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Stable isotope analysis shows limited assimilation of carbohydrates in muscle tissue of an omnivorous fish

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Abstract Stable isotope analysis is key to understanding trophic relationships, but how different diet components are incorporated into tissues remains unclear. Understanding trophic relationships for omnivorous species is particularly challenging because they consume both plants and animal material that vary in nutritional quality, isotopic composition, and metabolic routing. Carbohydrates can fuel metabolism but may not be incorporated into tissues, leading to underrepresentation in isotope signatures of muscle tissue. This study examined how stable isotopes (δ13C, δ15N, δ2H) in channel catfish (*Ictalurus punctatus*) muscle reflect contributions from carbohydrate-rich Russian olive fruit (*Elaeagnus angustifolia*) and protein-rich black soldier fly larvae (Stratiomyidae). Over a 44-day trial, catfish were fed mixed diets ranging from 100% olive to 100% fly meal, plus a commercial feed control. Muscle tissue from each treatment was analyzed for isotopic ratios after the feeding period. While treatment feeds differed in isotopic value, muscle tissue only reflected the assimilation of fly larvae, not olive. Fish fed 100% olive showed no significant isotopic difference from pre-trial controls, indicating minimal assimilation. Hydrogen isotopes showed no dietary effect, suggesting limited incorporation into muscle tissue. These results suggest carbohydrate-rich diet items that are metabolized for energy may be underrepresented by stable isotope analysis of muscle tissue.

Keywords δ13C · δ15N · δ2H · Channel catfish (*Ictalurus punctatus*) · Russian olive (*Elaeagnus angustifolia*) · Controlled feeding experiment · Isotopic routing

Introduction

Stable isotope analysis has advanced ecological research by providing insight into food web dynamics (Brett et al. 2017) and ecosystem processes (Douglas and Lohrer 2024). Stable isotope analysis provides the ability to distinguish chemically identical compounds based on their isotope content (Rodrigues et al. 2013). Isotopes have been readily adopted by aquatic researchers and managers to discriminate among prey sources (Peterson and Fry 1987; DeNiro and Epstein 1978; Polito et al. 2019), tracing the flow

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of energy through aquatic food webs (Wada et al. 1991; Layman et al. 2012), determining trophic level (Czermak et al. 2006; Layman et al. 2007; Madigan et al. 2012), evaluating the impact of invasive species (Britton et al. 2010), and determining natal origin or migration patterns (Hobson 1999). Stable isotope analysis can provide inference that direct observations may not detect, provide a nonlethal approach to diet evaluation, and enable the reconstruction of historical diets and food web structure from museum specimens (Post 2002). However, stable isotope analyses are not without biases, and although widely adopted, gaps remain in our understanding of the processes related to the incorporation of isotopes into consumer tissues.

To reduce the biases and misinterpretation of isotopic analyses, researchers must account for differences in how isotopes behave, the time it takes for isotopes to reach equilibrium, variations in baseline isotope levels, and the similarity between isotopic patterns. Turnover rates, the time or mass change required for a consumer's tissue to reflect a diet shift, can also vary based on temperature, metabolic rate, and tissue selection influencing interpretation (Boecklen et al. 2011; Hesslein et al. 1993; Tieszen et al. 1983). Spatiotemporal variability in baseline isotope values can muddle dietary analyses and may be influenced by seasonal changes, species invasion, and anthropogenic pollution (Cabana and Rasmussen 1994; Strayer et al. 1999; Post 2002; Vander Zanden and Rasmussen 2001). Even when accounting for these biases, overlapping isotopic signature among prey items and habitats often reduces the inference provided by the analyses (Stewart et al. 2017). Despite these limitations, isotope analysis provides a useful and cost-effective tool for researchers and managers to understand aquatic food webs.

Understanding trophic relationships of omnivorous fishes is particularly challenging due to their diverse diets that span multiple trophic levels. Isotopic signatures of prey items often have considerable overlap, blending isotopic signatures and complicating the distinction between dietary components (Post 2002; Bearhop et al. 2004; Griffen and Mosblack 2011). With dietary diversity, there is the potential for biases in how the prey or forage items are assimilated into tissue often sampled in isotope analysis (e.g., muscle, fin, blood). In addition, the macronutrient composition of diet items can determine how forage items are reflected by tissue isotopes. For example, nitrogen isotopes (15N) reflect the consumption of proteins, whereas carbohydrates lack nitrogen, and therefore will have minimal effect on nitrogen signatures (Villar-Salvador et al. 2015). In contrast, carbohydrates may be rapidly metabolized and therefore may not be incorporated into tissue or reflected in isotope signatures. Lipids tend to be depleted in Carbon (13C) compared to proteins and carbohydrates but may be corrected for in isotope analyses (Goring-Hartford et al. 2018). Isotopic signatures, specifically muscle tissue, may underestimate the contribution of carbohydrates to energy intake (Hobson and Sease 2006). This bias may be particularly important in omnivorous fishes that consume diet items that widely vary in nutritional composition.

