caution because size-related trends need to be checked (Hanisch *et al.* 2010; Jardine *et al.* 2011). There are some cases of correlation between fin–muscle differences in δX values ($\Delta\delta X_{f-m}$, following Willis *et al.* 2013; notation where f is fin and m is muscle) and fish size (e.g. *Salmo salar* and *Salvelinus fontinalis*, Jardine *et al.* 2005; *Oncorhynchus tshawytscha* and *O. mykiss*, Sanderson *et al.* 2009; *Parapercis colias*, Willis *et al.* 2013), but such effects were considered large enough to be relevant in ecological studies by some authors (Sanderson *et al.* 2009; Hanisch *et al.* 2010).

Fin samples could be used as surrogates for muscle samples if both tissues reflect the diet of the fish over a similar time period; otherwise, fin isotope ratios could be directly related to the food web but those results are not comparable to the results published in the copious literature based on muscle samples. Although there are a few studies that estimate fin tissue turnover rates, the results show that both fin and muscle integrate comparable time periods (Suzuki *et al.* 2005; German and Miles 2010; Heady and Moore 2013) (summarised in Table 1). According to Heady and Moore (2013) the faster turnover rates for fin tissues was 9 days; however, it was estimated only on the basis of the distal portion of the caudal fin of early juvenile *O. mykiss* (and not homogenising the entire fin clip, as it was done in the other studies).

Table 1. Published carbon and nitrogen turnover rates of fin and muscle tissues
HL, half-life (days)

Reference	Tissue	Carbon HL	Nitrogen HL
German and Miles (2010)[A]	Fin	14	22
	Muscle	–	–
Suzuki *et al.* (2005)[B]	Fin	26	21
	Muscle	22	19
Heady and Moore (2013)[C]	Fin	–	9
	Muscle	–	28

[A]Half-lives were calculated from the equations presented in the fig. S1 of German and Miles (2010).
[B]Half-lives were derived from the equations in figs 2 and 3 of Suzuki *et al.* (2005).
[C]This reference used only the distal portion of the caudal fin for stable isotope analysis, instead of the entire clip, as was done in the other two references.

Given that fin-clipping is a non-lethal easy-to-implement sampling technique, the aim of this study was to assess the suitability of caudal fin isotopic composition as a replacement for white muscle isotopic composition in trophic studies by determining whether the differences between tissues ($\Delta\delta X_{f-m}$) vary with fish size in four species of temperate reef fish. In investigating the strength of those relationships for different species, how the size range of fish sampled affects them, and by simulating scenarios for interpretation of trophic data, this study also provides guidance for sampling design to other studies aiming to elucidate trophic relationships using fin δX values.

Materials and methods

Studied species

The four fish species selected for this study were: silver porgy, *Diplodus argenteus* (Valenciennes, 1830); red porgy, *Pagrus pagrus* Linné, 1758; Argentinean sea bass, *Acanthistius patachonicus* (Jenyns, 1842) and Brazilian sandperch, *Pinguipes brasilianus* Cuvier, 1829. These species are commonly found in the coastal rocky-reefs of northern Patagonia (40–43°S) (Galván *et al.* 2005, 2009*b*). *D. argenteus* is a sparid that feeds mainly on benthic invertebrates but also eats macroalgae (Dubiaski-Silva and Masunari 2006), although its digestibility and contribution to fish nutrition is doubtful (Dubiaski-Silva and Masunari 2006). *P. pagrus* is another sparid that presumably occupies a higher trophic level than *D. argenteus*, feeding on mobile macroinvertebrates such as crabs or large polychaetes and small fish (Brankevich *et al.* 1990). *A. patachonicus* and *P. brasilianus* are demersal species associated with structured habitats such as caves and crevices, and with distinct diets. Whereas the former feeds mainly in crabs, polychaetes and small fish, the second feeds principally on grazers (e.g. sea urchins and snails), and on

Table 2. Carbon and nitrogen isotopic composition of both tissues, and results of the comparisons between them, in the four species studied
L_F, fork length range (for sparids) (cm); L_T, total length range (for *Pinguipes brasilianus* and *Acanthistius patachonicus*) (cm); L_{max}, maximum length; range, the percentage of the local size spectra that was covered. Maximum length of each species was defined as the maximum length registered in northern Patagonia following Irigoyen and Galván 2010

Species	L_T or L_F (Range, L_{max})	Isotope	Tissue	Mean (s.d.)	Mean $\Delta\delta X_{f-m}$ (s.d.)	*t*-test
Diplodus argenteus	13.5–25	$\delta^{13}C$	Muscle	−17.3 (0.5)	1.3 (0.4)	$t_{17} = -13.59$, $P < 0.001$
($n = 18$)	(~40%, 30)	$\delta^{13}C$	Fin	−15.9 (0.6)		
		$\delta^{15}N$	Muscle	18.7 (0.7)	−0.2 (0.3)	$t_{17} = 2.81$, $P < 0.05$
		$\delta^{15}N$	Fin	18.5 (0.7)		
Pagrus pagrus	10.9–33	$\delta^{13}C$	Muscle	−15.8 (0.4)	1.0 (0.6)	$t_{21} = -7.12$, $P < 0.001$
($n = 22$)	(~50%, 45)	$\delta^{13}C$	Fin	−14.8 (0.8)		
		$\delta^{15}N$	Muscle	19.4 (0.3)	−0.4 (0.4)	$t_{21} = 5.47$, $P < 0.001$
		$\delta^{15}N$	Fin	18.9 (0.4)		
Pinguipes brasilianus	13.8–36.8	$\delta^{13}C$	Muscle	−17.2 (0.3)	0.5 (0.6)	$t_{12} = -2.29$, $P < 0.05$
($n = 13$)	(~60%, 40)	$\delta^{13}C$	Fin	−16.7 (0.6)		
		$\delta^{15}N$	Muscle	18.4 (0.1)	0.6 (0.4)	$t_{12} = -5.19$, $P < 0.001$
		$\delta^{15}N$	Fin	19.0 (0.5)		
Acanthistius patachonicus	24–55.7	$\delta^{13}C$	Muscle	−16.2 (0.4)	1.5 (0.3)	$t_{15} = -17.23$, $P < 0.001$
($n = 16$)	(~50%, 65)	$\delta^{13}C$	Fin	−14.7 (0.5)		
		$\delta^{15}N$	Muscle	19.9 (0.5)	0.2 (0.3)	$t_{15} = -2.6$, $P < 0.05$
		$\delta^{15}N$	Fin	20.1 (0.5)		

