



Technical contribution

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

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Summary

Traditional techniques for stable isotope analysis (SIA) generally require sacrificing animals to collect tissue samples; this can be problematic when studying diets of endangered species such as the pallid sturgeon *Scaphirhynchus albus*. Our objectives were to (i) determine if pectoral fin tissue (non-lethal) could be a substitute for muscle tissue (lethal) in SIA of juvenile pallid sturgeon, and (ii) evaluate the influence of preservation techniques on stable isotope values. In the laboratory, individual juvenile pallid sturgeon were held for up to 186 day and fed chironomids, fish, or a commercially available pellet diet. Significant, positive relationships ($r^2 \geq 0.8$) were observed between fin and muscle tissues for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$; in all samples isotopes were enriched in fins compared to muscle tissue. Chironomid and fish based diets of juvenile pallid sturgeon were distinguishable for fast growing fish (0.3 mm day^{-1}) using stable $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopes. Frozen and preserved fin tissue $\delta^{15}\text{N}$ isotopes were strongly related ($r^2 = 0.89$) but $\delta^{13}\text{C}$ isotopes were weakly related ($r^2 = 0.16$). Therefore, freezing is recommended for preservation of fin clips to avoid the confounding effect of enrichment by ethanol. This study demonstrates the utility of a non-lethal technique to assess time integrated food habits of juvenile pallid sturgeon and should be applicable to other threatened or endangered species.

Introduction

Since the construction of dams on the Mississippi and Missouri rivers in the late 1930s, pallid sturgeon have declined throughout much of their native range (Kallemeyn, 1983; Dryer and Sandvol, 1993), and in 1990 they were placed on the federal endangered species list (USFWS 1990). In the Missouri River natural recruitment has not been documented for over 20 years (Dryer and Sandvol, 1993), and the population is now supplemented by stocking juveniles (Krentz et al., 2005; USFWS, 2008). Recruitment bottlenecks for pallid sturgeon are hypothesized to occur during early life stages as in other

sturgeon species (Bergman et al., 2008). Traditional diet studies have been successful in documenting prey composition for juvenile pallid sturgeon (Gerrity et al., 2006; Hoover et al., 2007; Wanner et al., 2007; Grohs et al., 2009). However, small sample sizes, limited size ranges and 'empty' stomachs make it difficult to quantify the relative importance of different prey taxa to pallid sturgeon growth. Further, diet research on small (< 200 mm) juvenile pallid sturgeon is non-existent; a non-lethal technique to assess food habits would aid in recovery efforts.

Stable isotope analysis (SIA) is a reliable tool used to assess time integrated feeding patterns and trophic interactions (Overman and Parrish, 2001). Carbon isotopes ($\delta^{13}\text{C}$) can be used to determine energy sources (autochthonous v. allochthonous) in lotic fishes (Rosenfeld and Roff, 1992), whereas $\delta^{15}\text{N}$ provides information on the trophic position of consumers (Peterson and Fry, 1987; Cabana and Rasmussen, 1996; Vander Zanden et al., 1997). Values of $\delta^{15}\text{N}$ in consumers tend to be 'enriched' relative to their prey, with a difference of approximately 3.4‰ generally considered to represent one trophic level increase.

Traditional SIA generally requires sacrificing the organism to obtain tissue samples (e.g. muscle, liver, whole fish, etc.), and can be problematic when studying endangered species. Diets of lake sturgeon *Acipenser fulvescens* (Stelzer et al., 2008) and the endangered Snake River sockeye salmon *Oncorhynchus nerka* (Selbie et al., 2007) have been successfully evaluated with stable isotope techniques, but necessitated sacrificing the fish. Non-lethal techniques have been recently developed for several species; however, the relationships among tissue types for carbon and nitrogen signatures were species-specific (see Kelly et al., 2006a for a review). Development of non-lethal SIA techniques for pallid sturgeon may provide an additional tool which could be used to help characterize feeding history or ontogeny of this rare species.