Fruits can provide a locally and seasonally abundant resource for herbivorous and omnivorous fishes, and fish frugivory is a common plant-animal interaction in floodplain rivers. Fish frugivory provides a nutritional subsidy to the fish consumers, and fish have the potential to disperse seeds of floodplain plants (ichthyochory) (Anderson et al. 2011; Pollux 2011). Frugivorous fish also contribute to nutrient cycling by breaking down plant material and enriching aquatic habitats with organic matter (Anderson et al. 2009). Jepsen and Winemiller (2002) were able to show that stable isotope analysis can be a valuable tool for understanding the linkages between fishes and plants, providing valuable insight into their role in floodplain ecosystems. Yet, carbohydrate-rich fruits may primarily contribute to metabolic energy, and isotope signatures may under-represent their contributions to energetic demands (Brett et al. 2012).

Fish frugivory is a common adaptation in tropical ecosystems but is likely a less common strategy in temperate regions, although frugivory remains understudied (Corlett 2017). However, channel catfish [*Ictalurus punctatus* (Rafinesque, 1818)], hereafter, simply "catfish," have been observed consuming the fruits of riparian trees including mulberries (*Morus rubra*) (Linnaeus, 1753) and Russian olive [*Elaeagnus angustifolia* (Linnaeus, 1753)] hereafter, simply "olive" (Cheek et al. 2025; Hedden et al. 2022; Chick et al. 2003). Interactions between catfish and olive have been shown to be particularly common in the San Juan River (NM/UT, USA) in the Southwest US, where olive fruits make up 44% of stomach content mass and 35% of energy consumed (Cheek et al. 2024). However, isotope analyses of food web structure from the San Juan River do not clearly reflect the contribution of olive to catfish diets (Gido et al.

2006). Isotopic ratios of muscle tissue may not accurately reflect the energetic contribution of carbohydrate-rich olive fruit to catfish diets due to the rapid metabolism and preferential use of carbohydrates for metabolic energy (Martínez del Rio et al. 2009; Newsome et al. 2007). Metabolic energy demands make up 25–60% of the total energy budget for catfish, representing a sizable portion of energy requirements (Brett and Groves 1979). Underrepresentation of metabolic energy sources in isotopic signatures of muscle tissue may minimize the importance of these diet items in fish energy budgets. To better understand the importance of fish frugivory, there is a need to better understand how fish assimilate fruits and estimate turnover times of isotopic tracers (Lucas and Baras 2008).

Given the importance of frugivory as a feed strategy in freshwater fishes, it is important to consider the ability of carbon, nitrogen, and hydrogen isotopes to infer the dietary contribution of carbohydrate-rich fruits. In this study, we compare the isotopic signatures of muscle tissue between catfish fed known mixes of fruit and invertebrate prey. Our study imitates a natural interaction that occurs between two invasive species: catfish and olive in the San Juan River (NM/UT, USA). We hypothesized that isotopic signatures would underrepresent a carbohydrate-rich feed item in the muscle of catfish. The specific objective of this study is to determine the contribution of olive fruit to stable isotope signatures of the catfish muscle tissue.

Methods

This experiment was conducted simultaneously with catfish growth, energy density, and lipid deposition data presented in Cheek et al. (2025). The individual fish used in this study shared experimental units and were subjected to identical experimental conditions and feed treatments as those described in Cheek et al. (2025). However, separate individuals from each experimental unit were used to generate the stable isotope data presented here, and no isotope data from the experiment has been previously published. We clearly indicate instances where data from Cheek et al. (2025) are referenced for context or comparison and have summarized the experimental methods in the following sections.

