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Variation in carbon and nitrogen isotopic ratios of fin and muscle tissues of Longnose Gar (*Lepisosteus osseus*) and Smallmouth Buffalo (*Ictiobus bubalus*)

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Abstract

Fin clips have been proposed as a non-lethal and non-invasive alternative to dorsal muscle samples in stable isotope analysis. However, potential differences in elemental composition and turnover rates can bias inferences when different tissues are combined. Here, we tested the average difference and correlation of the isotopic signature of $\delta^{13}C$ and $\delta^{15}N$ between muscle and fin samples in two arge-bodied fishes: Longnose Gar (*Lepisosteus osseus*) and Smallmouth Buffalo (*Ictiobus bubalus*). We found that $\delta^{15}N$ signatures of muscle and fin tissues were strongly and positively correlated for both species, although the muscle tissue for Smallmouth Buffalo was slightly more enriched in δ15N. $\delta^{13}C$ signatures of both tissues were significatively different and not strongly correlated for Longnose Gar, but similar and strongly correlated for Smallmouth Buffalo. Our results suggest that fin and muscle tissue can be combined for analyses of $\delta^{15}N$, but correction for higher enrichment of muscle tissue may be necessary for Smallmouth Buffalo. Conversely, combining fin and muscle tissue for analysis of $\delta^{13}C$ requires more caution due to their weaker correlation and dependence of species identity.

1 | INTRODUCTION

Stable isotope analysis (SIA) has become a popular tool in ecology, with applications to animal movement (Cunjak et al., 2005) and trophic ecology (Layman et al., 2012). SIA of animal tissues normally requires invasive and often lethal methods. For example, a sample of dorsal muscle tissue is frequently obtained from fish specimens for SIA (Tronquart, Mazeas, Reuilly-Manenti, Zahm, & Belliard, 2012). Harvest of large numbers of individuals for SIA is undesirable for large, long-lived species that have relatively low demographic resilience (Winemiller, 2005). A non-lethal and relatively non-invasive alternative to the use of muscle tissue is removal of fin clips for SIA (Tronquart et al., 2012). Because animal tissues have different elemental compositions and turnover rates, combining isotopic data from multiple tissue types could bias inferences (Heady & Moore, 2013). Therefore, for studies that analyze isotopic data from fin clips of large fishes and muscle tissue of small fishes lacking sufficient fin mass, it is important to document the degree of isotopic divergence between fin and muscle tissue. Here, we test whether istopic ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) vary between fin and muscle tissues of two large freshwater fishes, Longnose Gar *Lepisosteus osseus* (Linnaeus 1758) and Smallmouth Buffalo *Ictiobus bubalus* (Rafinesque 1818), and discuss the feasibility of combining fin and muscle samples for analyses of trophic ecology.

2 | MATERIAL AND METHODS

Ten Longnose Gar and ten Smallmouth Buffalo were collected from the lower Guadalupe River near Victoria, Texas (USA), during March and June of 2016. From each specimen following euthanasia (TAMU AUP #2015-0290), a sample of muscle tissue was removed from the flank approximately 5 cm below the base of the dorsal fin, and a sample of soft fin tissue also was clipped from the distal margin

of the superior edge of caudal fin in an attempt to minimize potential effects from variation in tissue location (Hayden et al., 2015). Each tissue sample was placed in a separate plastic bag and labeled. Following the protocol described by Arrington and Winemiller (2004), samples were preserved in NaCl for storage until processed in the lab 10–60 days later. For processing for SIA, samples were soaked in distilled water for 5 hr, rinsed in distilled water, dried in an oven at 60°C for 48 hr, and then ground to a fine powder using mortar and pestle. From each sample, 15–20 mg of material was packed into Ultra-Pure tin capsules (Costech Analytical). Samples were sent to the Analytical Chemistry Laboratory of the Institute of Ecology at the University of Georgia (USA) for analysis of $\delta^{13}C$ and $\delta^{15}N$ using mass spectrometry. Isotope ratios were reported in parts per thousand (‰) standardized in relation to Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The analytical precision for stable isotope measurements was ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$.

