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Stable isotope enrichment in muscle, liver, and whole fish tissues of brown-marbled groupers (*Epinephelus fuscoguttatus*)

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Abstract

Introduction: The purpose of this study was to assess enrichments in stable carbon and nitrogen isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in brown-marbled groupers (*Epinephelus fuscoguttatus*), a marine fish that has been widely used in aquaculture. Stable isotope analysis has been used to evaluate dietary sources and the trophic position of fish. There is the need to better understand the pattern of isotope enrichment between consumers and diets under laboratory conditions.

Methods: We studied the changes in stable carbon and nitrogen isotopes of juvenile brown-marbled groupers during a feeding experiment in 2009. Fish were grown in aquaria and fed a sole source of protein for 56 days and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios in whole fish, muscle, and liver tissues.

Results: At the end of the 56-day feeding experiment, fish grew to an average of 55.6 g from an average of 22.5 g. Compared to the dietary isotope compositions, whole fish and muscle tissues of the juvenile groupers were enriched in $\delta^{13}\text{C}$ by 1.6 and 0.5‰, while the liver was depleted by 1.3‰. The $\delta^{15}\text{N}$ enrichments were 1.6‰ for whole fish, 1.3‰ for muscle, and 1.0‰ for liver. Except for liver, $\delta^{15}\text{N}$ isotope values increased significantly with time.

Conclusions: The small change in $\delta^{13}\text{C}$ between the diet and fish suggests that little isotope alteration is occurring during the assimilation of dietary carbon. This provides a basis for estimates of the importance of different sources of dietary components when contrasted with the isotope values from a formulated diet with known isotope values of the different components. The smaller than expected $\delta^{15}\text{N}$ enrichment in all tissue suggests that isotope values from a wild fish sample may not always reach isotope equilibrium with the current diet, and an inference about recent dietary sources and an estimate of the consumer's trophic position should be evaluated with caution.

Keywords: Stable isotopes, Diet shift, Fish, Isotope enrichment

Introduction

Carbon and nitrogen stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) are increasingly used to evaluate the relative contributions of different food sources and the trophic position of fish (Anderson et al. 1987; Lochman and Phillips 1996; Gu et al. 1996a, b; Gamboa-Delgado et al. 2008). Because isotope compositions reflect the organic compounds that have been incorporated into the bodies of consumers, the measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ provide insightful information on the dietary component assimilated by the consumers. At the

same time, conventional studies such as feeding observations or gut content analyses reveal only the materials ingested and not necessarily those assimilated by the consumers. These approaches can also be labor intensive and time-consuming while providing little information on the sources of energy and nutrients for animal growth (Bitterlich and Gnaiger 1984; Gu et al. 1996a, b).

The use of stable isotope analysis in trophic ecology is based on the premise that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of consumers reflect those of the assimilated diet and that the alteration of isotope compositions during the assimilation of food by consumers can be followed in a predictable manner (DeNiro and Epstein 1978, 1981; Minagawa

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and Wada 1984; Post 2002). Isotope enrichment in consumers takes place during the assimilation of carbon and nitrogen from the diet. A review of the literature indicates that the average isotope enrichment during each trophic transfer is 0.5‰ for $\delta^{13}\text{C}$ and 3.4‰ for $\delta^{15}\text{N}$, but with large variations in both stable isotopes (Post 2002). These data are largely derived from field studies with many unknown and uncontrolled factors such as food sources (Gu et al. 1996a, b; Post 2002) and differences in growth rate, age, and food quality, which may affect the magnitude of isotope fractionation. Muscle tissue of fish has been widely used to represent the whole fish isotope characteristic. However, previous and current studies also indicate wide variations in isotope composition among consumer tissues (DeNiro and Epstein 1978, 1981; Tieszen et al. 1983; Pinnegar and Polunin 1999; Miller 2006). More studies are needed to validate the use of a certain tissue type during a food web study to establish the precise trophic relationship between consumers and their diets.

This study was designed to analyze the magnitude of isotope enrichment in fish and thus to provide the basis for a better understanding of the dietary sources and trophic position of fish. The experiment utilized brown-marbled groupers, *Epinephelus fuscoguttatus*, a carnivorous fish widely distributed in the subtropical and tropical marine regions and grown as a high quality human food in Southeast Asia.