Experimental design

Olive fruits were gathered from the floodplains of the San Juan River across New Mexico and Utah, USA, during the peak periods of fruit drop in late September and early October of 2017. Fruits were bagged and frozen at a temperature of −20 °C to preserve them for subsequent analysis and processing. Once thawed, the seeds were extracted through manual pressure applied over wire mesh, isolating the fruit pulp. The pulp was dried at 60 °C for 48 h and was finely ground. A commercially available insect meal derived from black soldier fly larvae (Stratiomyidae family), (Repashy Superfoods Inc.) was chosen for its nutritional resemblance to the aquatic and terrestrial insect diet of catfish in the San Juan River. These two dietary components were then proportionally mixed into five feeds by weight, creating a series that included 100% olive, a gradation of mixed meals (75% olive:25% fly meal, 50% olive:50% fly meal, 25% olive:75% fly meal), 100% fly meal, and a commercial aquaculture feed (Purina Aquamax 500). To these blends, carboxymethyl cellulose was added as a non-nutritive binder to aid in the formation of cohesive and stable food pellets. The dry ingredients underwent a thorough mixing process using a v-blender (Patterson-Kelly Lab 8 qt V-blender) for 5 min before being combined with water. This mixture was then extruded into 8 mm diameter strands, dried over a 24-h period, broken into pellets, and stored at −20 °C until needed. We conducted stable isotope analysis on all feed ingredients to determine signatures of the individual feed items.

We used a total of 27 experimental units in an unbalanced design in which the 100% olive, 50% olive, and commercial feed treatments had five replicates, and the 75% olive, 25% olive, and 0% olive treatments were represented by four replicates. Experimental units, 113.6 L aquariums, shared a common recirculating water system; therefore, the isotopic contribution of the water source was the same for all treatments. Feed treatments were fed for 44 days to provide sufficient turnover time for feed diets to be represented in tissues (Winter et al. 2019; Cheek et al. 2024). In addition, a longer feeding period was not possible due to the size and holding capacity of the experimental units as fish size increased during the trial. To accurately replicate the environmental conditions experienced by catfish in the San Juan River, as per Cheek et al. (2024), the study

was situated within an environmental chamber. This allowed for precise control over both water temperature and photoperiod, emulating the conditions based on averaged mean daily temperatures from USGS gauges (Shiprock, Four Corners, and Mexican Hat) from the fall of 2016 and day-length hours calculated from sunrise and sunset times courtesy of the National Oceanic and Atmospheric Administration Solar Calculator for Four Corners, UT. Specific adjustments were made to decrease water temperatures from 22 °C to 14 °C incrementally at 0.18 °C/day and shorten day length from 14 to 10 h by 5.4 min per day. Despite a malfunction in the temperature control system leading to fluctuations within the first 10 days, temperatures were closely monitored and adjusted to maintain consistency within a 0.5 °C range for the remainder of the study. Key water quality parameters, including dissolved oxygen, pH, and conductivity, were monitored daily, while nitrate, nitrite, and ammonia levels were checked weekly to ensure they remained within safe thresholds.

Catfish fingerlings were sourced from Andry's Fish Farm LLC and acclimated at the Purdue Aquaculture Research Laboratory, where they were raised on commercial feed until they achieved a size indicative of fruit consumption from field observations (mean total length = 254 mm) (Cheek et al. 2024). Following a seven-day fasting period aimed at reducing stress from handling, three catfish were stocked into each experimental unit. Initially, all fish were acclimated to the experimental conditions on uniform commercial feed for 2 weeks, after which they were transitioned to their respective experimental feed. The weight of feed consumed by each tank was documented on a weekly basis, following a daily feeding schedule that aligned with simulated sunrise times. Fish were fed to satiation, with any feed remaining in the tanks after 30 min removed, dried, and weighed. Upon the completion of the experimental period, the fish were euthanized for analysis.

During the acclimatization phase, fish that showed signs of stress or failed to feed were removed and replaced with fish from the communal grow-out tanks. Following the acclimatization period, any experimental fish that displayed signs of stress or died were recorded and removed from the experiment without replacement. During this time, three fish were removed from three separate experimental units—two from the 50% olive treatment and one from the 25% olive treatment—ensuring that at least one fish remained in each experimental unit by the end of the acclimatization period (Table S1). One fish was removed from each experimental unit at the beginning of the feeding period and was used for lipid and energy density estimates presented in Cheek et al. (2025), leaving two fish in all but three experimental units where fish were removed due to stress. No mortality was observed during the subsequent 44-day trial.

Only one fish from each experimental unit was randomly selected for isotope sampling, and the other two fish were used in the Cheek et al. (2025) experiment. Fish muscle tissue samples were removed from a portion of the fish near the dorsal fin and dried at 60 °C for 48 h. Once dry, muscle samples were ground using a mortar and pestle. Ground samples were weighed to 0.9–1.0 mg for carbon and nitrogen and 0.1–0.3 mg for hydrogen. Weighed tissue samples were encapsulated in 3 × 5 mm tin capsules (Costech Analytical Technologies Inc., Valencia, CA, USA) for carbon and nitrogen isotopes and 3 × 5 mm silver capsules (Costech Analytical Technologies Inc., Valencia, CA, USA) for hydrogen. Treatment feeds were collected after pelletizing and samples were dried, ground, weighed, and encapsulated using the same methods as muscle tissue.