filter feeders, mainly the bivalve *Aulacomya atra atra* (Galván *et al.* 2009*a*). These fishes also exhibit different size-related feeding behaviour: whereas *A. patachonicus* and *P. pagrus* increased the trophic level with size, *D. argenteus* and *P. brasilianus* do not show dietary trends with size (Galván *et al.* 2009*a*; Funes *et al.* 2014).

Sampling

Specimens were collected in shallow reefs (5–15 m) located near Puerto Lobos beach (San Matías Gulf, Argentina, 42°04′S, 65°01′W) using spear guns or hook and lines in February 2012 (*P. pagrus* and *P. brasilianus*) and April 2012 (*D. argenteus* and *A. patachonicus*). Sampling efforts were directed to cover the population size range homogeneously, minimising spatial and temporal variation. Given that mean half-life of both elements in muscle and fin are ~20 days (Table 1) it was important to concentrate sampling events and prevent possible diet shifts that might obscure results. Thus, the whole sample for each species was obtained in the nearby reefs, on the same day and covering 40–60% (Table 2) of the observed local size range (Irigoyen and Galván 2009).

Stable isotope analysis

All fish were kept on ice following collection until being processed the same day of their capture. Pieces of dorsal white muscle (Pinnegar and Polunin 1999) and caudal fin clips of the distal portions (~1 cm^2) (Sanderson *et al.* 2009), including only soft rays, were cut and oven-dried at 60°C for 48 h. Dried samples were ground to a fine powder using a hand mortar and pestle before their dispatch for analysis. Using previous results for the elementary composition of muscle and fin (D. E. Galván, unpubl. data), the optimal sample weight to conduct the analyses were respectively estimated as ~1.2 and ~1.6 mg. In order to estimate analytical precision eight samples were analysed in duplicate. Stable isotope analysis was performed by a mass spectrometer in the stable isotope facility of the University of California, Davis. The stable isotope ratios are expressed as δ values as per mille (‰):

$$\delta X = 10^3 (R_{sample} - R_{standard}) R_{standard}^{-1}$$

where X is ^{13}C or ^{15}N and R the corresponding ratio $^{13}C : {}^{12}C$ or $^{15}N : {}^{14}N$. Standards used were Vienna Peedee belemnite for C and N_2 for N. Laboratory internal standards used were G11 (Nylon), G13 (Bovine Liver), G17 (USGS-41 Glutamic Acid) and G9 (Glutamic Acid). Analytical precision, as standard deviations (s.d.), was: for $\delta^{13}C$ measurements (‰), $s.d._{G11} = 0.08$, $s.d._{G13} = 0.29$, $s.d._{G17} = 0.04$, and $s.d._{G9} = 0.09$; and for $\delta^{15}N$ measurements, $s.d._{G1} = 0.09$, $s.d._{G13} = 0.01$, $s.d._{G17} = 0.10$, and $s.d._{G9} = 0.05$. Lipid content in muscle samples was assessed by computing the elemental C to N ratios (C : N). Sparids had muscle samples with C : N > 3.5 (see Results), thus lipid-controlled $\delta^{13}C$ data were derived for both entire muscle datasets (Sweeting *et al.* 2006), with the modification proposed by Greenwood *et al.* (2010).

Statistical analysis

To test the reliability of fin as a muscle replacement, isotope ratios of both tissues (hereafter δX_{fin} and δX_{mus}) and C : N values were compared, for each species, by means of paired Student's *t*-tests (Crawley 2007). In addition, linear regressions ($\delta X_{mus} = a + b\delta X_{fin}$) were fitted (1) to examine whether the relationships among tissues were directly proportional; (2) to check whether slopes (*b*) differed significantly from 1.0; and (3) to generate predictive models of muscle isotopic composition from fin samples (hereafter δX_{pred}). Regressions were fitted

Table 3. Magnitudes of change of trophic level with fish size (ΔTL) and effect of fish size on the relationship between muscle and fin tissue signatures (offset $\Delta\delta X_{f-m}$), between the smaller and the bigger individual in the five simulated datasets (A, B, C, D and E)

Population	ΔTL	$\Delta\delta X_{f-m}$
A	No	No
B	No	No
C	No	1‰
D	2‰	No
E	2‰	1‰

separately for each species. Finally, to test for possible effects of fish growth on the relationship between δX_{fin} and δX_{mus} values, which could mislead ecological interpretations of δX_{fin} or δX_{pred} datasets, $\Delta\delta X_{f-m}$ offsets were calculated and regressed against fish size. Assumptions of normality and homocedasticity and the importance of influential points were verified using regressing residuals and absolute residuals against fitted values (Jiao *et al.* 2004), and then inspecting diagnostic plots and comparing models with and without possible influential data (Crawley 2007). In addition, total C and N percentage composition, as well as C : N ratios, were used to retrocalculate optimal sample weights. All tests and regression analyses were done with R software V.2.15. (R Development Core Team 2012).