Stable isotope analysis of preserved specimens or tissue may also provide a means to investigate historic food webs. Preservation techniques (i.e. ethanol, formalin, freezing) can influence stable isotope ratios in preserved samples, necessitating the development of species-specific correction models (Kelly et al., 2006b; Schmidt et al., 2009). In particular, samples preserved in ethanol have been shown to provide reasonable estimates of isotopic signatures, but correction

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factors must be developed on a species specific basis (Sweeting et al., 2004; Schmidt et al., 2009). Understanding how preservation techniques might affect isotopic signatures of pallid sturgeon tissue would be useful given the widespread and varied research efforts focused on this species.

In this study, we evaluate the use of non-lethal sampling for quantifying isotopic composition of juvenile pallid sturgeon and explore the effects of tissue preservation techniques on isotope values. Our objectives were to (i) determine if pectoral fin tissue (non-lethal) could be a substitute for muscle tissue (lethal) in SIA of juvenile pallid sturgeon, and (ii) evaluate the influence of preservation techniques on stable isotope values.

Materials and methods

Lethal and non-lethal tissue comparisons

Juvenile pallid sturgeon ($n = 34$; fork length [FL] = 145–186 mm) were obtained from Gavins Point National Fish Hatchery (US Fish and Wildlife Service, Yankton, SD) and transported to the wet laboratory at South Dakota State University (SDSU). Prior to arrival at SDSU the diet of all hatchery reared pallid sturgeon consisted of fishmeal (42% protein) formulated pellets (Silver Cup Trout Chow[®], Nelson and Sons Inc., Murray, UT). In the laboratory, fish were randomly assigned to one of three diet treatments and each fish was held in an individual 38 L tank, resulting in 11 or 12 replicate fish per diet treatment. Diet treatments were selected to reflect the potential diets of juvenile pallid sturgeon in the Missouri River (i.e. invertebrates and fish; Gerrity et al., 2006; Wannier et al., 2007; Grohs et al., 2009) as well as a hatchery-based diet of commercial fish feed. Pallid sturgeon in the invertebrate treatment ($n = 11$) were fed commercially available frozen chironomidae (Hikari Inc., Hayward, CA); individuals in the fish treatment ($n = 11$) were fed fathead minnow *Pimephales promelas* tissue. Fatheads were obtained from a local bait distributor, frozen, cut into small pieces and fed to the sturgeon. Pallid sturgeon in the hatchery diet treatment ($n = 12$) were fed a diet of commercial fish feed. This treatment allowed us to determine if diet shifts away from the hatchery-based diet were identifiable over a relatively short time frame (186 day). Because of mortality from refusal to feed on hatchery pellets, juvenile pallid sturgeon in this diet treatment were switched to the chironomidae diet on day 14 of the experiment. Hereafter, this group will be referred to as the ‘mixed diet’ treatment. Pallid sturgeon were fed *ad libitum* rations one to two times per day and excess food was removed daily. All experiments were conducted at ambient room temperature (approximately 15°C). Any dead fish were removed from the aquaria, placed in individually labeled plastic bags, and frozen at –20°C. After 186 day all remaining fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222). At the end of feeding trials, all fish were weighed (g) and measured to FL (mm) and dorsal muscle and pectoral fin tissues were excised from each fish and then frozen.

Frozen and preserved tissue comparison

In addition to our tank trials, we wanted to assess the impacts of ethanol preservation on stable isotope signatures in juvenile pallid sturgeon. Pelvic fin tissue samples from 29 juvenile pallid sturgeon (FL = 135–185 mm) were obtained from the US Fish and Wildlife Service Bozeman Fish Technology Center (Bozeman, MT). Paired pelvic fin samples were obtained from

individual fish, with one sample frozen and the other preserved in a 95% ethanol solution. Samples were preserved using both methods for 18 months before analysis.

Stable isotope analysis

Prior to SIA, all samples were rinsed with deionized water, placed in individual aluminum trays and dried at 60°C for 72 h. After drying, samples were homogenized into a fine powder using a mortar and pestle, and placed into individually labeled glass scintillation vials. Approximately 0.08 mg (± 0.002) of the homogenized tissue was placed into individual tin capsules. All samples were then sent to the Cornell Isotope Laboratory (COIL, Ithaca, NY; <http://www.cobsil.com/>) where they were analyzed for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) using a Thermo Delta V Isotope Ratio Mass Spectrometer interfaced to a Carlo Erba NC2500 elemental analyzer.