Isotope samples were analyzed at the University of New Mexico Center for Stable Isotopes (Albuquerque, NM). Carbon and nitrogen isotopes were measured using a Costech 4010 elemental analyzer coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer. Hydrogen samples were also analyzed using a Thermo Scientific Delta V mass spectrometer with a dual inlet and Conflo IV interface connected to a high-temperature conversion elemental analyzer. Stable isotope data are expressed as δ values using the equation $\delta X = [(R_{Sample} - R_{Standard})/R_{Standard}] \times 1000$, where R_{Sample} and $R_{Standard}$ are the ratios of $^{15}N/^{14}N$, $^{13}C/^{12}C$, $^{2}H/^{1}H$. The internationally accepted standards for $\delta^{2}H$ and $\delta^{13}C$ are Vienna Standard Mean Ocean Water (VSMOW) and Vienna Pee Dee Belemnite (VPDB), respectively, while $\delta^{15}N$ uses atmospheric N^2 as the standard.

Analytical methods

Differences in nitrogen, carbon, and hydrogen isotopic signatures of feed and catfish muscle tissue were evaluated between feed treatments using a one-way analysis of variance. All models met the assumptions

Table 1 Analysis of variance for carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and hydrogen ($\delta^{2}H$) isotopes in feed treatments Russian olive (*Elaeagnus angustifolia*) fruit and black soldier fly meal (*Stratiomyidae*: Repashy Superfoods, Inc.) were used as diet treatments alone with three additional diet mixes (75% olive: 25% fly meal, 50% olive: 50% fly meal, 25% olive: 75% fly meal) and channel catfish (*Ictalurus punctatus*) fish muscle tissue with *p* value significance of 0.01

Isotope	*F*-value	df	*p*-value
Feed C	309.9	5:47	**<0.001**
Feed N	595.2	5:47	**<0.001**
Feed H	376.4	5:47	**<0.001**
Fish C	19.5	5:21	**<0.001**
Fish N	4.791	5:21	**<0.001**
Fish H	0.274	5:21	0.922

of normality and variance, tested using the Shapiro–Wilk test and Levene's test, respectively, with an alpha of 0.05. Tukey's Honest Significant Difference (HSD) was performed post-hoc to identify specific pairwise differences between our six treatments using the "HSD.test" function with the "agricolae" package (de Mendiburu and de Mendiburu 2019). We tested differences in nitrogen, carbon, and hydrogen in treatment feeds and muscle tissue using a permutational multivariate analysis of variance (PERMANOVA) based on Euclidean distance and 10,000 permutations. PERMANOVA models were implemented using the "adonis2" function in package "vegan" (Oksanen et al. 2007). We included all isotopes as responses variables and treatment as the fixed effect. Multivariate pairwise comparisons between treatments were conducted with the "pairwise.adonis2" (Martinez Arbizu 2020) function in the pairwiseAdonis package; analysis was performed in R version 4.4.1 (R Core Team 2021).

Results

Catfish growth varied significantly between treatment diets (Cheek et al. 2025). As expected, catfish fed the optimum commercial (control) diet had the greatest weight gain during the trial (Table S1). Catfish fed the mixed diets all increased in weight and total length during the trial, but catfish fed the 100% olive diet only maintained or slightly lost weight (mean -1 ± 1.5 g). For the mixed diets, growth increased as the proportion of fly meal in the diet increased. Feed ration was not significantly different between treatments.

