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ARTICLE

Nonlethal Fin Clip Model Validation for Stable Isotope Analysis of Spotted Gar and Alligator Gar

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Abstract

Stable isotope analysis (SIA) can be a powerful tool for investigating trophic ecology and energy fluxes through an ecosystem. In fish, muscle tissue is generally preferred for SIA due to its intermediate turnover rate, even though sampling muscle is often lethal. Here, we evaluated the feasibility of a fin clip model using fin tissue as a nonlethal alternative to using muscle tissue for SIA (nitrogen, δ^{15}N and carbon, δ^{13}C) in Spotted Gar *Lepisosteus oculatus* and Alligator Gar *Atractosteus spatula*. We also investigated the effects of locality, age, and size of Spotted Gars on the robustness of the fin clip model. Muscle tissue plugs and fin tissue clips were collected from Spotted Gars ($n = 104$) and Alligator Gars ($n = 16$) at four localities in southeastern Louisiana in 2019. Both Spotted Gars and Alligator Gars showed strong positive Pearson's correlations in δ^{15}N and δ^{13}C between the fin and muscle tissues, and a linear regression analysis confirmed that the correlations were significant (Spotted Gar; δ^{15}N: $R^2 = 0.88$; δ^{13}C: $R^2 = 0.95$, $P < 0.001$; Alligator Gar; δ^{15}N: $R^2 = 0.74$; δ^{13}C: $R^2 = 0.94$, $P < 0.001$). The fin clip model for Spotted Gar was affected by locality but not by size or age. Overall, our results suggest that fin tissue can be used as a nonlethal surrogate for muscle tissue for SIA for both Spotted Gars and Alligator Gars.

Understanding the trophic ecology of animal populations can be a powerful tool for the conservation of complex ecosystems (Link and Browman 2014). Accurate estimations of trophic level are a useful tool for many applications in biology, including calculating energy transfer and tracking the accumulation of contaminants within a system (Finlay and Kendall 2007). Previous studies have used stomach content analysis to assess resource use and understand the trophic level of an organism (Jacobsen and Bennet 2013). However, stomach

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content analysis is often unreliable given that a stomach may not be full when it is sampled, content may be too digested to accurately identify consumed prey items, and full stomachs only provide a small snapshot of what an organism eats (Peterson and Fry 1987; Pinnegar and Polunin 1999). Stable isotope analysis (SIA) is an alternative to stomach content analysis and can be used to track trophic levels in food webs (Post 2002), assess dietary sources of carbon within a system (Layman et al. 2011), and track animal migrations (Hoppe and Koch 2007). When conducting SIA, muscle is generally the preferred tissue due to its relatively slow turnover rate compared with other tissues such as blood or liver (Thomas and Cahoon 1993). Additionally, muscle tissue is homogenous, which gives it low isotopic variation (Pinnegar and Polunin 1999). However, sampling of muscle tissue is often lethal and has led to a growing need for alternative tissues (Kelly et al. 2006). Several noninvasive surrogates for muscle tissue in fish have been explored, such as fin, blood, scale, and mucus (Church et al. 2009; Busst et al. 2015; Winter et al. 2019).

Fin tissue has been widely proposed as an ideal substitute for muscle because both tissues have a similar isotopic turnover rate (Sanderson et al. 2009; Fincel et al. 2011; Vander Zanden et al. 2015). Muscle tissue can be depleted or enriched compared with the fin tissue in an individual, and this relationship is different depending on the species (Kelly et al. 2006; Fincel et al. 2011; Willis et al. 2013).

Previous work has focused on establishing species-specific correction factors (Sanderson et al. 2009) that can use fin tissue to accurately predict muscle tissue isotope signatures. Other studies have further investigated whether factors such as fork length (Willis et al. 2013), fish length (Hanisch et al. 2010), or sex (Vašek et al. 2016) can influence the relationship between fin and muscle tissue isotope signatures. Sanderson et al. (2009) investigated isotopic variability in Chinook Salmon *Oncorhynchus tshawytscha* that were captured from different streams and found that the isotope ratios in the fin and muscle tissues had a consistent relationship and could be used interchangeably. Kelly et al. (2006) found that Slimy Sculpin *Cottus cognatus* from different streams had significantly different isotope ratios, and individuals from each location needed to be sacrificed to calibrate an accurate model.