Statistical analysis

We used paired *t*-tests to test the null hypothesis that differences between muscle and fin isotope signatures and between frozen and ethanol preserved fin isotope signatures were zero. When differences between muscle isotope values and those from fins were significant at $P < 0.10$, we used linear regression analysis to determine relationships between tissue types. For the non-lethal tissue comparison, we regressed muscle isotopic values on fin isotopic values; for the preservation evaluation we regressed frozen fin isotopic values on ethanol preserved isotopic values. Regression parameters (slope, intercept) were used to develop a predictive model for estimating muscle isotope values from fin isotope values.

Results

Lethal and non-lethal tissue comparison

A total of 15 pallid sturgeon (FL = 154–258 mm) yielded adequate amounts of both muscle and fin tissue for SIA and were used to compare lethal (muscle) to non-lethal (fin) isotopic values. Of these sturgeon, three were maintained on the fish diet, seven were on the chironomid diet, and five were fed the mixed diet treatment. Twelve of fifteen fish exhibited positive growth with average total growth (FL mm \pm SE) of 0.7 mm (± 4.8), 90.5 mm (± 5.9), or 33.3 mm (± 5.3) for sturgeon maintained on the fish, chironomid, or mixed diets. Three sturgeon on the chironomidae diet exhibited no growth.

We found significant differences between juvenile pallid sturgeon muscle and pelvic fin tissue isotope values for both $\delta^{13}\text{C}$ ($P < 0.01$) and $\delta^{15}\text{N}$ ($P < 0.01$). However, the relationship between muscle and fin tissue isotope values was positive for both $\delta^{15}\text{N}$ (slope = 0.86, intercept = 2.27, $r^2 = 0.79$, $P < 0.001$; Fig. 1a) and $\delta^{13}\text{C}$ (slope = 0.89, intercept = –1.02, $r^2 = 0.97$, $P < 0.001$; Fig. 1b) isotopes.

Pallid sturgeon on the chironomid diet treatment had the highest variation in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in both tissue types (Fig. 2a,b), and the highest growth rates. Pallid sturgeon fed the fish diet treatment exhibited the slowest growth of the three diets and had the least amount of variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (Fig. 2a,b). Pallid sturgeon fed a mixed diet exhibited growth rates greater than sturgeon on the fish diet, but slower growth than the majority of the sturgeon on the chironomidae diet. After 186 days, isotopic values from

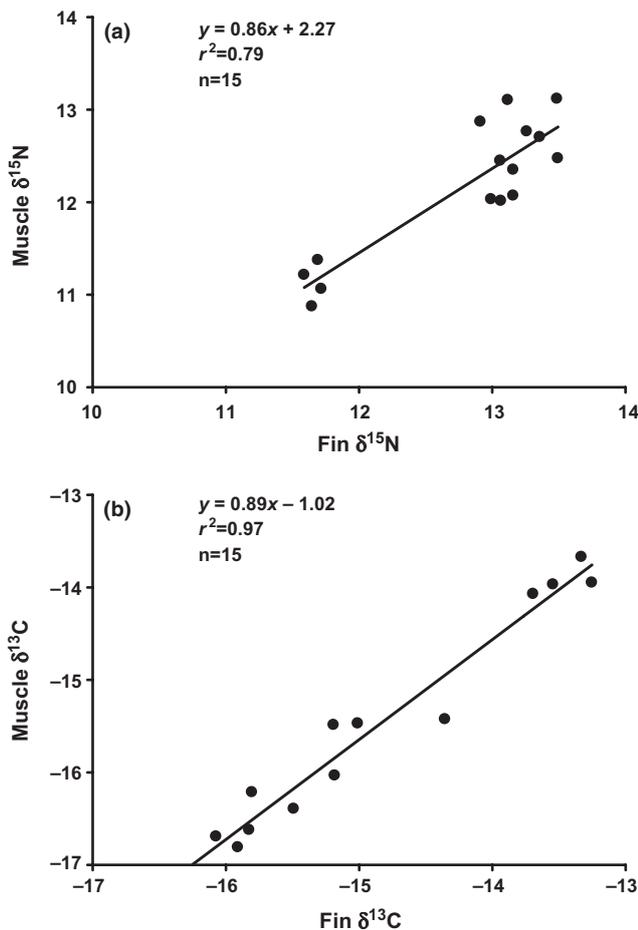


Fig. 1. Scatterplot comparing (a) $\delta^{15}\text{N}$ isotope values and (b) $\delta^{13}\text{C}$ isotope values in muscle and fin tissues from 15 juvenile pallid sturgeon fed known diets over a 186 day period. Black line = linear regression model

sturgeon on different diet treatments were uniquely identifiable (Fig. 2).