Data simulation

Simulation was conducted to achieve a better description and understanding of the implications for sampling design and posterior ecological interpretation of δX values, derived from fin samples. The simulation experience consisted of the generation of five different datasets (hereafter populations A, B, C, D and E) and the paired comparisons of these populations in order to represent three commonly found scenarios when predicting muscle signatures from fin samples. Hence, simulated populations varied in their size-based feeding behaviour and magnitude of the effect on the relationship between muscle and fin tissue stable isotope values ($\Delta\delta X_{f-m}$). Populations A, B and C exhibited no change in δX with size, whereas Populations D and E exhibited an increase in δX with size (i.e. change in trophic level with size, 2‰ difference between the smaller and larger fish). Population B had mean δX values 1‰ bigger than those for Population A. Populations C and E simulated an effect of size on $\Delta\delta X_{f-m}$ of 1‰ between the smaller and bigger individual (note that it is smaller than the trophic change simulated) whereas Populations A, B and D showed no trend (Table 3). Scenario 1 compared and combined Populations A and B to illustrate the error incorporated when using fish from more than one population to predict the isotope composition of muscle from fin samples. Scenario 2 compared Populations A and C and Scenario 3 compared D and E to illustrate the bias introduced when applying correction models to populations with size-related trends on $\Delta\delta X_{f-m}$ that were generated for species without such trends.

Each dataset consisted in 10 individuals randomly sampled from simulated populations with normally distributed δX_{mus} values ($\mu_{A,C,D,E} = 1‰$ and $\mu_B = 2‰$; $\sigma_{A,B,C,D,E} = 0.25‰$). In these simulations σ represents the variation among individuals due to physiology plus the analytical error. Similar analytical errors were assumed for muscle and fin samples, for simplicity, and sampling of body size was assumed to be uniform throughout the entire size range. All simulated populations had the same fin–muscle linear relationship described by the slope ($b = 0.7$).

Results

Data analyses showed that fin and white muscle δX values were different for both C and N in all species (Table 2). Muscle samples showed higher $\delta^{13}C$ values than fin samples for all four species whereas $\delta^{15}N$ values did not show clear trends (Table 2; Fig. 1). Reinforcing the result that δX_{fin} and δX_{mus} values were not directly comparable in most cases, only two 95% CI of the regression slopes included 1.0 as a possible result (Table 4; Fig. 1*e*, *h*), whereas in all other cases estimated slopes were smaller than 1.0. However, in seven of the eight tested relationships it was possible to estimate significant parameters to derive muscle isotopic composition (δX_{pred}) from δX_{fin} values (Table 4; Fig. 1). Results also showed that fish size influenced the relationship between fin and muscle isotope ratios in two species; $\Delta\delta X_{f-m}$ values displayed a linear and significant relationship against size in *P. brasilianus* for both elements and for C in *D. argenteus* (Table 4; Fig. 2*a*, *c*, *g*). The estimated magnitude of the offset $\Delta\delta C_{f-m}$, along the size-spectra sampled, was 1.2‰ for *D. argenteus* and 1.7‰ for *P. brasilianus*; whereas the estimated offset $\Delta\delta N_{f-m}$ was 1.1‰ for *P. brasilianus.*

The mean difference between duplicated muscle samples were 0.11‰ for $\delta^{15}N$ and 0.12‰ for $\delta^{13}C$, whereas fin had respective differences of 0.55 and 0.43‰. These analytical errors expressed as a percentage of the trophic fractionation ($\Delta^{13}C = 1.5‰$ and $\Delta^{15}N = 3.2‰$, following Sweeting *et al.* 2007*a*, 2007*b*) represented ~8 and ~3.5% in muscle samples but ~29 and ~17% in fin samples.

All muscle C : N ratios ranged between 3.10 and 4.50 whereas fin samples ranged between 3.23 and 4.10 (*D. argenteus*: muscle, from 3.26 to 4.49; fin, from 3.23 to 3.49; *P. pagrus*: muscle, from 3.26 to 3.75; fin, from 3.16 to 3.47; *P. brasilianus*: muscle, from 3.11 to 3.29; fin, from 3.30 to 4.08; and *A. patachonicus*: muscle, from 3.15 to 3.29; fin, from 3.34 to 3.61). In muscle samples, total C and N represented between 49 and 67% of the sample mass, whereas in fin samples it represented between 27 and 50%. These results, although statistically different ($t_{68} = 13.2182$, $P < 0.001$), did not represent a substantial practical difference when the mean optimal sample weights were retrocalculated (1.12 mg for muscle samples and 1.75 mg for fin samples). Dry samples of fin clips weighted, on average, 0.11 g, which is more than 50 times the amount of sample required for C and N dual stable isotope analysis.

Data simulation

Scenario 1 showed that pooling individuals from different populations might lead to errors in predicting muscle–fin relationship. The combination of the simulated datasets A and B led to slope estimation closer to 1.00, $b = 0.945$