To date, many studies have evaluated the ability of fin tissue to accurately predict muscle tissue for a variety of species. The purpose of our study was to further investigate this relationship between tissues by evaluating whether locality and difference in habitat could significantly alter the accuracy of a species-specific fin clip model (FCM) in large river fish that have access to seasonally available floodplain habitats. Areas that experience an annual flood pulse are especially prone to interannual and seasonal changes in δ^{15}N and δ^{13}C due to resource variability (Post 2002; Akin and Winemiller 2006; Cullen et al. 2019). Therefore, validation of the FCM is especially important when studying floodplain fishes, as floodplain inundation timing, magnitude, and duration can influence large-river fish ecology (King et al. 2003; Akin and Winemiller 2006; Bonvillain et al. 2008).

Spotted Gar *Lepisosteus oculatus* and Alligator Gar *Atractosteus spatula* are two large, long-lived, primarily piscivorous fishes that often use seasonally available floodplain habitat (Ferrara 2001; Love 2004; Bonvillain et al. 2008). Nonlethal sampling of Spotted Gars is important for conservation, with threatened or endangered populations in Ohio, Pennsylvania, and Canada (COSEWIC 2005; Ohio Department of Natural Resources 2010; Pennsylvania Code 2011). Alligator Gar is the largest and longest-lived species in the family Lepisosteidae, with some individuals reaching over 2.5 m long and over 60 years old (Ferrara 2001; Garcia de Leon et al. 2001; Daugherty et al. 2020). Alligator Gars historically ranged from Illinois to Mexico (Jelks et al. 2008); however, the species is now considered rare throughout its current distribution in the United States, except in Louisiana and Texas (Buckmeier et al. 2016; Smith et al. 2019).

We hypothesized that fin tissue could be used as a surrogate for muscle tissue for both Spotted Gars and Alligator Gars based on successful studies on other large freshwater fishes (Hanisch et al. 2010; Busst et al. 2015; Keppeler et al. 2019). We also hypothesized that Spotted Gars that inhabit areas with annual access to floodplain habitat would have different δ^{15}N and δ^{13}C in both fin and muscle tissues when compared with Spotted Gars that inhabit areas without floodplain access; therefore, a general species-level FCM may not be suitable for gars that have access to seasonal floodplain habitats. Cullen et al. (2019) showed that annual floodplain inundation can increase isotopic variability within a system; therefore, we predicted that floodplain inundation would affect the applicability of the FCM to Spotted Gars from an area that experiences an annual flood pulse and Spotted Gars from a locality that does not. If a difference exists, it would suggest that fish species that experience an annual flood pulse may need correction equations on a population-by-population basis as opposed to a general fin clip model. We also tested whether the FCM was affected by the size or age of Spotted Gars.

METHODS

A total of 104 Spotted Gars were sampled from three areas in Louisiana: the Atchafalaya River basin (ARB; 29°47'44.8"N, 91°10'29.3"W), the upper Barataria Estuary (UBE; 29°59'35.4"N, 90°53'09.2"W), and a marsh in Chauvin, Louisiana (CHA; 29.440925°, −90.581122°; Figure 1). Spotted Gars were collected from the UBE and

ARB using electrofishing. Alligator Gar samples ($n = 13$) were collected from fish that were caught by anglers who were participating in a local fishing tournament on Lake Pontchartrain in Louisiana, on August 5, 2019. Additional Spotted Gars ($n = 5$) and Alligator Gars ($n = 3$) were collected near Chauvin, Louisiana, using gill nets. The Spotted Gars were weighed (g) and measured (cm) for total length, and length for Spotted Gars was categorized into three size-classes, 40–50 cm, 50–60 cm, and 60–80 cm.

The sagittal otoliths from the sampled fish were removed, rinsed, and blotted dry for aging. One sagittal otolith from each fish was sent to the University of Minnesota Duluth. Otoliths that were sent to the University of Minnesota Duluth were thin-sectioned following Smylie et al. (2016) and Lackmann et al. (2019). The otoliths were read following the established growth pattern that is found in sectioned and ground sagittal otoliths of gar (DiBenedetto 2009; Buckmeier et al. 2012; Frenette and Snow 2016). The remaining otoliths were ground using sandpaper until the annuli were visible. The visible annuli were counted by two separate readers, beginning from the proximal edge, to estimate age based on King et al. (2018).