Treatment feeds were significantly different in isotopic ratios for carbon (Table 1: $F_{5,47} = 309.9$, $p < 0.001$), nitrogen (Table 1: $F_{5,47} = 595.2$, $p < 0.001$), and hydrogen (Table 1: $F_{5,47} = 376.4$, $p < 0.001$). Tukey's pairwise comparisons indicate that all treatment feeds were significantly different in carbon isotopic signature (Fig. 1a: Table S2). However, there was less variation between treatment feeds for nitrogen, with olive being depleted in 15N. There was no difference in nitrogen isotopic value between the commercial feed and the 50% olive treatment and only a slight enrichment in 15 N in the 25% and 0% olive treatments (Fig. 1b: Table S1). The hydrogen isotope values of treatments showed no significant differences when comparing commercial to 25% olive treatment and 100% olive treatment to the 75% olive treatment, but all other Tukey's pairwise comparisons were significantly different (Fig. 1c: Table S2). Biplots of carbon and nitrogen values of feed treatments (Fig. 2a) show distinct groupings of all treatment diets. The PERMANOVA analysis also reveals that all feed treatments were significantly different in multivariate space and all pairwise comparisons were significant (Table 2: $R^2 = 0.97$, $p < 0.001$). Multivariate pairwise comparisons show that Russian Olive (100%) and Fly Meal (0%) exhibited the greatest distances from the means (Table S2, $p = 0.004$), while our mixed treatments show a closer distance to the means in isotopic signatures (Table S3, $p = 0.002$). There was a difference in the percentage of nitrogen ($F_{1,48} = 717.1$, $p < 0.001$), carbon ($F_{1,48} = 340.11$, $p < 0.001$), and hydrogen ($F_{1,48} = 63.22$, $p < 0.001$) in treatment feeds showing differences between treatments (Table 3).

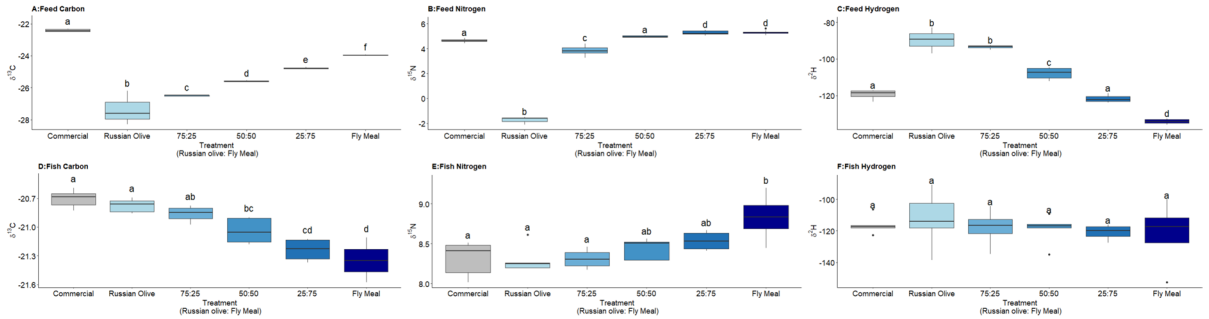


Fig. 1 Isotope carbon (δ13C), nitrogen (δ15N), and hydrogen (δ2H) signatures of feed (top) and channel catfish (*Ictalurus punctatus*) fish muscle tissue (bottom) from a 44-day feeding trial with six feed treatments. Commercial feed was used as a positive control and represents pre-trial feed conditions. Russian olive (*Elaeagnus angustifolia*) fruit and black soldier fly meal (Stratiomyidae: Repashy Superfoods, Inc.) were used as diet treatments alone with three additional diet mixes (75% olive: 25% fly meal, 50% olive: 50% fly meal, 25% olive: 75% fly meal). Letter represents Tukey HSD-test for pairwise differences. The median (black bar), interquartile range (shaded box), range (vertical lines), and included outliers (black dots) illustrate the variation by treatment