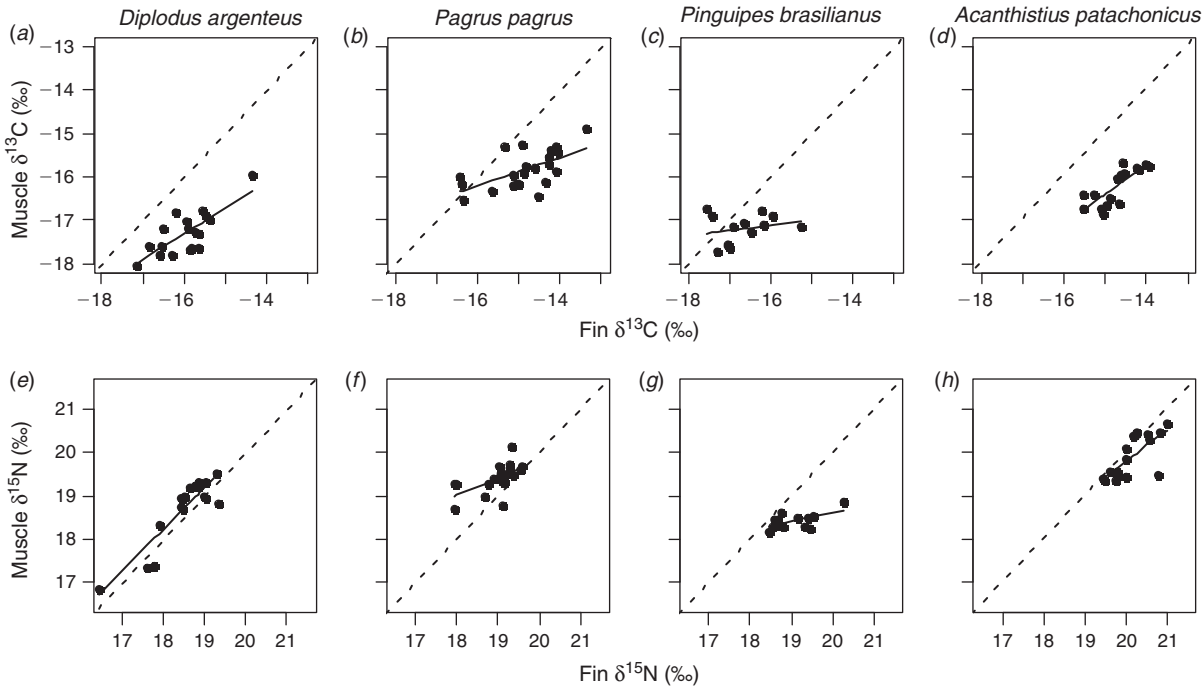

Fig. 1. Relationships between muscle and fin $\delta^{13}C$ and $\delta^{15}N$ values in *Diplodus argenteus*, *Pagrus pagrus*, *Pinguipes brasilianus* and *Acanthistius patachonicus*. Dashed lines represent the 1 : 1 relationship between tissues.

Table 4. Results of the regression models between δX_{mus} and δX_{fin} values and between fin-muscle offsets ($\Delta\delta X_{f-m}$) and fish size

P values indicate the degree to which the estimated regression parameters were significantly different from zero (H0). Slopes that were not significantly different from 1.0 are shown in bold

Species	Isotope	Muscle *v.* fin			$\Delta\delta X_{f-m}$ *v.* size	
		Intercept (H0, $a = 0$)	Slope (H0, $b = 0$)	Slope 95% CI	Intercept	Slope (H0, $b = 0$)
Diplodus argenteus	$\delta^{13}C$	−7.76 ($P < 0.01$)	0.596 ($P < 0.001$)	0.33–0.86	0.66	−0.099 ($P < 0.001$)
($n = 18$)	$\delta^{15}N$	1.07 ($P > 0.05$)	**0.954** ($P < 0.001$)	**0.70–1.20**	0.88	−0.033 ($P > 0.05$)
Pagrus pagrus	$\delta^{13}C$	−10.94 ($P < 0.001$)	0.329 ($P < 0.01$)	0.13–0.53	−0.99	0.001 ($P > 0.05$)
($n = 22$)	$\delta^{15}N$	11.82 ($P < 0.001$)	0.400 ($P < 0.01$)	0.14–0.65	0.08	0.013 ($P > 0.05$)
Pinguipes brasilianus	$\delta^{13}C$	−15.22 ($P < 0.001$)	0.118 ($P > 0.05$)	−0.22–0.45	1.39	−0.073 ($P < 0.01$)
($n = 13$)	$\delta^{15}N$	14.40 ($P < 0.001$)	0.211 ($P < 0.05$)	0.02–0.40	−1.88	−0.048 ($P < 0.01$)
Acanthistius patachonicus	$\delta^{13}C$	−6.09 ($P < 0.05$)	0.687 ($P < 0.001$)	0.38–0.99	−1.36	−0.003 ($P > 0.05$)
($n = 16$)	$\delta^{15}N$	4.99 ($P > 0.05$)	**0.740** ($P < 0.001$)	**0.36–1.12**	−0.56	0.008 ($P > 0.05$)

(95% CI = 0.896–0.993), which was higher than the actual value, $b = 0.7$ (Fig. 3*a*). Scenarios 2 and 3 (datasets A and C, and D and E) showed that muscle–fin relationships can vary between populations just because of the different effect that size has on $\Delta\delta X_{f-m}$. Small size-related trends in $\Delta\delta N_{f-m}$ (1‰ between the smaller and the bigger individual simulated for Population C but not for Population A) could lead to large differences in correction models (Fig. 3*b*, *c*).

The estimated slopes for Populations C and E were $b_C = 0.373$ (95% CI = 0.012–0.734) and $b_E = 0.563$ (95% CI = 0.516–0.610) instead of $b = 0.7$ for populations A and D. In addition, variation in the offset $\Delta\delta X_{f-m}$ with size introduced an extra source of variation in species that do not exhibit ecologically meaningful size-based trophic trends with ontogeny ($r^2_C = 0.34$ and $r^2_A = 1$) (Fig. 3*b*) because fish of different length may have similar δX_{mus} values but very different δX_{fin} values (Fig. 3*b*).

Discussion

In summary, the main results showed that fin and white muscle δX values were different for both C and N in all species but had similar C : N ratios. Muscle samples showed higher $\delta^{13}C$ values than fin samples for all four species whereas $\delta^{15}N$ values did not show clear trends. In seven of the eight tested relationships it was possible to estimate models to derive muscle isotopic composition from fin values and in most cases the estimated regression slopes were smaller than 1.0. The results also showed that fin–muscle relationship varied with fish-size in two of four species.