The Spotted Gars were transported alive from the field and then euthanized by using a clove oil-ethanol solution. White muscle tissue (1–2 g) was sampled from above the lateral line, and fin tissue was removed from the caudal fin (Pinnegar and Polunin 1999; Figure 2). All of the tissue samples were stored at −18°C until they were rinsed with distilled water then dried at 60°C for 24 h by using a drying oven. Individual samples of muscle and fin were pulverized into a fine powder and measured to the optimal SIA sample weight for animal matter (1.00–1.50 mg). The weighed powder was placed into tin capsules and measured on a Finnigan MAT Delta Plus IRMS that was coupled to an elemental analyzer (Carlo Erba NC2500) at the Cornell University Stable Isotope Laboratory (Ithaca, New York). The samples were compared against the International Atomic Energy Agency standard (atmospheric nitrogen for $\delta^{15}N$ and Vienna Pee-Dee Belemnite for $\delta^{13}C$). The data were not corrected for lipid content because mean C:N ratios for Spotted Gar ($C{:}N_{fin} = 2.86$; $C{:}N_{muscle} = 3.61$) and Alligator Gar ($C{:}N_{fin} = 3.13$; $C{:}N_{muscle} = 3.74$) were below four, which indicates nonfatty tissue (McConnaughey and Roy 1979; Sanderson et al. 2009).

To improve the normality of the data for the analyses, weight (g), length (cm), and $\delta^{13}C$ were log-transformed for Spotted Gars. After the log transformation, all of the data conformed to the assumptions for parametric tests. We

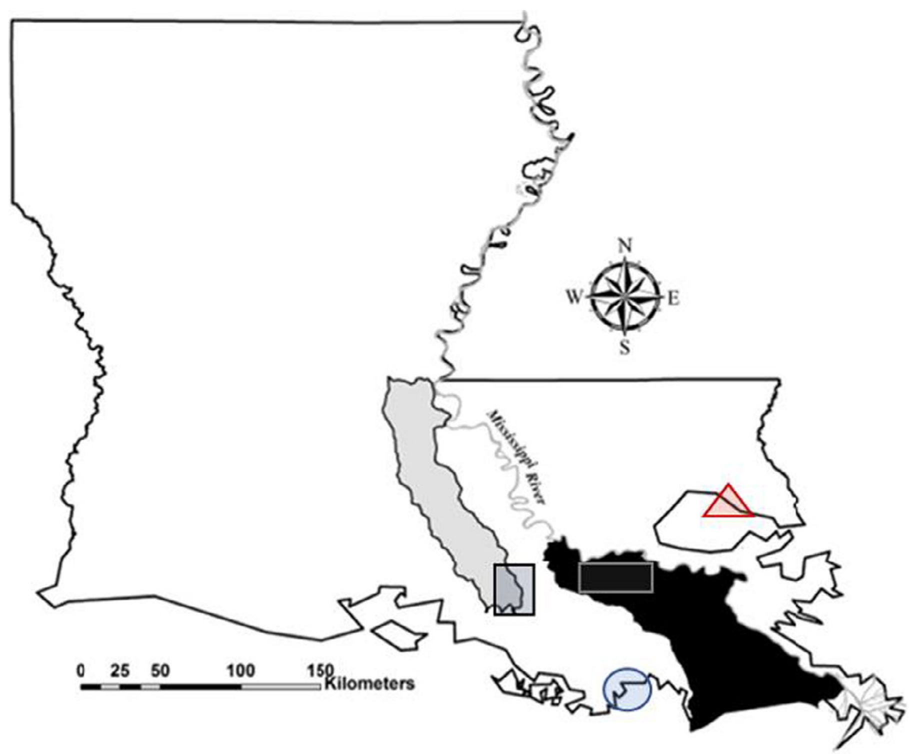

FIGURE 1. The Spotted Gars were collected from the Atchafalaya River basin (black box), the Barataria basin (gray rectangle), and Chauvin, Louisiana (blue circle). The Alligator Gars were collected from Chauvin, Louisiana, and from Lake Pontchartrain (red triangle). Modified from Ballinger (2018).

FIGURE 2. Locations on Spotted Gars where muscle (white circle with vertical lines) and fin (white circle with black diamonds) tissues were sampled for the stable isotope analysis.

used paired *t*-tests to determine whether there was a significant difference in $\delta^{15}N$ and $\delta^{13}C$ ratios between the fin and muscle tissue samples. Pearson's correlation was used to test whether the results for fin and muscle tissues were correlated in Spotted Gars and Alligator Gars. Linear regression was used to determine whether a predictable relationship existed between fin and muscle for $\delta^{15}N$ and $\delta^{13}C$ for Spotted Gars and Alligator Gars. A linear mixed model was used to determine whether locality, size, and age affect the isotopic relationships between fin and muscle tissues in Spotted Gars. Age, size, and locality were included as fixed effects, as well as their interaction with fin tissue's ability to predict muscle tissue isotope signatures ($\delta^{15}N$ and $\delta^{13}C$). Season nested within basin was included as a random effect to account for locality and seasonal variability within the model. The statistical analyses were completed using R version 3.6.1 (The R Foundation for Statistical Computing: www.r-project.org) and the linear mixed models were performed using the nlme package, with alpha set at 0.05 for all of the tests.

