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Dietary histories of herbivorous loricariid catfishes: evidence from $\delta^{13}C$ values of otoliths

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Abstract The ecology of many Neotropical fishes is difficult or often impossible to study during rainy seasons. Thus, ecological studies of tropical fishes are usually performed on fish captured only during dry seasons. Because otoliths preserve a record of life history, this study evaluated the utility of otolith stable isotope values for the investigation of trophic ecology of Neotropical fishes (specifically herbivorous loricariid catfish) throughout their lives. Because plant dietary materials have $\delta^{13}C$ values that are determined by their photosynthetic pathways, metabolism and environmental conditions, different plants may impart different isotope values on fish otoliths that reflect consumption of these plants. The $\delta^{13}C_{(otolith)}$ values of xylophagous *Panaque nigrolineatus* captured in the field were significantly lower than those of algivorous *Hypostomus regani* from a nearby region. A laboratory experiment wherein *Hypostomus sp.* had $\delta^{13}C_{(otolith)}$ values that reflected the $\delta^{13}C$ values of their plant diet and additional evidence indicate that $\delta^{13}C_{(otolith)}$ values in loricariid catfish otoliths can record dietary history.

Keywords Otolith · Loricariidae · Panaque · Hypostomus · $\delta^{13}C$ · Herbivorous

Introduction

Loricariid catfish are found exclusively in freshwater ecosystems of the Neotropics (Schaefer 1987; Nelson 1994). Although this family is one of the most diverse fish families in the world (with at least 100 genera and more than 680 described species (Isbrücker 2002)), little is known about their phylogenetic relationships, physiology, population biology or trophic ecology (Power 1984; Schaefer 1987; Agostinho et al. 1995; Mol 1995; Armbruster 1997; Nelson 2002). This taxonomic diversity, that appears to have occurred almost exclusively at lower trophic levels, coupled with regionally high loricariid densities (Vanni et al. 2002), indicates that this loricariid radiation is an interesting evolutionary event. Morphological novelties such as dermal plates, ventral sucker-like lips, jaw musculature, and tooth morphology

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were certainly important to the evolutionary success of the loricariids (Schaefer and Lauder 1986, Schaefer and Stewart 1993). However, studies of loricariid trophic ecology will be necessary to better understand this interesting radiation as well as to understand nutrient dynamics in many tropical stream systems (Flecker et al. 2002).

Most herbivorous fish derive their energy from autochthonous carbon that is fixed in the water column (Opuszynski and Shireman 1995), but some loricariids may specialize in allochthonous carbon from terrestrial vegetation (Araujo-Lima et al. 1986; Yossa and Araujo-Lima 1998, 1999) and at least one genus, *Panaque* (Eigenmann and Eigenmann 1889), may utilize only allochthonous carbon sources. Both field observations and laboratory experiments have shown that *Panaque* actively utilize wood in their diet and have shown positive growth on wood only diets (Schaefer and Stewart 1993; Nelson et al. 1999; Nelson 2002). Fish specializing on allochthonous carbon have been described, but generally these fishes target specialized lower-fiber plant tissues such as leaves, fruits, and nuts. Digestion of wood as a trophic strategy has not been described in other bony fishes. Therefore, because wood-eating loricariid catfishes like *Panaque sp.* (and perhaps *Cochliodon sp.)*, occupy unique niches in Neotropical ecosystems, studies of their trophic ecology will undoubtedly yield biological insight. In addition, because loricariids often exert considerable influence on tropical stream nutrient dynamics (Vanni et al. 2002), study of their trophic ecology may yield greater understanding of tropical stream ecology.

Plant materials have distinct stable carbon isotope values that are determined by their photosynthetic pathways (e.g. Wickman 1952; Craig 1954), and a variety of environmental factors (e.g. Ehleringer et al. 1993). Plants that rely on the C_4 carbon fixation pathway display a range of values that are centered around –12 to –14‰ VPDB, compared to plants that utilize the C_3 carbon fixation pathway that generates tissue with lower values (–25 to –28‰ VPDB). Aquatic particulate organic carbon (POC) and phytoplankton in the Amazon have $\delta^{13}C$ values that may be even lower than those of C_3 macrophytes (i.e. –33.3‰VPDB, Araujo-Lima et al. 1986). Metabolically inert otoliths store information in the ratios of trace elements and isotopes that provide records of the environment and physiology throughout the entire lifetime of the fish (e.g. Campana 1999). Because fish otoliths have been demonstrated to preserve a record of diet and metabolism (e.g. Radtke et al. 1996; Weidman and Millner 2000; Carpenter et al. 2003; Wurster and Patterson 2003; Wurster et al. 2005; Solomon et al. 2006, Jeff P. Chanton [Florida State University] personal communication), it is proposed that loricariid otoliths could store a record of dietary history. Otolith formation is regulated by macular cells that secrete calcium into the endolymphatic fluid surrounding the otoliths (e.g. Campana and Neilson 1985). Both metabolically-derived carbon and dissolved-inorganic carbon (DIC) are incorporated during calcium deposition (Kalish 1991a; Wurster and Patterson 2003; Wurster et al. 2005; Solomon et al. 2006). Thus, inorganic components of fish otoliths may serve as both an inter- and intra-annual secular record of the fish's biochemical status. Milling of this structure can provide time-specific samples that yield a secular $\delta^{13}C$ record of animal metabolism and/or diet. Heterotrophic tissue values tend to be consistent with, though higher than $\delta^{13}C_{(diet)}$ values (Minson and Ludlow 1975; DeNiro and Epstein 1978; Araujo-Lima et al. 1986). This relationship has been used in a diverse and growing number of trophic physiological and ecological studies (Kelly 2000). Amazonian phytoplankton, macrophytes and terrestrial materials tend to differ from each other in $\delta^{13}C$ values (Araujo-Lima et al. 1986) indicating that differences in loricariid catfish diets could be recorded in their $\delta^{13}C_{(otolith)}$ values. Because many species are inaccessible for study during lengthy rainy seasons, otoliths provide the potential for yielding trophic information unavailable from traditional stomach content analysis or soft-tissue $\delta^{13}C$ analysis.