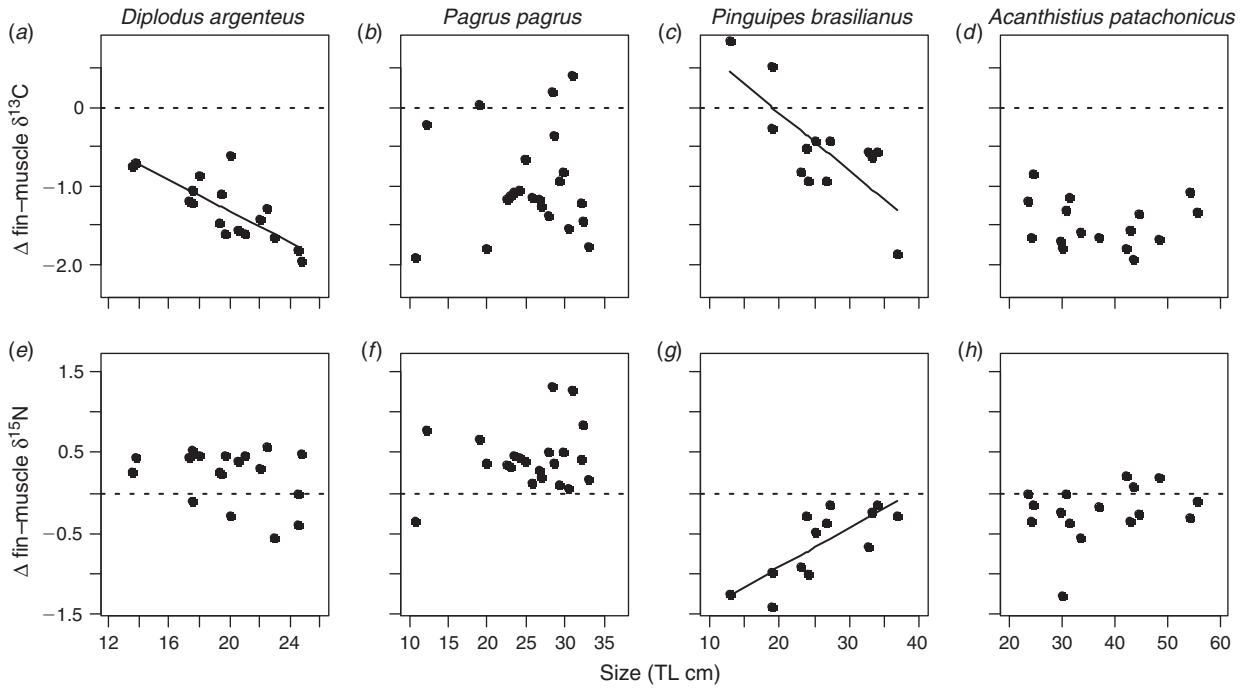

Fig. 2. Relationships between fish length and fin–muscle offsets ($\Delta\delta X_{f-m}$) in *Diplodus argenteus*, *Pagrus pagrus*, *Pinguipes brasilianus* and *Acanthistius patachonicus*. Regression lines are included only for the significant regressions.

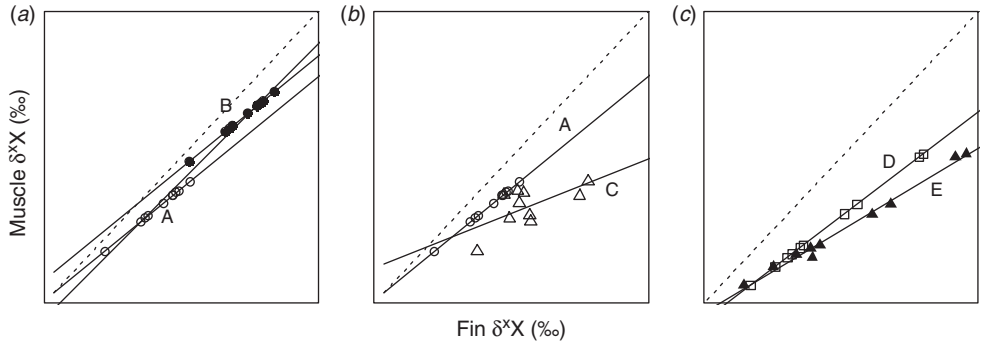

Fig. 3. Relationship between muscle and fin δX values in the five simulated datasets: A, B, C, D and E, representing situations commonly found in nature. A, open circles; B, filled circles; C, open squares; D, open triangles; E, filled triangles. Dashed lines represent the 1 : 1 relationship between tissues. (*a*) Scenario 1, (*b*) Scenario 2, and (*c*) Scenario 3.

The development of non-lethal techniques to obtain fish samples for stable isotope analysis is critical to address the increasing public concern on fish welfare and obviously to study endangered species (Huntingford *et al.* 2006). Small clips of the caudal fin (<1 cm^2) were enough to obtain sufficient tissue for numerous analytical determinations and corroborated the results of Sanderson *et al.* (2009), supporting fin clips as a more practical and non-lethal method for fish bigger than 65 mm. In addition, the present results partially supported the use of pieces of fin as a surrogate for white-muscle in stable isotope studies, showing that although interconversion between δX_{fin} and δX_{mus} values seems rare, it was possible to build robust models to estimate δX_{pred} values. However, the relationships between fish size and $\Delta\delta X_{f-m}$ values reported here showed that samplings to estimate correction models should be designed with caution because size could be a confounding factor on ecological interpretation of stable isotope signatures of fin tissue.