Methods

The experiment was conducted at the aquaculture facility of Long Hai Tian Science Garden, Dong Hai Island, Guangdong, China, between July and October 2009. The experimental units were plastic aquaria with the dimensions of 70 × 50 × 70 cm (length × width × height). Each aquarium was filled with continuously aerated natural seawater prefiltered using a sand medium. Water quality was monitored following the standard methods (APHA 1998). The experiment was conducted indoors using a natural dark:light cycle. The fish diet was of trash fish (*Engraulis ringens*) powder imported from Peru, which was formed into pellets. The composition of the formulated pellets is presented in Table 1. The average stable isotope values of the diet were 16.4‰ for $\delta^{15}\text{N}$ and -20.4‰ for $\delta^{13}\text{C}$ (Table 1).

Newly hatched larval fish were obtained from the Guangdong Ocean University hatchery and raised in a large cement tank with a commercial feed. After reaching an average weight of 22.5 ± 3.2 g, the fish were transferred to six aquaria stocked with 12 fish per aquarium. Fish were fed with the formulated pellets as the only protein source twice daily at 0900 and 1300 local time. Samples for stable isotope analysis were taken at day 0, 3, 7, 14, 28, 42, and 56. Prior to sampling, all fish were

Table 1 Chemical composition and stable isotope values of the diet (trash fish) used in the current experiment

Component	Values
Crude protein (% dry mass)	50.17
Crude fat (% dry mass)	10.32
Crude fiber (% dry mass)	5.6
Gross energy (kJ/g)	19.25
$\delta^{13}\text{C}$ (‰)	-20.4
$\delta^{15}\text{N}$ (‰)	16.4

starved for 24 h to expunge the food content of their stomachs and intestines. All fish from a randomly selected aquarium were harvested during each sampling event. Three individuals were used for whole fish, muscle, and liver ($n = 9$ fish), respectively. The remaining fish served as backup alternates. For whole fish, the entire fish was cut into several pieces. For muscle and liver tissue, the dorsal muscle tissue and liver were removed from each fish and washed with deionized water to remove blood. Fish feces were collected by siphoning from the bottom of the aquarium. All samples were dried at 70°C and ground to a fine power.

For stable isotope analysis, about 1 mg of the sample was loaded into a tin capsule and shipped to the Stable Isotope Facility of the University of California, Davis for ^{13}C and ^{15}N analysis using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK). Samples were combusted at 1,000°C in a reactor packed with chromium oxide and silvered cobaltous/cobaltic oxide. During analysis, samples were interspersed with several replicates of at least two different laboratory standards. The long term standard deviation is 0.2‰ for ^{13}C and 0.3‰ for ^{15}N . The heavy to light isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) are reported as the conventional delta notation (δ) defined as $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000$, where X is ^{13}C or ^{15}N and R is the ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. Isotopic ratios are expressed relative to VPDB (Vienna Pee Dee belemnite) for $\delta^{13}\text{C}$ and to atmospheric nitrogen for $\delta^{15}\text{N}$.

Results

During the experimental period, water temperature ranged from 24 to 28°C, salinity from 29 to 31‰, pH from 7.9 to 8.2, dissolved oxygen was > 6 mg/L, ammonium < 0.02 mg/L, and nitrate < 2.0 mg/L. At the end of the experiment, fish grew to an average of 55.6 g from an average of 22.5 g, more than doubling their initial weight (Table 2). The average growth rate (k) of fish was calculated to be 0.023.

Changes in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of whole fish, muscle, and liver tissues during the experimental period

Table 2 Body length, total length and wet weight of brown-marbled groupers before and during the experimental period

Time	Body length (cm)		Total length (cm)		Wet weight (g)		Sample number
	Mean	SD	Mean	SD	Mean	SD	
Before	9.0	0.6	10.7	0.7	22.5	3.8	10
3	9.1	0.5	10.7	0.4	24.5	3.8	10
7	9.4	0.5	11.6	0.9	31.1	5.1	10
14	10.7	0.4	12.8	0.5	35.4	5.1	10
28	11.6	0.6	13.4	0.6	42.7	5.9	10
42	12.7	0.6	14.4	0.8	45.6	7.5	10
56	10.5	1.2	13.6	1.5	55.6	14.1	10

are presented in Figure 1. The initial whole fish $\delta^{13}\text{C}$ value (-21.1‰) was below the dietary value (-20.4‰) and increased linearly to -18.8‰ by day 56 (Figure 1A). In contrast, the initial muscle $\delta^{13}\text{C}$ value (-18.6‰) was above the dietary value and increased to -17.5‰ by day

56 (Figure 1B). The initial liver $\delta^{13}\text{C}$ value (-23.2‰) was well below the dietary value and reached a peak value (-21.3‰) at day 28 and then slightly decreased and increased at day 42 and day 56, respectively (Figure 1C). At the end of the experiment, whole fish and muscle tissue of the juvenile groupers were enriched in $\delta^{13}\text{C}$ by 1.6 and 0.5‰ while the liver was depleted by 1.3‰. All regression models showed significant increases in $\delta^{13}\text{C}$ values during the experimental period.