RESULTS

The results for fin and muscle tissue were significantly correlated for $\delta^{15}N$ ($r = 0.94$, $P < 0.001$) and for $\delta^{13}C$ ($r = 0.96$, $P < 0.001$) for all of the Spotted Gars ($n = 104$). The linear regression of $\delta^{15}N_{fin}$ by $\delta^{15}N_{muscle}$ yielded an R^2 value of 0.88 ($P < 0.001$), with an equation of $\delta^{15}N_{muscle} = 0.9182(\delta^{15}N_{fin}) + 1.0438$ (Figure 3). A paired *t*-test showed that there was no difference between $\delta^{15}N_{fin}$ and $\delta^{15}N_{muscle}$ ($t_{103} = 0.53$, $P = 0.59$). The linear regression of $\delta^{13}C_{fin}$ by $\delta^{13}C_{muscle}$ resulted in an R^2 value of 0.95 ($P < 0.001$), with an equation of $\delta^{13}C_{muscle} = 1.0041(\delta^{13}C_{fin}) - 2.7906$ (Figure 3). However, a paired *t*-test indicated that there was a significant difference between $\delta^{13}C_{fin}$ ($-26.23 \pm 1.76‰$ [mean ± SD]) and $\delta^{13}C_{muscle}$ ($-29.17 \pm 1.64‰$; $t_{103} = -33.22$, $P < 0.001$), indicating that a correction equation may be necessary to substitute the two tissues.

The results for the fin and muscle tissue samples from Alligator Gar ($n = 16$) were significantly correlated for $\delta^{15}N$ ($r = 0.86$, $P < 0.001$) and $\delta^{13}C$ ($r = 0.97$, $P < 0.001$). The linear regression of $\delta^{15}N_{fin}$ by $\delta^{15}N_{muscle}$ for Alligator Gar yielded an R^2 value of 0.74 ($P < 0.001$), with an equation of $\delta^{15}N_{muscle} = 1.028(\delta^{15}N_{fin}) - 0.0939$ (Figure 3). A paired *t*-test showed no difference between $\delta^{15}N_{fin}$ and $\delta^{15}N_{muscle}$ ($t_{15} = 1.45$, $P = 0.16$), indicating that a correction equation may not be necessary. The regression for $\delta^{13}C_{fin}$ by $\delta^{13}C_{muscle}$ yielded an R^2 value of 0.94 ($P < 0.001$), with an equation of $\delta^{13}C_{muscle} = 0.9865(\delta^{13}C_{fin}) - 2.2316$ (Figure 3). A paired *t*-test indicated that the $\delta^{13}C$ values for Alligator Gar fin and muscle tissue were significantly different ($t_{15} = -6.46$, $P < 0.001$).

Basin had a significant effect on the FCM for Spotted Gar $\delta^{15}N$ ($t_{99} = 3.54$, $P < 0.001$), but not for $\delta^{13}C$ ($t_{99} = -0.70$, $P = 0.70$; Figure 4). A linear mixed model showed that $\delta^{15}N_{fin}$ and $\delta^{15}N_{muscle}$ for the ARB Spotted Gars was significantly higher than it was for the UBE Spotted Gars ($P < 0.001$). The UBE and ARB Spotted Gars did not have significantly different $\delta^{13}C$ signatures in fin ($P = 0.23$) or muscle ($P = 0.18$; Table 1). The results for the fin and muscle tissue linear regression was not significantly affected by length ($\delta^{13}C$: $t_{103} = -0.02$, $P = 0.51$; $\delta^{15}N$: $t_{99} = -0.41$, $P = 0.17$), or age ($\delta^{13}C$: $t_{70} = 1.17$, $P = 0.53$; $\delta^{15}N$: $t_{70} = 0.29$, $P = 0.49$); Figure 5).