Only two of the eight tested relationships between δX_{fin} and δX_{mus} values could be used as direct surrogate for muscle without applying a correction factor. The rest of the tested relationships (Fig. 1) were directly proportional with slopes smaller than most published relationships (Tronquart *et al.* 2012; Willis *et al.* 2013). The present study pays special attention to reduced spatial and temporal variation in the sampling design, whereas other studies have tried to amplify that variation (e.g. Jardine *et al.* 2011; Tronquart *et al.* 2012),

attempting to obtain a broad range of stable isotopic values associated with different source baselines (Jardine *et al.* 2011). However, given that the results of this and other studies (e.g. Hanisch *et al.* 2010) showed an intrapopulation tendency for a proportional relationship between δX_{fin} and δX_{mus} with slopes <1, the estimated regression parameters relating to more than one population could lead to estimation of erroneous regression slopes with the tendency to be closer to 1.0 (Fig. 3*a*). This error leads researchers to propose erroneous correction factors (Willis *et al.* 2013). Although there is consensus about that the formulation of a general model to estimate muscle isotopic composition from fin values is difficult to achieve; the formulation of regional models was proposed as an appropriate tool (Jardine *et al.* 2011; Tronquart *et al.* 2012). In particular, Tronquart *et al.* (2012) found no differences between species-specific and regional models, but the species-specific models used fish from different populations, which biases regression slopes towards 1.0 (Fig. 3*a*) (Willis *et al.* 2013). Given that correction models should be specific, to minimise fish killing it is central to understand how many fish are necessary in order to estimate reliable models. To design an appropriate sampling and calculate a minimum sample size with enough power to detect differences between tissues it is necessary to know the analytical errors typical for both tissues (present study and Sanderson *et al.* 2009), the potential spread of intrapopulation differences (which could be taken from the literature or calculated by a pilot sampling using only fin clips), and conduct a power analysis (Galván *et al.* 2010). Considering that the spread of intrapopulation differences varies between species and systems it is not possible to give a generic number and the minimum sampling size should be calculated for each case.

The results presented here also indicate that size range of fish sampled to estimate fin–muscle correction models is another issue that could introduce noise into the ecological interpretation of stable isotope datasets. As noted in the Introduction, previous studies have drawn the attention of researchers to possible or significant size-related trends within $\Delta\delta X_{f-m}$ values (Sanderson *et al.* 2009; Hanisch *et al.* 2010), although the authors considered that such trends were not large enough to alter ecological interpretation of the results. In contrast, it was proposed that, given that relationship between size and $\Delta\delta X_{f-m}$ values, fins are more sensitive than muscle in detecting size-based feeding changes in some fish species using stable isotopes (Willis *et al.* 2013); however, this is not supported by actual data. Although stable isotopic turnover differs between the two tissues (Suzuki *et al.* 2005; Watanabe *et al.* 2005) they are not sufficiently different to show differences based on feeding history (estimated half life ranging from 19 to 26 days for both elements and tissues, Suzuki *et al.* 2005) when compared with fish life span. Both studies (the present and Willis *et al.* 2013) sampled temperate reef fish (families Pinguipedidae, Sparidae and Labridae), which have slow growth rates as a feature in common. Thus, sampling design integrated more than 3–4 years of feeding history, which is several times longer than published turnover rates for muscle or fin (Suzuki *et al.* 2005; Watanabe *et al.* 2005). For this reason, it is plausible that the reported differences are only due to physiological differences between tissues, like routing of essential amino acids or fatty acids, than size-related changes in fish diet. If size-related trends in $\Delta\delta X_{f-m}$ values do not show feeding differences, it is important to cover a representative size range in studies aiming to estimate fin–muscle correction factors; otherwise, an important bias could be introduced in the δX_{pred} values (Fig. 3*b*, *c*) with the subsequent erroneous ecological interpretation.

Another important concern, highlighted by the results presented here, is that $\Delta\delta X_{f-m}$ size-related trends introduce an extra source of variation in species that do not exhibit ecologically meaningful ontogenetic dietary shifts, as fish of different lengths may have similar δX_{mus} values but very different δX_{fin} values (Fig. 3*b*). The latter, coupled with increased error sources associated with higher analytical error reported for fin tissues (Sanderson *et al.* 2009; present results), makes fin tissue a poor replacement for muscle to study size-related feeding behaviour (contrary to Willis *et al.* 2013) because fin sampling clearly needs larger sample size than muscle to achieve enough power to detect size-related feeding behaviour (Galván *et al.* 2010). Conversely, $\Delta\delta X_{f-m}$ values varied around 30% of the mean trophic fractionation factor for N isotopes. That level of variation makes fin tissues an acceptable replacement for white muscle that could be used without corrections for broad interspecific trophic comparisons, when the goal is to describe general patterns or the identification of trophic guilds rather than to test slight intraspecific differences. In contrast, the present results support the use of fin clip isotope ratios directly relative to the other food web components and highlights the need for experimental work to estimate fin-specific trophic fractionation factors and to develop a better understanding of turnover rates.

Summarising, our results support the contention that caudal fin tissue is a useful non-lethal surrogate for muscle tissue, being easy to collect in quantities sufficient for mass spectrometry analyses and with C : N ratios similar to those of white muscle with low lipid content. However, its usefulness relies on the following sampling precautions.

- Fin–muscle correction models should be estimated on a species-by-species basis. Sampling should be conducted in a way to reduce spatial and temporal variation.
- Species-specific correction factors may not be valid and applicable to other populations of the same species if the presumed range of δX values differs from those of the population used to estimate the correction models.
- Before tracking size-related dietary shifts using fin samples it is necessary to test whether there are any significant trends between $\Delta\delta X_{f-m}$ and body size.

Although the previous recommendations improve the usefulness of fin tissue as a non-lethal surrogate for muscle given that size-related differences reflect possible physiological differences between tissues, the understanding of such uncertainties could help to elucidate reliable, powerful and general models in order to achieve the final goal of reducing lethal sampling.