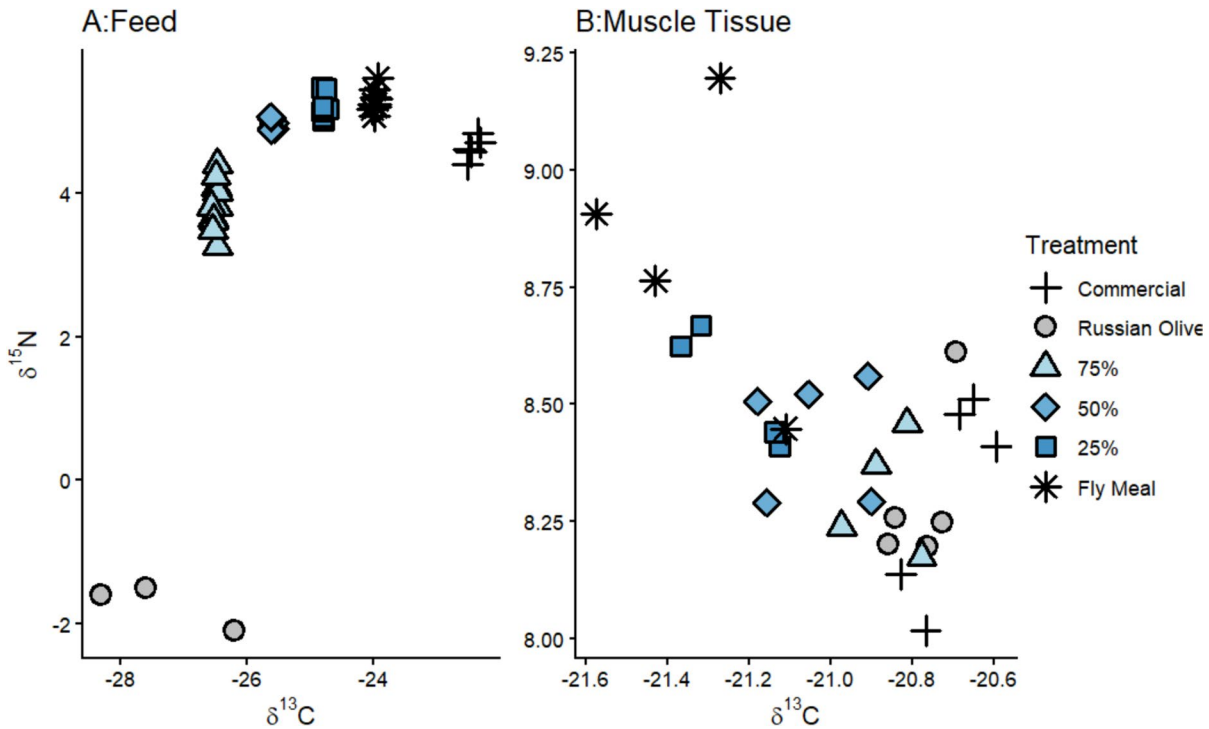


Fig. 2 Biplot of isotopic composition carbon (δ13C), nitrogen (δ15N), for feed items Russian olive (*Elaeagnus angustifolia*) fruit and black soldier fly meal (*Stratiomyidae*: Repashy Superfoods, Inc.) were used as diet treatments alone with three additional diet mixes (75% olive: 25% fly meal, 50% olive: 50% fly meal, 25% olive: 75% fly meal) (left panel) and channel catfish (*Ictalurus punctatus*) fish muscle tissue (right panel) across different treatments. Biplot illustrates the variation in isotopic signatures among treatments

Table 2 PERMANOVA results (adonis, vegan package) for feed items and fish muscle tissue based on 10,000 permutations Fish CNH is PERMANOVA with all isotopes and Fish CN is only with carbon and nitrogen. Significant results are indicated by a p-value < 0.05

Source	df	SS	R^2	Pseudo-F	p-value
Feed	5	11,447.3	0.97	377.42	<0.001
Residual	42	254.8	0.02		
Total	47	11,702.1	1		
Fish (C, N, H)	5	250.6	0.06	0.27	0.9248
Residual	21	3802.2	0.93		
Total	26	4052.9	1		
Fish (C, N)	5	2.34	0.68	9.03	<0.001
Residual	21	1.09	0.31		
Total	26	3.44	1		

Table 3 Mean percent of carbon, nitrogen, hydrogen for experimental feed and channel catfish muscle tissue after being fed six treatment feeds for 44-days

Treatment	Feed						Muscle					
	%C		%N		%H		%C		%N		%H	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
100:0	41.4	0.19	.7	<0.1	55.5	1.58	47.4	3.1	14.0	0.9	5.0	0.8
75:25	43.2	0.26	2.3	<0.1	18.5	0.48	48.1	2.9	14.2	0.9	5.1	0.6
50:50	45.8	0.28	4.0	<0.1	11.4	<0.1	47.6	2.9	14.0	0.9	5.2	0.5
25:75	47.7	0.16	5.6	<0.1	8.5	<0.1	48.2	3.5	13.9	1.4	5.3	0.8
0:100	49.4	0.44	7.2	<0.1	6.8	<0.1	49.2	0.6	14.4	0.2	5.1	0.7
Commercial	48.2	0.23	6.7	0.10	7.2	<0.1	49.0	0.9	14.4	0.2	5.0	0.6