DISCUSSION

Our study provides evidence that fin tissue can be used as an appropriate nonlethal surrogate for muscle tissue in SIA for both Spotted Gars and Alligator Gars. Our results are comparable with those of other studies that have combined fish populations from multiple localities into a single linear species regression (Sanderson et al. 2009; Tronquart et al. 2012; Hayden et al. 2017). We did not detect any significant difference between $\delta^{15}N_{fin}$ and $\delta^{15}N_{muscle}$ for Spotted Gars or Alligator Gars, which suggests that a fin clip could replace muscle tissue without the need for a correction. However, significant differences between $\delta^{13}C_{fin}$ and $\delta^{13}C_{muscle}$ in Spotted Gars and Alligator Gars suggests that a correction equation (Spotted Gar: $\delta^{13}C_{muscle} = 1.0041(\delta^{13}C_{fin}) - 2.7906$; Alligator Gar: $\delta^{13}C_{muscle} = 0.9865(\delta^{13}C_{fin}) - 2.2316$) is necessary to interpret the $\delta^{13}C$ values from fin tissue samples. Fin–muscle relationships can fluctuate spatially (Hayden et al. 2017), and the Spotted Gars that were separated by basin displayed different R^2 values. This variability could be due in part to the lower sample size and lower variability when Spotted Gars