Acknowledgements

We thank A. Irigoyen, G. Trobbiani and P. Fiorda, who provided invaluable assistance in the field and fish capture. This work was financed by grants from ANPCyT (PICT 01272) and CONICET (PIP 174), both to O. O. Iribarne; and from ANPCyT (PICT 2008-1433 and 2010-2461) to

D. E. Galván. Part of the fieldwork was conducted within a World Natural Heritage Site and the project was approved by the Dirección General de Conservación de Areas Protegidas del Chubut.

References

Andvik, R. T., VanDeHey, J. A., Fincel, M. J., French, W. E., Bertrand, K. N., Chipps, S. R., Klumb, R. A., and Graeb, B. D. S. (2010). Application of non-lethal stable isotope analysis to assess feeding patterns of juvenile pallid sturgeon *Scaphirhynchus albus*: a comparison of tissue types and sample preservation methods. *Journal of Applied Ichthyology* **26**, 831–835. doi:10.1111/J.1439-0426.2010.01527.X

Böckelmann, P. K., and Bechara, I. J. (2009). The regeneration of the tail fin actinotrichia of carp (*Cyprinus carpio*, Linnaeus, 1758) under the action of naproxen. *Brazilian Journal of Biology* **69**, 1165–1172. doi:10.1590/S1519-69842009000500022

Brankevich, A., Roux, A., and Bastida, R. (1990). Relevamiento de un banco de besugo (*Spagrus pagrus*) en la plataforma bonaerense. Carcterísticas fisiográficas generales y aspectos ecológicos preliminares. *Frente Marítimo* **7**, 75–86.

Church, M. R., Ebersole, J. L., Rensmeyer, K. M., Couture, R. B., Barrows, F. T., and Noakes, D. L. G. (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. doi:10.1139/F08-206

Crawley, M. J. (2007). 'The R book.' (Wiley: Chichester, UK.)

DeNiro, M. J., and Epstein, S. (1976). You are what you eat (plus a few ‰) the carbon isotope cycle in food chains. *Geological Society of America* **6**, 834–835.

Dietrich, J. P., and Cunjak, R. A. (2006). Evaluation of the impacts of Carlin tags, fin clips, and Panjet tattoos on juvenile Atlantic salmon. *North American Journal of Fisheries Management* **26**, 163–169. doi:10.1577/M05-032.1

Dubiaski-Silva, J., and Masunari, S. (2006). Ontogenetic and seasonal variation in the diet of Marimbá, *Diplodus argenteus* (Valenciennes, 1830) (Pisces, Sparidae) associated with the beds of *Sargassum cymosum* C. Agardh, 1820 (Phaeophyta) at Ponta das Garoupas, Bombinhas, Santa Catarina. *Journal of Coastal Research* **SI**(39), 1190–1192.

Estrada, J. A., Lutcavage, M., and Thorrold, S. R. (2005). Diet and trophic position of Atlantic bluefin tuna (*Thunnus thynnus*) inferred from stable carbon and nitrogen isotope analysis. *Marine Biology* **147**, 37–45. doi:10.1007/S00227-004-1541-1

Fincel, M. J., Vandehey, J. A., and Chipps, S. R. (2012). Non-lethal sampling of walleye for stable isotope analysis: a comparison of three tissues. *Fisheries Management and Ecology* **19**, 283–292. doi:10.1111/J.1365-2400.2011.00830.X

Funes, M., Liberoff, L., and Galván, D. E. (2014). Cambios tamaño-dependientes en la dieta de peces marinos y su estudio mediante análisis de isótopos estables. *Ecología Austral* **24**, 118–126.

Galván, D. E., Venerus, L., Irigoyen, A., Parma, A. M., and Gosztonyi, A. (2005). Extension of the distributional range of the silver porgy, *Diplodus argenteus* (Valenciennes 1830), and the red porgy, *Pagrus pagrus* (Linnaeus 1758) (Sparidae) in northern Patagonia, south-western Atlantic. *Journal of Applied Ichthyology* **21**, 444–447. doi:10.1111/J.1439-0426.2005.00621.X

Galván, D. E., Botto, F., Parma, A. M., Bandieri, L., Mohamed, N., and Iribarne, O. (2009*a*). Food partitioning and spatial subsidy in shelter-limited fish species inhabiting patchy reefs of Patagonia. *Journal of Fish Biology* **75**, 2585–2605. doi:10.1111/J.1095-8649.2009.02453.X

Galván, D. E., Venerus, L. A., and Irigoyen, A. J. (2009*b*). The reef-fish fauna of the Northern Patagonian gulfs, Argentina, southwestern Atlantic. *The Open Fish Science Journal* **2**, 90–98. doi:10.2174/1874401X00902010090

Galván, D. E., Sweeting, C. J., and Reid, W. D. K. (2010). Power of stable isotope techniques to detect size-based feeding in marine fishes. *Marine Ecology Progress Series* **407**, 271–278. doi:10.3354/MEPS08528

German, D. P., and Miles, R. D. (2010). Stable carbon and nitrogen incorporation in blood and fin tissue of the catfish *Pterygoplichthys disjunctivus* (Siluriformes, Loricariidae). *Environmental Biology of Fishes* **89**, 117–133. doi:10.1007/S10641-010-9703-0

Greenwood, N. D. W., Sweeting, C. J., and Polunin, N. V. C. (2010). Elucidating the trophodynamics of four coral reef fishes of the Solomon Islands using $\delta^{15}N$ and $\delta^{13}C$. *Coral Reefs* **29**, 785–792. doi:10.1007/S00338-010-0626-1

Hanisch, J. R., Tonn, W. M., Paszkowski, C. A., and Scrimgeour, G. J. (2010). $\delta^{13}C$ and $\delta^{15}N$ signatures in muscle and fin tissues: nonlethal sampling methods for stable isotope analysis of salmonids. *North American Journal of Fisheries Management* **30**, 1–11. doi:10.1577/M09-048.1

Heady, W. N., and Moore, J. W. (2013). Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. *Oecologia* **172**, 21–34. doi:10.1007/S00442-012-2483-9

Hesslein, R., Hallard, K., and Ramial, P. (1993). Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in responce to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 2071–2076. doi:10.1139/F93-230

Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandoe, P., and Turnbull, J. F. (2006). Current issues in fish welfare. *Journal of Fish Biology* **68**, 332–372. doi:10.1111/J.0022-1112.2006.001046.X

Irigoyen, A., and Galván, D.E. (Eds) (2009). 'Peces de Arrecife Argentinos.' (Proyecto Arrecife: Puerto Madryn, Argentina.)