The initial $\delta^{15}\text{N}$ values of whole fish, muscle, and liver tissues were all below the dietary value and increased linearly as fish grew (Figure 1). The whole fish $\delta^{15}\text{N}$ value increased from 15.7‰ at day 0 to 18.0‰ at day 56 (Figure 1D). The muscle $\delta^{15}\text{N}$ value increased from 16.1‰ at day 0, reached its peak value (18.1‰) at day 42, and then decreased slightly to 17.8‰ at day 56 (Figure 1E). The initial liver $\delta^{15}\text{N}$ value (14.5‰) was well below the dietary value and remained little changed between days 3 and 27 before rapidly increasing above the dietary value during the final two sampling dates (Figure 1F). The $\delta^{15}\text{N}$ enrichments were 1.6‰ for whole fish, 1.3‰ for muscle, and 1.0‰ for liver. Except for

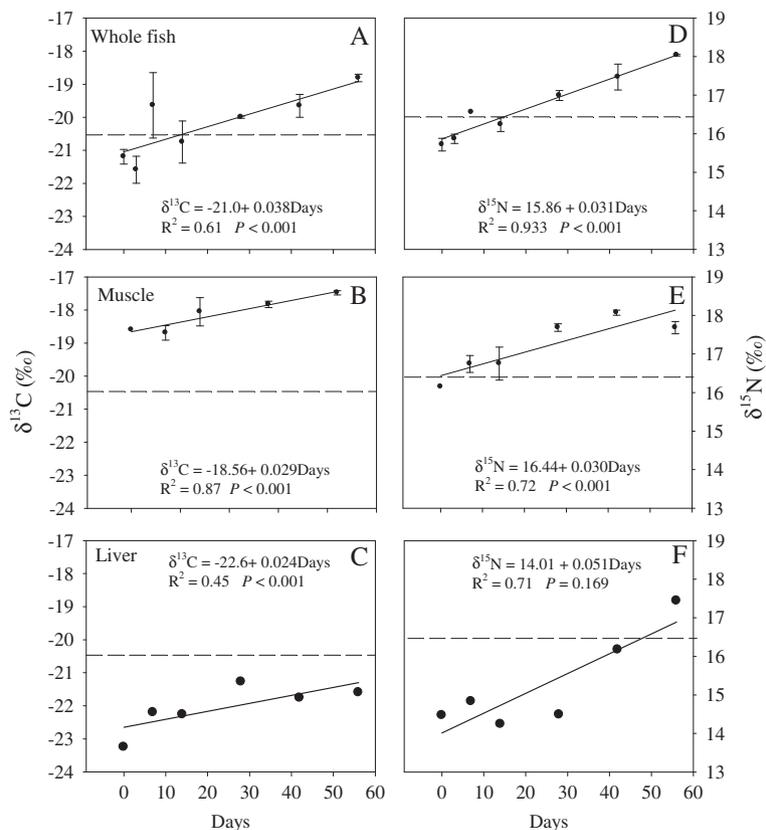


Figure 1 Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for whole fish, muscle, and liver tissues of juvenile brown-marbled groupers during the 56-day experimental period. The dashed lines represent the dietary values of $\delta^{13}\text{C}$ (-20.4‰) or $\delta^{15}\text{N}$ (16.4‰).

liver, regression models for whole fish and muscle tissue showed significant increases in $\delta^{15}\text{N}$ values during the experimental period.

Discussion and conclusions

Previous studies have shown various magnitudes of isotope enrichment in whole animals and individual tissues such as muscle, liver, kidney, blood, and hair (DeNiro and Epstein 1978, 1981; Tieszen et al. 1983; Pinnegar and Polunin 1999; Miller 2006). Isotope enrichments were the result of preferential retention of the heavy isotopes ^{13}C and ^{15}N during assimilation of dietary carbon and nitrogen (DeNiro and Epstein 1978, 1981). This was also confirmed in this experiment by noting that the feces were isotopically depleted (Table 3), leading to ^{13}C and ^{15}N enrichment in the tissues of juvenile groupers. Our results also show that, compared to the dietary isotope value, liver was depleted in ^{13}C . This is likely attributed to lipid storage in the liver that is depleted in ^{13}C .