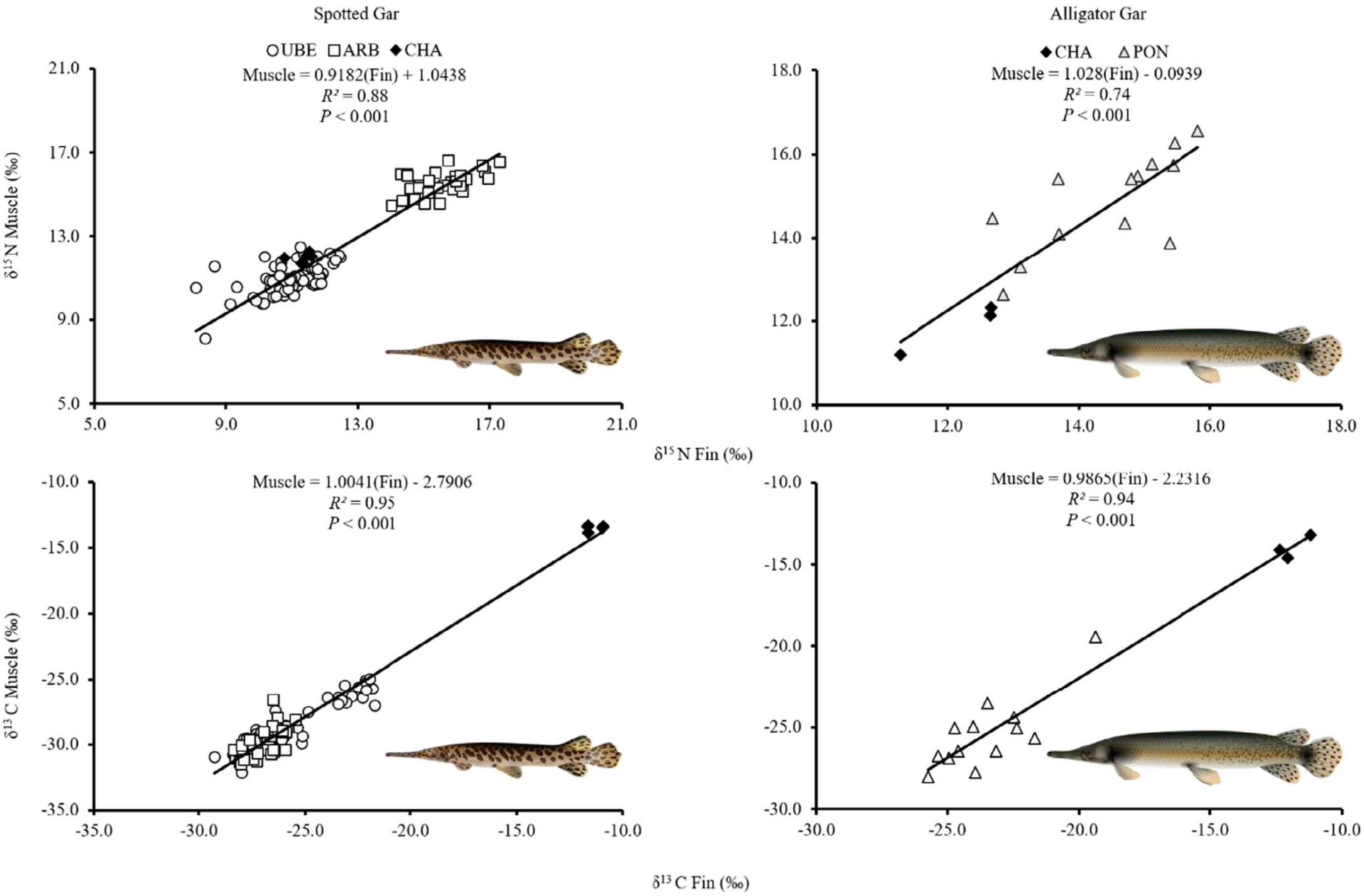

FIGURE 3. Linear regression of $\delta^{15}N$ and $\delta^{13}C$ between fin and muscle tissue for Spotted Gars (left panels) and Alligator Gars (right panels) from the upper Barataria Estuary (UBE) from the Atchafalaya River basin (ARB), from Lake Pontchartrain (PON), and from a marsh near Chauvin, Louisiana (CHA).

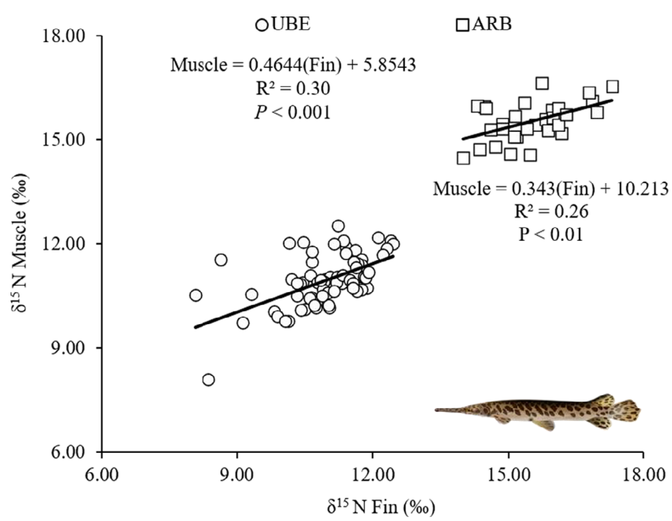

FIGURE 4. Linear regression for UBE ($n = 69$) and ARB ($n = 30$) for Spotted Gars $\delta^{15}N$ fin and muscle tissues. The linear mixed model showed that fin–muscle relationships were significantly affected by basin for $\delta^{15}N$ but not for $\delta^{13}C$.

are separated by locality. The variability could also be due to specific tissue turnover rates for members of Lepisosteidae, which to date are unknown. Two to 4 months is considered the average tissue turnover rate for muscle and fin in medium–large fishes; however, these numbers vary by species (Vander Zanden et al. 2015). The Common Barbel *Barbus barbus* has an average muscle turnover rate of 84 d, whereas the Pacific Bluefin Tuna *Thunnus orientalis* has a turnover rate of 167 d (Madigan et al. 2012; Busst and Britton 2017). To better understand variability, we suggest that future work conduct isotope diet switch studies to determine specific tissue turnover rates for Lepisosteidae.

Basin significantly affected the FCM for $\delta^{15}N$ in Spotted Gars, a result that is consistent with the significant enrichment of $\delta^{15}N$ signatures in the ARB Spotted Gars compared with the UBE Spotted Gars. Hanisch et al. (2010) suggested that the strongest 1:1 relationship in fin and muscle tissue is obtained by combining different populations that have a wide range of isotopic values.

TABLE 1. Mean (±SD) and range of δ^{15}N (‰), δ^{13}C (‰), total length (cm) and Age (years) for Spotted Gars from the UBE ($n = 69$), ARB ($n = 30$), CHA ($n = 5$) and for Alligator Gars from CHA ($n = 3$) and PON ($n = 13$).

			δ^{15}N (‰)		δ^{13}C (‰)			
Species	Basin	Tissue	Mean	Range	Mean	Range	Total length (cm) range	Age (years) range
Spotted	UBE	Fin	11.18 ± 0.58	9.89–12.30	−25.57 ± 2.04	−27.97–21.74	41.2–64.0	2–23
		Muscle	10.85 ± 0.57	9.77–12.18	−28.58 ± 1.83	−31.18–24.99		
	ARB	Fin	15.72 ± 0.79	14.36–17.30	−26.67 ± 0.71	−27.89–25.48	45.1–76.0	3–23
		Muscle	15.59 ± 0.60	14.58–16.55	−29.85 ± 1.30	−31.25–26.59		
	CHA	Fin	11.32 ± 0.33	10.77–11.60	−11.36 ± 0.39	−11.67–10.91	56.5–64.5	4–5
		Muscle	12.00 ± 0.21	11.69–12.27	−13.47 ± 0.22	−13.86–13.31		
Alligator	CHA	Fin	12.20 ± 0.80	11.27–12.67	−11.86 ± 0.61	−12.37–11.18	70.0–78.5	2–3
		Muscle	11.88 ± 0.60	12.63–16.56	−13.98 ± 0.71	−14.59–13.20		
	PON	Fin	14.34 ± 1.09	12.69–15.82	−23.52 ± 1.74	−25.72–19.38	119.4–213.4	
		Muscle	14.87 ± 1.18	12.63–16.56	−25.42 ± 2.23	−28.03–19.42		