The effect of the treatment feed on isotope signatures of muscle tissue was significant for carbon (Table 1: $F_{5,21}=19.5$, $p<0.001$) and nitrogen (Table 1: $F_{5,21}=4.791$, $p<0.001$), but not hydrogen (Table 1: $F_{5,21}=0.274$, $p=0.922$). We found no significant difference in the variation of isotopic ratios between treatments for carbon ($F_{5,21}=1.90$, $p=0.137$), nitrogen ($F_{5,21}=0.68$, $p=0.637$), or hydrogen ($F_{5,21}=1.038$, $p=0.4215$). The isotopic signatures of catfish muscle tissue fed diets of 100% olive and 75% olive treatments were not significantly different from the commercial feed for carbon, nitrogen, or hydrogen isotopes (Fig. 1d–f: Table S4). Catfish were fed the commercial feed prior to the initiation of the trial feeds; therefore, isotopic signatures of catfish fed 100% olive and 75% olive treatments did not vary from the pre-trial conditions. Isotopic signatures of catfish fed 100% fly meal were significantly different from all other treatments for carbon and nitrogen, indicating a change in tissue isotopes from the pre-trial conditions (Fig. 1d, e: Table S4). For carbon (Fig. 1d), isotopic values of muscle tissue reflected the assimilation of fly meal with increased depletion of 13C as the proportion of fly meal in the diet increased. For nitrogen (Fig. 1e), all treatments that included olive (25%, 75%, 50%, and 100% olive) were not significantly different from the commercial feed at the end of the trial (Table S4). There was no significant difference for any Tukey's pairwise comparisons in the hydrogen isotopic signature of catfish muscle tissue (Fig. 1f). Biplots of carbon and nitrogen isotope values (Fig. 2b) indicate that muscle tissue signatures show differences between 100% fly meal and commercial feeds, but little difference between 100% olive and commercial feed. However, when evaluating the difference in muscle tissue isotopes signatures (C, N, and H) in multivariate space, the effect of treatment feed was not significant (Table 2: PERMANOVA: $R^2=0.06$, $p=0.924$). However, there

were significant differences between treatments when considering only carbon and nitrogen (Table 2: PERMANOVA: $R^2=0.68$, $p<0.001$). Adonis pairwise comparisons indicate that fish fed 100% olive and 75% olive did not differ in carbon and nitrogen from the commercial pretrial conditions (Table S4). In addition, multivariate pairwise comparisons indicate that carbon and nitrogen signatures of fish fed 100% fly meal were significantly different from all treatments except the 25% olive treatment (Table S4). There was no difference in the percentage of nitrogen ($F_{1,50}=1.446$, $p=<0.235$), carbon ($F_{1,50}=2.698$, $p=0.107$), and hydrogen ($F_{1,50}=0.33$, $p=0.857$) in muscle tissue shows between treatments (Table 3).

Discussion

In our study, carbon and nitrogen isotopes analysis of bulk muscle tissue accurately reflected consumption of commercial feed and soldier fly larvae meal but failed to accurately represent the contribution of olive fruit to the diet of catfish. Our results support our hypothesis that the carbohydrate-rich forage items are underrepresented by isotope analysis of muscle tissue when the energy is used for metabolic processes and not assimilated into tissue. Olive fruit is composed primarily of carbohydrates and has a large proportion of indigestible material (Cheek et al. 2024). Catfish fed a diet of 100% olive can maintain weight, but fruits are not nutritionally sufficient for muscle growth assimilation. It was also found that these fish had the potential to lose weight as well (Cheek et al. 2025). Olive fruits represent a significant energetic subsidy to catfish populations in the San Juan River, accounting for an estimated 35% of annual energy consumed by catfish populations (Cheek et al. 2024). However, diet composition derived from field estimates of C and N in muscle tissue of catfish does not reflect the contribution of olive fruit (Gido et al. 2006).

Isotopic composition of muscle tissue in catfish may not accurately represent carbohydrate intake due to the metabolic processes involved. Plant-based carbohydrates are often rapidly utilized for energy and are less likely to be incorporated directly into muscle tissue (Martino et al. 2019). In contrast, proteins and lipid-derived carbon are preferentially incorporated into structural tissues (Perkins et al. 2014). Isotopic signatures of carbohydrates may also be modified through gluconeogenesis, where non-carbohydrate precursors are converted into glucose, skewing the isotopic profile toward lipids and proteins (Bequette et al. 2006). Concurring with our results, isotopic signatures of bulk muscle tissue may lead to an incomplete representation of dietary intake of catfish. Correa and Winemiller (2014), however, were able to show that the contribution of fruits to isotopic signatures of frugivorous fishes can be due to the study species' ability to crush the seeds of the fruits or even the fruits' composition itself. Our findings align with existing scientific literature that highlights the challenges of detecting dietary contributions from these rich carbohydrate diet items. Martinez de Rio et al. (2009) and Newsome et al. (2007) were able to show that carbohydrates can rapidly metabolize and are preferentially used for energy and were not incorporated into tissue, this in turn can lead to the misrepresentation of the unique isotopic signature.