Jardine, T., Gray, M., Mc William, S., and Cunjak, R. (2005). Stable isotope variability in tissues of temperate stream fishes. *Transactions of the American Fisheries Society* **134**, 1103–1110. doi:10.1577/T04-124.1

Jardine, T. D., Hunt, R. J., Pusey, B. J., and Bunn, S. E. (2011). A non-lethal sampling method for stable carbon and nitrogen isotope studies of tropical fishes. *Marine and Freshwater Research* **62**, 83–90. doi:10.1071/MF10211

Jiao, Y., Chen, Y., Schneider, D., and Wroblewski, J. (2004). A simulation study of impacts of error structure on modeling stock recruitment data using generalized linear models. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 122–133. doi:10.1139/F03-149

Kelly, M. H., Hagar, W. G., Jardine, T. D., and Cunjak, R. A. (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. doi:10.1577/M05-084.1

McCarthy, I. D., and Waldron, S. (2000). Identifying migratory *Salmo trutta* using carbon and nitrogen stable isotope ratios. *Rapid Communications in Mass Spectrometry* **14**, 1325–1331. doi:10.1002/1097-0231(20000815)14:15<1325::AID-RCM980>3.0.CO;2-A

Miller, T. (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. doi:10.1007/S10641-006-9020-9

Perga, M. E., and Gerdeaux, D. (2005). 'Are fish what they eat' all year round? *Oecologia* **144**, 598–606. doi:10.1007/S00442-005-0069-5

Pinnegar, J., and Polunin, N. V. C. (1999). Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. *Functional Ecology* **13**, 225–231. doi:10.1046/J.1365-2435.1999.00301.X

R Development Core Team (2012). R: A language and environment for statistical computing. (R Foundation for Statistical Computing: Vienna, Austria.) Available at: http://www.R-project.org [Verified February 2012].

Rodgers, K., and Wing, S. (2008). Spatial structure and movement of blue cod *Parapercis colias* in Doubtful Sound, New Zealand, inferred from $\delta^{13}C$ and $\delta^{15}N$. *Marine Ecology Progress Series* **359**, 239–248. doi:10.3354/MEPS07349

Sanderson, B. L., Tran, C. D., Coe, H. J., Pelekis, V., Steel, E. A., and Reichert, W. L. (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. doi:10.1577/T08-086.1

Suzuki, K. W., Kasai, A., Nakayama, K., and Tanaka, M. (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. doi:10.1139/F04-231

Sweeting, C. J., Jennings, S., and Polunin, N. V. C. (2005). Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. *Functional Ecology* **19**, 777–784. doi:10.1111/J.1365-2435.2005.01019.X

Sweeting, C. J., Polunin, N. V. C., and Jennings, S. (2006). Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry* **20**, 595–601. doi:10.1002/RCM.2347

Sweeting, C. J., Barry, J. P., Barnes, C., Polunin, N. V. C., and Jennings, S. (2007*a*). Effects of body size and environment on diet-tissue $\delta^{15}N$ fractionation in fishes. *Journal of Experimental Marine Biology and Ecology* **340**, 1–10. doi:10.1016/J.JEMBE.2006.07.023

Sweeting, C. J., Barry, J. P., Polunin, N. V. C., and Jennings, S. (2007*b*). Effects of body size and environment on diet-tissue $\delta^{13}C$ fractionation in fishes. *Journal of Experimental Marine Biology and Ecology* **352**, 165–176. doi:10.1016/J.JEMBE.2007.07.007

Tronquart, N. H., Mazeas, L., Reuilly-Manenti, L., Zahm, A., and Belliard, J. (2012). Fish fins as non-lethal surrogates for muscle tissues in freshwater food web studies using stable isotopes. *Rapid Communications in Mass Spectrometry* **26**, 1603–1608. doi:10.1002/RCM.6265

Valladares, S., and Planas, M. (2012). Non-lethal dorsal fin sampling for stable isotope analysis in seahorses. *Aquatic Ecology* **46**, 363–370. doi:10.1007/S10452-012-9407-Y

Watanabe, Y., Seikai, T., and Tominaga, O. (2005). Estimation of growth and food consumption in juvenile Japanese flounder *Paralichthys olivaceus* using carbon stable isotope ratio $\delta^{13}C$ under laboratory conditions. *Journal of Experimental Marine Biology and Ecology* **326**, 187–198. doi:10.1016/J.JEMBE.2005.05.020

Willis, T., Sweeting, C., Bury, S., Handley, S., Brown, J., Freeman, D., Cairney, D., and Page, M. (2013). Matching and mismatching stable isotope ($\delta^{13}C$ and $\delta^{15}N$) ratios among fish species in fin and muscle tissue: a critical review. *Marine Biology* **160**, 1633–1644. doi:10.1007/S00227-013-2216-6

Wills, A. A., Kidd, A. R. III, Lepidina, A., and Poss, K. D. (2008). Fgfs control homeostatic regeneration in adult zebrafish fins. *Development* **135**, 3063–3070. doi:10.1242/DEV.024588