Frozen and ethanol preserved tissue comparison

A total of 29 paired samples (i.e. individual pallid sturgeon) were used to compare isotopic values of frozen and ethanol-preserved fin tissue. We observed significant differences between frozen and preserved fin tissue for both $\delta^{13}\text{C}$ ($P < 0.01$) and $\delta^{15}\text{N}$ ($P < 0.01$) isotopes in juvenile pallid sturgeon. Both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope values in ethanol-preserved samples were enriched relative to frozen samples. We observed positive linear relationships between preservation methods for both $\delta^{15}\text{N}$ (slope = 0.66, intercept = 4.24, $r^2 = 0.89$, $P < 0.001$ Fig. 3a) and $\delta^{13}\text{C}$ (slope = 0.57, intercept = -7.67, $r^2 = 0.16$, $P = 0.03$, Fig. 3b).

Discussion

Stable isotope values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) obtained from pectoral fin tissue in juvenile pallid sturgeon provided a reasonable surrogate for lethally obtained values from muscle tissues. Similar to previous studies, we found a correlation between tissues collected by lethal and non-lethal means (Perga and Gerdeaux, 2003; Jardine et al., 2005; Kelly et al., 2006a; Church et al., 2009). Relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values in fin and muscle tissue of pallid sturgeon

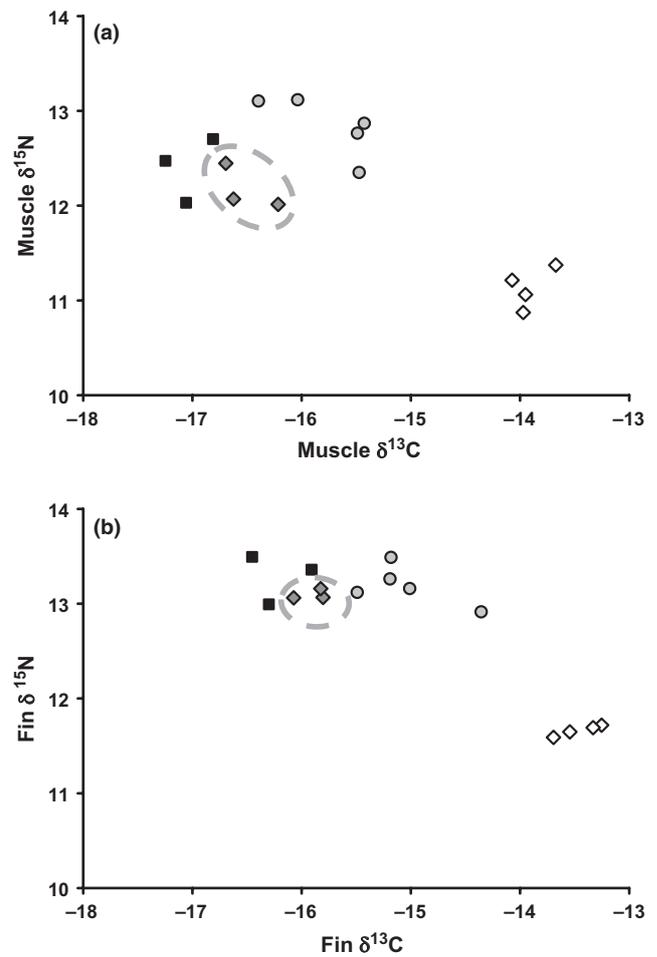


Fig. 2. Scatterplot comparing $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of (a) muscle vs muscle and (b) fin vs fin tissue for 15 juvenile pallid sturgeon fed known diets over a 186 day period. Diet treatments: fish diet = black squares; chironomid diet = white diamonds; chironomid diet fish exhibiting no growth = dark gray diamonds; mixed diet = gray circles. Mixed diet fish were initially fed commercial pellets then switched to chironomids due to mortality. The gray oval encircles fish from the chironomid diet treatment that exhibited no growth and which retained their original hatchery based diet signature