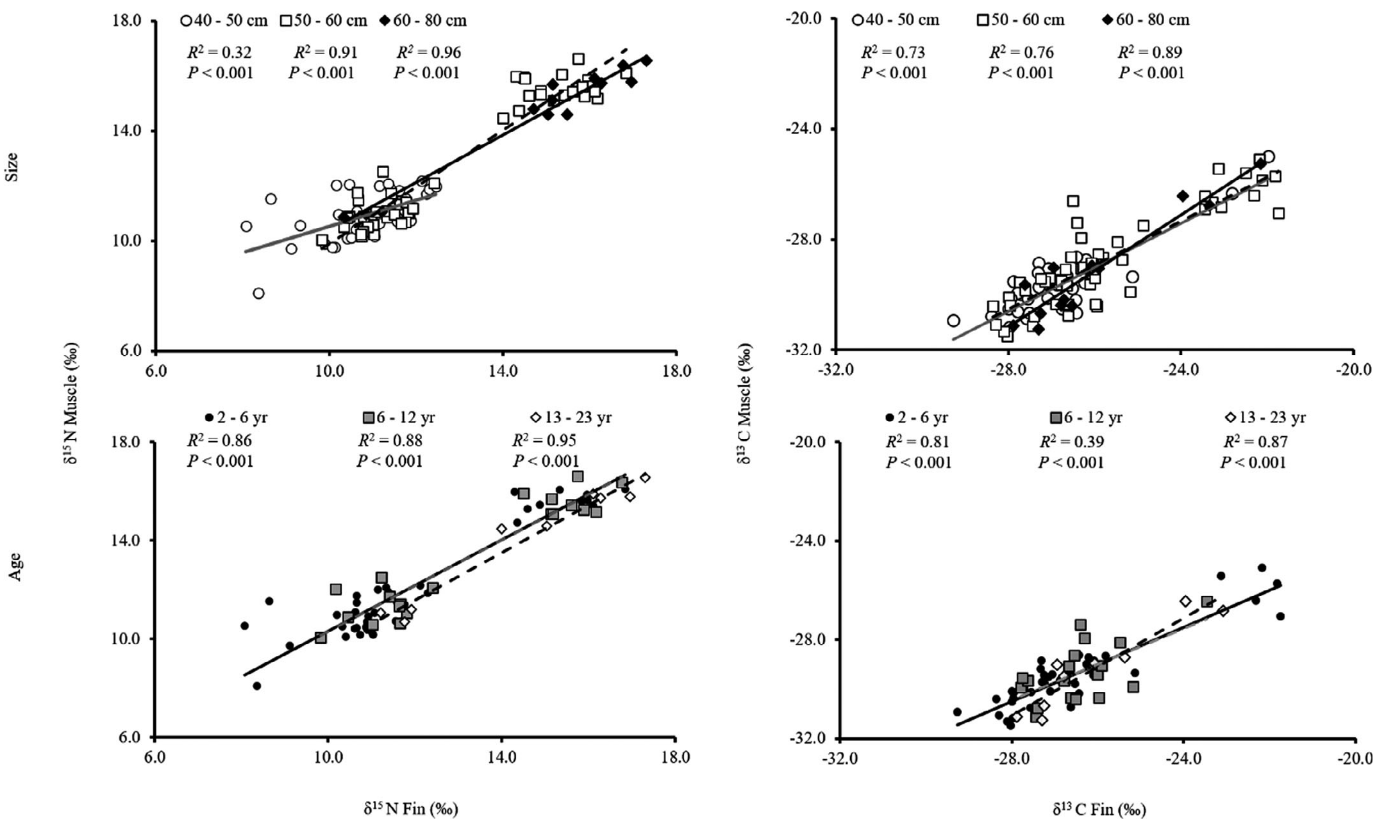

FIGURE 5. Linear regression results for all UBE and ARB Spotted Gars ($n = 99$). The left-hand panels show the values for δ^{15}N, and the right-hand panels show the values for δ^{13}C. The values are grouped by total length (top panels) and by age (bottom panels). The length lines correspond as follows: solid gray 40–50 cm, dashed black 50–60 cm, solid black 60–80 cm. The age lines correspond as follows: solid black 2–6 years, dashed gray 6–12 years, and dashed black 13–23 years.