Being omnivorous, catfish differ from herbivorous fishes in their ability to assimilate plant material into muscle tissue due to their digestive anatomy and mechanical processing ability. Catfish have a relatively short gut length compared to herbivorous fishes, which often possess elongated digestive tracts adapted to maximize the breakdown and fermentation of plant material such as cellulose (Bakke et al. 2010; Duque-Correa et al. 2024). Herbivorous fishes may also host specialized gut microbiomes that produce enzymes to aid in complex carbohydrate digestion, which are unlikely to occur in the digestive tract of catfish (Ganguly and Prasad 2012). In addition, catfish lack adaptations for masticating and grinding food items, such as pharyngeal teeth, which can increase the digestibility of tough plant material. These adaptations may allow herbivorous or omnivorous species to better digest and assimilate olive fruit or seeds into muscle tissue. For example, Heinrich et al. (2021) observed isotopic signatures of olive fruit consumption in the muscle tissue of common carp, *Cyprinus carpio* Linnaeus, 1758, a species with large crushing pharyngeal teeth (Sibbing et al. 1986) that may allow them to digest both fruit and seed from olives. Seeds are typically composed of more protein and lipids compared to carbohydrate-rich fruit flesh (Jordano 2000); therefore, adaptations that improve digestion of both the fruit and seed are likely to change how muscle tissue isotopes reflect the diet item. Seeds consumed and passed by catfish remain viable for germination, indicating that little, if any,

nutrition is gained from the seed itself (Cheek et al. 2025). Therefore, muscle tissue samples from fishes with adaptations for feeding on plant material may be more likely to reflect the isotopic signatures of carbohydrates found in their diet.

Carbohydrates are a key source of hydrogen in diet and therefore have the potential to reflect the dietary contribution to isotopic signatures. Yet, our results suggest that even though feed treatments were significantly different in hydrogen isotope composition, they were not reflected in bulk muscle samples for any feed treatment. Unlike carbon and nitrogen, a significant proportion of hydrogen in animal tissues is absorbed or ingested through water intake and may be particularly pronounced in aquatic organisms (Solomon et al. 2009; Soto et al. 2013). The contribution of hydrogen from environmental water can mix with hydrogen ingested through diet and reduce the correspondence between dietary and muscle isotopic signatures (Soto et al. 2016; Whitledge et al. 2006). Our results indicate that the hydrogen signature did not reflect dietary hydrogen contribution, and the effect of the feed treatment was overwhelmed by the influence of the shared water source both prior to and during the trial.

Our study was designed to better understand existing isotope data from muscle tissue collected from catfish in the San Juan River; therefore, we did not evaluate alternative isotopes or tissues that may better reflect the contribution of fruits to metabolic energy. However, our results suggest that one of the most common methods of collecting bulk C and N isotope samples from muscle tissue can considerably underestimate the contribution of carbohydrate-rich diet items to energy consumption in an omnivorous fish. Sampling metabolically active tissues with faster turnover rates, such as liver and blood plasma, would be more likely to reflect short-term isotopic signatures of metabolized carbohydrates (Felip et al. 2012). Compound-specific stable isotope analysis targeting carbohydrate metabolites or glucose may also help to isolate isotopic signatures related to carbohydrate consumption and metabolism (Blanke et al. 2017; Whiteman et al. 2019). Researchers should consider these approaches that will limit the confounding effects of macronutrient composition and metabolic processes to enable more accurate tracking of dietary energy sources in aquatic consumers. In the future, researchers should consider conducting this experiment on individuals of sexual maturity and consider other species as well.

Understanding the inherent assumptions and biases of stable isotope analysis through controlled laboratory experiments is essential for developing tools in isotope ecology and for a better understanding of ecological questions that rely on these analyses. In our experiment, the consumption of a carbohydrate-rich diet item by an omnivorous freshwater fish was not reflected by bulk isotope samples of carbon, nitrogen, or hydrogen in muscle tissue. Researchers and managers should be aware that consumption estimates that rely on bulk muscle tissue for estimating diet composition could be significantly underestimating the contribution of carbohydrates if they are present in stomach contents, and it should not be assumed that they are not contributing to the energetic demand of the consumer.

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Author contribution All authors contributed to the study conception and design. Material preparation and data collection were performed by Christopher Cheek, Brandon Peoples, and Reuben Goforth, and analysis was performed by Justin Sturtz. The first draft of the manuscript was written by Justin Sturtz, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Data will be available upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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