Various factors have been proposed to account for the differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between animal tissues and their diet. Earlier studies showed that different individuals of a species differ in isotope fractionation when fed the same diet, but that isotope fractionation differs more between species than among individuals of the same species (DeNiro and Epstein 1978, 1981). Our results also showed differing isotope enrichments or depletions compared with the dietary $\delta^{13}\text{C}$. However, in general, the $\delta^{13}\text{C}$ enrichment of the juvenile groupers was less than 2‰. The muscle tissue of the juvenile groupers was enriched by 0.5‰, nearly identical to the reported average enrichment (Post 2002). These results suggest that muscle tissue is the most consistent proxy for carbon isotope fractionation when compared to the findings of many previous studies on fish (Hesslein et al. 1993; Pinnegar and Polunin 1999).

Our results also show that the $\delta^{15}\text{N}$ enrichment of muscle and liver tissues or whole fish is only 1.0–1.6‰, which is far below the reported average value (3.4‰). Several mechanisms may explain this discrepancy. First,

different consumers may have their intrinsic isotope fractionation during assimilation of protein. For example, Hesslein et al. (1993) found a maximum value of 3.0 of $\delta^{15}\text{N}$ enrichment for broad white fish (*Coregonus nasus*). Pinnegar and Polunin (1999) reported $\delta^{15}\text{N}$ enrichment in rainbow trout (*Oncorhynchus mykiss*) of 2.6‰. Second, consumers might assimilate a specific dietary component isotopically different from the whole diet, especially when they have access to a variety of diets in nature or are fed a formulated diet that is made from several sources of protein (Robbins et al. 2005a, b). This is not the case in our study because only a single source of protein was used. Third, dietary nutritional quality (Robbins et al. 2005a, b) and growth conditions (Hobson et al. 1993; Gaye-Siessegger et al. 2004) may also affect the isotope compositions of the consumers. These cannot be applied to our study because the formulated diet was high in nutritional value (Table 1), and good water quality was maintained during the experimental period. Lastly, it is also likely that the consumers had not reached isotopic equilibrium with the new diet during this study. This is evident from the linear relationship between time and the isotope values of the liver tissue and whole fish tissues (Figure 1), which never attained asymptotic values for either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$. It appears that the muscle $\delta^{15}\text{N}$ likely reached isotope equilibrium, but the experimental period was not long enough to provide sufficient data to confirm the asymptotic isotope stage. This finding is important when the measured values are used to determine the trophic position of fish that may switch diets due to migration or growth or that are affected by rapid changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the dietary organisms over a seasonal cycle (Gu 2009; Gu et al. 2011).

Results from this experiment have important implications for the studies of animal dietary composition and trophic positions. The small change in $\delta^{13}\text{C}$ between the diet and fish suggests that little isotope alteration is occurring during the assimilation of dietary carbon. This provides the basis for the estimates of the importance of different sources of dietary components when contrasted with the isotope values from a formulated diet with known isotope values of the different components (Lochman and Phillips 1996; Gamboa-Delgado et al. 2008). The data from this study also suggest that isotope values from a wild fish sample may not reach isotope equilibrium with the current diet and hence an inference about recent dietary sources or trophic position should be evaluated with caution because the isotope values are likely the results of previous and current dietary uptake. This is especially true in aquatic ecosystems where environmental changes or fish migration often result in a shift in diet or changes in the isotope composition of dietary organisms such as plankton (see review by Gu 2009; Gu et al. 2011).

Table 3 Average (standard deviation) of stable carbon and nitrogen isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and percent carbon (%C) and nitrogen (%N) in the feces of brown-marbled grouper

Day	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	%N	%C	Sample number
0	-21.9 (0.2)	10.3 (0.0)	2.2 (0.2)	23.1 (2.0)	4
7	-22.3 (0.6)	15.5 (0.5)	1.9 (0.4)	19.9 (3.1)	5
14	-21.5 (0.3)	14.8 (0.8)	1.9 (0.3)	17.7 (3.3)	5
28	-21.2 (0.6)	14.8 (0.9)	1.8 (0.3)	18.0 (4.0)	4
42	-21.6 (0.3)	14.3 (0.2)	1.8 (0.1)	18.0 (0.7)	2
56	-20.1	18	3.3	20.6	1

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GC, HZ and BG designed the study; HZ and DJ oversaw the experiment; BG drafted the manuscript. All authors read and commented on the manuscript.

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