($r^2 = 0.97$ and 0.79 , respectively) were in the range found for slimy sculpin *Cottus cognatus* ($r^2 = 0.84$ and 0.90 ; Kelly et al., 2006a), sunfish *Lepomis* spp. ($r^2 = 0.94$ and 0.97 ; Kelly et al., 2006a), Atlantic salmon *Salmo salar* ($r^2 = 0.97$ and 0.80 ; Jardine et al., 2005) and brook trout *Salvelinus fontinalis* ($r^2 = 0.94$ and 0.74 ; Jardine et al., 2005), indicating that isotopic relationships exist across taxa. For both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes all fin tissue values were enriched compared to muscle tissue values. This enrichment could result from different tissue turnover rates (i.e. faster turnover in fin tissue; Church et al., 2009), differential fractionation between tissue types (Pinnegar and Polunin, 1999), lower lipid concentrations in fin tissues (Post et al., 2007), or a combination of these possibilities. Given more time (> 186 days) the fin values might not have been enriched in all cases. During the juvenile stages, high somatic growth rates could result in differential fractionation of heavy isotopes into different tissues, or differential turnover rates for various tissues (Vander Zanden et al., 1998). Regardless of the mechanism, the strong correlation between isotope values (fins vs muscle) shows that non-lethal tissues (fins) provide a reasonable approximation of $\delta^{13}\text{C}$

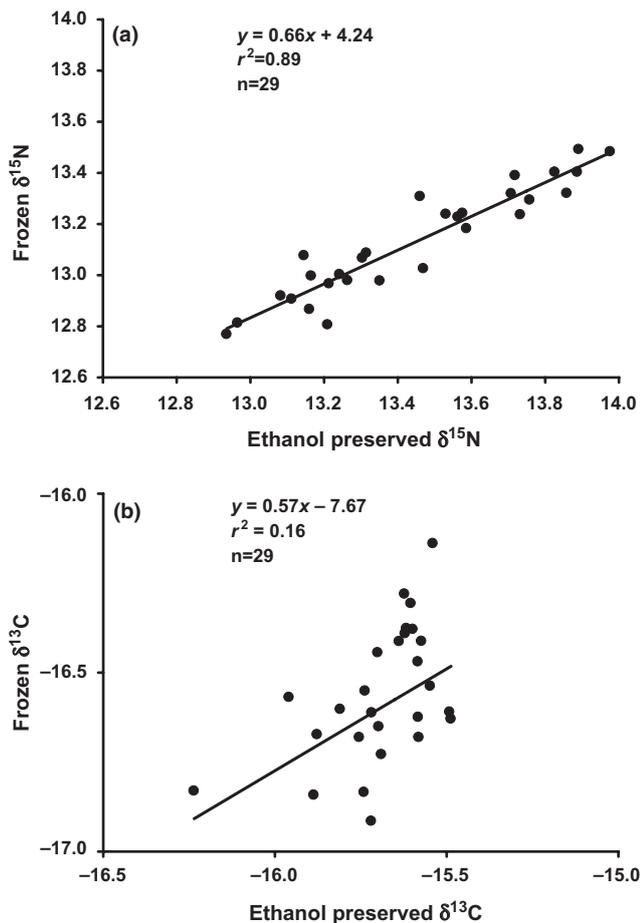


Fig. 3. Scatterplot comparing (a) $\delta^{15}\text{N}$ and (b) $\delta^{13}\text{C}$ of pelvic fin tissue from 29 juvenile pallid sturgeon preserved by freezing vs ethanol. Black line = linear regression model

and $\delta^{15}\text{N}$ values for juvenile pallid sturgeon, a first for Acipenseridae.