Paired *t*-tests were performed for each species to determine if $\delta^{13}C$ and $\delta^{15}N$ values differ significantly between fin and muscle tissue. Paired *t*-tests were used because fin and muscle tissues were obtained from the same specimens. Model II simple linear regression using ranged major axis (RMA) was used to examine the relationship between isotopic signatures of fin and muscle tissue for each species separately. The ranged major axis method was used to account for uncertainties associated with measurements of both explanatory and response variables (Legendre & Legendre, 2012).

3 | RESULTS

Mean $\delta^{13}C$ was significantly different between the two tissue types for Longnose Gar ($t_{(9)}$ = 3.31, p < .01) but not for Smallmouth Buffalo ($t_{(9)}$ = 1.19, p = .26). For Longnose Gar, signatures of $\delta^{13}C$ for fin were on average 1.93% higher (*SD*: 0.66‰) than for muscle tissues. $\delta^{13}C$ signatures of muscle and fin tissues were poorly correlated for Longnose Gar, but strongly correlated for Smallmouth Buffalo (Table 1; Figure 1).

Mean $\delta^{15}N$ of Longnose Gar fin and muscle tissue was not statistically different ($t_{(9)}$ = −1.23, p = .25). Conversely, comparisons for Smallmouth Buffalo showed that $\delta^{15}N$ in fin samples were on average 0.93‰ lower (*SD*: 0.61‰; $t_{(9)}$ = −3.43, p < .001) than muscle samples. $\delta^{15}N$ signatures of muscle and fin tissues were strongly and positively correlated for both species (Table 1; Figure 1).

4 | DISCUSSION

The large variation associated with the regression slopes for Lognose Gar suggest that ^{13}C assimilation and/or turnover differ between fin and muscle. This result could reflect the higher similarity in C:N ratios between fin and muscle in Smallmouth Buffalo (~0.71 ± *SD* 0.61 C/N difference) than in Longnose Gar (~1.42 ± 1.53). Previous studies showed that variation in C:N ratios can be associated with variation in lipid content of tissue samples, which has the potential to introduce bias into $\delta^{13}C$ measurements (Post et al., 2007). Despite of that, we believe that this is unlikely to affect our findings, since preliminary analysis carried out with $\delta^{13}C$ data that was normalized by lipid content (see Post et al., 2007) generated virtually the same results.

TABLE 1 Parameter values from Model II simple linear regression (ranged major axis) of isotopic ratios ($\delta^{13}C$ or $\delta^{15}N$) of muscle vs. fin tissue. Bold values indicate statistically significant regressions (p < 0.05).

Isotope	Fish species	Model descriptors
		a [95% *CI*]
δ 13C‰	Longnose Gar	6.35 [-281.77, -23.68]
	Smallmouth Buffalo	2.99 [-16.18, 93.53]
δ 15N‰	Longnose Gar	5.54 [-9.79, 12.86]
	Smallmouth Buffalo	0.41 [-74.67, 13.34]
		b [95% *CI*]
δ 13C‰	Longnose Gar	1.35 [-10.99, 0.06]
	Smallmouth Buffalo	**1.13 [0.40, 4.55]**
δ 15N‰	Longnose Gar	**0.69 [0.26, 1.57]**
	Smallmouth Buffalo	**1.03 [0.06, 6.69]**
		R^2
δ 13C‰	Longnose Gar	0.09
	Smallmouth Buffalo	**0.43**
δ 15N‰	Longnose Gar	**0.30**
	Smallmouth Buffalo	**0.53**

Fin-muscle relationship have been shown to vary with size, space and time (Galván, Funes, Liberoff, Botto, & Iribarne, 2015), but these factors did not seem to affect our results given that body size variation was low in both species (Longnose Gar = mean SL of 66.5 ± 6.7 cm, Smallmouth Buffalo = mean SL of 49.25 ± 10.2 cm, respectively) and fishes were collected in the same period from the same river reach. Regarding $\delta^{15}N$, the regression slopes deviated slightly from a perfect 1:1 relationship (Table 1; Figure 1), suggesting that fin tissues assimilate N at rates similar to those of muscle tissue in these species. These results are similar to findings of previous studies that report strong correlations between $\delta^{15}N$ for muscle and fin tissues (Jardine, Hunt, Pusey, & Bunn, 2011; Tronquart et al., 2012).