Separating the UBE and ARB Spotted Gars by basin weakened the overall R^2 value for the general FCM, indicating that combining fish from different populations can increase the model's ability to accurately predict muscle isotope data from fin tissue. The fin–muscle linear regression slopes for Spotted Gar were not affected by age or size for δ^{15}N or δ^{13}C, indicating that the model can be applied to a variety of individual Spotted Gars.

In an earlier study, sample sizes that were greater than $n = 40$ in Chinook Salmon and greater than $n = 25$ in steelhead *Oncorhynchus mykiss* resulted in a narrowing of isotopic variability in $\delta^{15}N$ for fin and muscle (Sanderson et al. 2009), suggesting that each species may have an optimal sample size that is necessary to quantify $\delta^{15}N$ and $\delta^{13}C$. Although the sample size for Alligator Gar was smaller than that for the Spotted Gar, the strength of the fin–muscle relationship had similar R^2 values. Keppeler et al. (2019) found poor correlation between $\delta^{13}C_{fin}$ and $\delta^{13}C_{muscle}$ (R^2 = 0.09) in Longnose Gars *Lepisosteus osseus* and suggested caution when substituting fin for muscle. However, Keppeler et al. (2019) used a sample size of $n = 10$ with weak correlation, whereas our Alligator Gar sample size of $n = 16$ had strong correlation. Our results indicate that a minimum sample size of 15 fish is appropriate to decrease the risk of committing Type II error and to calibrate the model for individual variance for members of Lepisosteidae.

Spotted Gar length did appear to alter the intercept of the FCM, with the smaller Spotted Gar size-group having a lower intercept, which is consistent with previous work. Jardine et al. (2011) found similar results showing a decrease in $\delta^{15}N$ variability as fish size increased, with larger fish having less variability in the overall fin–muscle isotope relationship. Mature fish with slower growth rates will have less variability in tissue turnover rates as shown by Suring and Wing (2009) in Blue Cod *Parapercis colias*. Smaller fish still experiencing periods of rapid growth may have faster tissue turnover rates and higher $\delta^{15}N$ variability, which could explain the lower R^2 values in smaller Spotted Gars (Vašek et al. 2016). Larger individuals were used for the model calibration, so we suggest calibrating the model with smaller Spotted Gars and Alligator Gars (<30 cm) before using fin as a surrogate for muscle in smaller individuals.

Our study adds to the growing body of literature that investigates the relationship between fin and muscle tissue stable isotope signatures and how those relationships may change based on locality. Previous research found that a flood pulse and subsequent inundation of terrestrial systems can cause variation in isotope data for Alligator Gars and Spotted Gars (Cullen et al. 2019). Our results show that the Spotted Gar FCM is still robust with the varying factors of location, length, and age. The strength of the fin–muscle relationship in Alligator Gars further shows that large, long-lived species can be viable candidates for nonlethal sampling, with a relatively low number of individuals sacrificed for model calibration. Previous studies have emphasized the use of nonlethal fin sampling for at-risk species such as Atlantic Salmon *Salmo salar*, Chinook Salmon, Pallid Sturgeon *Scaphirhynchus albus*, and Lake Sturgeon *Acipenser fulvescens* (Sanderson et al. 2009; Andvik et al. 2010; Hanisch et al. 2010; Smith et al. 2015). Therefore, the results of this study could be applied to threatened and endangered populations of Spotted Gars in Pennsylvania, Ohio, and Ontario, Canada (COSEWIC 2005; Ohio Department of Natural Resources 2010; Pennsylvania Code 2011) and at-risk populations of Alligator Gars in Illinois, Kentucky, Arkansas, and Tennessee (Buckmeier et al. 2016; Smith et al. 2019). Our study provides additional support to the use of fin tissue as a viable, nonlethal surrogate for muscle tissue in SIA. The species-specific correction equations that we developed can be used for future stable isotope studies on Spotted Gars and Alligator Gars. By employing nonlethal stable isotope sampling, future studies can improve experimental designs by sampling the same individual over time, increasing sample sizes, and studying vulnerable populations. By using SIA and establishing the trophic positions of these primary predators now, ecosystem shifts may be easier to track. Understanding trophic dynamics in ecosystems is paramount to comprehending how those systems can be disrupted and, more importantly, how they can be conserved.

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