Diet differences among pallid sturgeon were clearly detectable in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values after 186 days. Moreover, both muscle and fin tissue responded similarly to differences in diet composition, thus lending further support that non-lethal tissue (fins) can track relative changes in diet composition of pallid sturgeon. Isotope signatures of both tissues showed consistent enrichment of ^{15}N and depletion of ^{13}C for pallid sturgeon fed fish compared to sturgeon fed chironomids. Juvenile pallid sturgeon fed chironomids had $\delta^{13}\text{C}$ isotope values indicative of a more benthic based diet relative to sturgeon on both the fish and hatchery diets (Fig. 2a; Peterson and Fry, 1987; Vander Zanden and Rasmussen, 1999). Relative to other trials, pallid sturgeon fed the fish diet had lower $\delta^{13}\text{C}$ isotope values and higher $\delta^{15}\text{N}$ values, indicative of feeding at higher trophic levels (Fig. 2a; Peterson and Fry, 1987; Vander Zanden et al., 1998). Fish fed a mixed diet had $\delta^{15}\text{N}$ isotope values intermediate to individuals fed chironomids and fish (Fig. 2b; Vander Zanden and Rasmussen, 1999). One exception to these results was the isotope signatures from juvenile pallid sturgeon fed the chironomid diet, which experienced no growth throughout the experiment (Fig. 2). These fish exhibited little to no growth and had $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values more reflective of their initial formulated pellet diet at the hatchery. These findings could be related to the lack of assimilation of their new diet, or potential reabsorption of

their own tissues prior to mortality (Vander Zanden et al., 1998).

The ability to differentiate pallid sturgeon diets based on non-lethal SIA could help identify size-dependent feeding patterns (Grohs et al., 2009), distribution (Gerrity et al., 2006), and potential origin (hatchery vs naturally recruited fish) of juvenile pallid sturgeon. Prior to stocking, all hatchery reared juvenile pallid sturgeon are fed a known, quantifiable diet. By obtaining a baseline isotope signature for the hatchery diet and comparing it with isotope signatures obtained from small sturgeon captured < 1 year post-stocking, biologists can gain knowledge on turnover and assimilation rates, evaluate ontogenetic diet shifts, and identify important prey items of juvenile pallid sturgeon following stocking. Additionally, diet information can be non-lethally collected from fish too small to safely remove stomach contents using gastric lavage.

We were able to account for a significant amount of the variation in $\delta^{15}\text{N}$ isotope values between frozen and preserved pectoral fin tissue with our linear regression model, indicating that preserved fin tissue can be used as a reasonable estimator for $\delta^{15}\text{N}$ isotope values. Although the model that explained the most variability in our $\delta^{13}\text{C}$ signatures from ethanol-preserved samples was statistically significant, the low r^2 value ($r^2 = 0.16$) indicates only modest value in application. Alcohol based preservation had a larger impact on $\delta^{13}\text{C}$ signatures than $\delta^{15}\text{N}$ signatures in juvenile pallid sturgeon, similar to other species (Edwards et al., 2002). The enrichment of $\delta^{13}\text{C}$ we observed was consistent with the patterns observed by Kelly et al. (2006b) and Sweeting et al. (2004), and can probably be attributed to the interaction of tissue lipids with the preservation media (DeNiro and Epstein, 1978; Bosley and Wainright, 1999; Sarakinos et al., 2002). Because ethanol contains no nitrogen, the enrichment in $\delta^{15}\text{N}$ following ethanol preservation was likely due to tissue hydrolysis or leaching (Sarakinos et al., 2002). Bosley and Wainright (1999) postulated that preservatives may prevent the loss of ^{15}N and ^{12}C enriched compounds, or that preservatives may promote the leaching of compounds that are enriched in ^{14}N and ^{13}C . However, our data did not permit us to test the mechanisms behind $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment.

Our study did have limitations. The range of sizes and isotopic signatures of pallid sturgeon were relatively narrow in our analysis; however, the relative consistency between muscle and fin tissue signatures across various diets highlights the utility of non-lethal SIA techniques for endangered species. Future studies should examine a larger size range of pallid sturgeon (e.g. adult fish) with differing diets and across a broader geographic range to better develop correction factors for both preserved tissues and non-lethally obtained fin tissues. Additionally, obtaining isotope values from known prey items would allow researchers to better assess turnover rates in various tissue types. Knowledge of pallid sturgeon dietary preference (i.e. fish vs benthic invertebrates) across a large size distribution and throughout their current range would allow managers to identify critical natural prey items and habitats key to their production to help sustain pallid sturgeon, especially during early life.

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