In conclusion, results indicate that combining results from fin and muscle tissue in SIA may depend on the isotope element and species identity. For analyses of $\delta^{15}N$, fin and muscle tissue can be combined for hypotheses testing. Such findings are particularly relevant for studies aiming to test hypothesis related to trophic position, where interspecific comparisons and large, long-lived and rare species are to be included. Combining fin and muscle tissue for analysis of $\delta^{13}C$, however, requires more caution, because the correlations between tissues were weaker, possibly because the

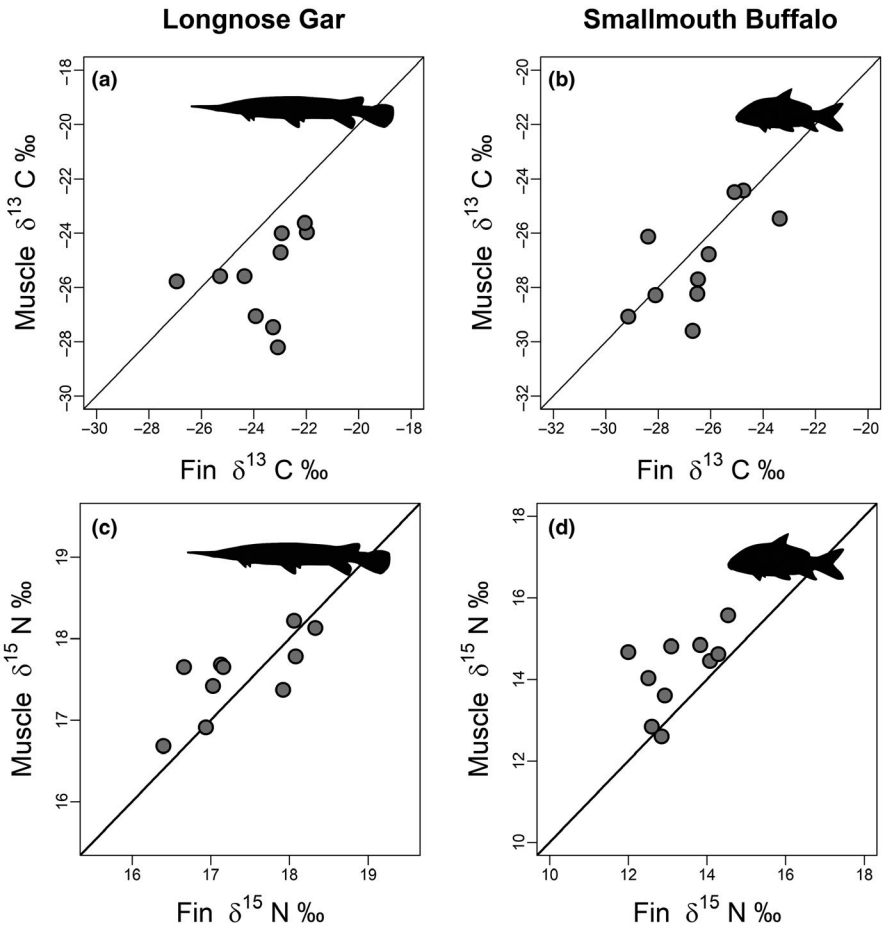

FIGURE 1 Relationship between isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) of tail fin versus muscle tissues of Longnose Gar (a and c, respectively) and Smallmouth Buffalo (b and d, respectively). 1:1 line is added to each panel for reference

dynamics of elemental assimilation and/or tissue turnover rates vary among tissue types.

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DATA AVAILABILITY STATEMENT

The script and data analyzed in this study will be made publicly available after paper acceptance.

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