One objective of this study was to determine whether otolith isotope chemistry could differentiate between species of loricariid catfish from similar geographic regions that were likely eating different items in the field. A second objective was to determine the correspondence of $\delta^{13}C_{(otolith)}$ values to various $\delta^{13}C_{(Diet)}$ values in laboratory-reared loricariids.

Materials and methods

Field-collected otolith analysis

All field-captured fish were sacrificed by an overdose of the anesthetics MS-222 or benzocaine. Otoliths were then extracted from individuals of the two loricariid species: (1) *Panaque nigrolineatus*, a known wood-eating loricariid, was recovered by hand from the wood it was foraging upon (22 fish; 5–9 July 2001, Rio do Peixe, Aruana, Goias, Brazil), and (2) algivorous *Hypostomus regani* captured by cast net (14 fish; 9 March 2001, Rio Mogi-Guaçu, Pirassununga, São Paulo, Brazil). After measuring total length and wet weight, sagittal otoliths were recovered from the fish by dissection, fixed to a standard glass slide, polished, and micromilled as described by Wurster et al. (1999). Estimated sample masses were ≥13 μg for *H. regani* and ≥20 μg for *P. nigrolineatus* otoliths.

Laboratory experiment

This experiment consisted of raising *Hypostomus* sp. on monotypic diets with distinctive $\delta^{13}C$ values. Populations of loricariids will consume and grow in the laboratory on virtually any commercially available algae, fruit, or vegetable (Nelson 2002). About 48 juvenile *Hypostomus* sp. ranging from 5 to 8 cm in total length were purchased from aquarium wholesalers and transported to the laboratory on 2 May 2 2001. About 12 individuals were randomly assigned to one of four dietary treatments: (1) maize, *Zea mays* (corn), a C_4 plant with relatively high $\delta^{13}C$ value of –11.4‰ VPDB; (2) commercially obtained freshwater algae, *Spirulina* sp., $\delta^{13}C$ = –23.8‰ VPDB; (3) broccoli, *Brassica oleracea*, $\delta^{13}C$ = –30.0‰ VPDB; and (4) a C_3 wood, red maple, *Acer rubrum* with $\delta^{13}C$ = –27.0‰ VPDB. In addition we determined $\delta^{13}C$ values of a coconut wood, *Scheelea phalerata* $\delta^{13}C$ = –26.0‰ VPDB. This was the actual wood all *P. nigrolineatus* were captured foraging upon and comprised the dominant gut content.

A total of 12 tanks (four fish/tank and three replicate tanks were used for each dietary treatment) were aerated continuously and water changed on a regular basis to minimize environmental differences among tanks other than diet. Naturally occurring algae were excluded from the diet by completely covering all the tanks with opaque material and muting the lights in the room. Temperature was kept at 28°C throughout the experiment. All *Hypostomus* sp. on the red maple diet gradually died over the first 5 months of the experiment. *Hypostomus* sp. were not expected to live long on a wood only diet since they were previously unable to exhibit positive growth on a wood-only diet in the laboratory (Nelson 2002). After 9 months on the monotypic diets, surviving fish were sacrificed by an overdose of anesthetic, and otoliths removed and prepared for isotope analysis. The four longest-lived *Hypostomus* sp. from the wood only diet treatment were also analyzed. The otoliths from the laboratory experiment were too small to micromill (less than 1 mm in diameter). Thus, whole otoliths from this experiment were subjected to stable isotope analysis.

Stable isotope analyses

Milled otolith samples or whole otoliths were placed in stainless steel capsules and roasted in vacuo for 1 h at 200°C to remove any volatiles that may interfere with analyses. Samples were reacted with 103% phosphoric acid at 70°C in a Finnigan ''Kiel-III'' carbonate preparation device directly coupled to a Finnigan MAT 252 gas ratio mass spectrometer. Calibration of $\delta^{13}C$ values was maintained by daily analysis of carbonate standards that bracket the sample values.

Dietary items were loaded into tin capsules and stable isotope values were obtained using a Thermo Finnigan Flash 1112 EA coupled to a Thermo Finnigan Delta Plus XL via a Conflo III interface. Samples are dropped under helium into an oxidation furnace packed with chromium (VI) oxide and silvered cobaltic/cobaltous oxide (to remove any halogens) at 1000°C. Organic material is oxidized to carbon dioxide and various nitrogen gases. This gas is then passed through a reduction furnace packed with elemental copper at 680°C to reduce all nitrogen bearing compounds to pure gaseous nitrogen. The resulting gases are then passed through a water trap to

eliminate moisture. A GC column at 50°C then follows to separate carbon dioxide and nitrogen gases for analysis in the mass spectrometer. Carbon isotope ratios are corrected for ^{17}O contribution and reported in per mil notation relative to the VPDB standard. Nitrogen isotope ratios are reported in per mil notation relative to AIR. Precision and calibration of data are monitored through routine analyses of in-house standards that are calibrated using IAEA standards. Accuracy of $\delta^{13}C$ and $\delta^{15}N$ measurements are 0.2‰ and 0.3‰, respectively, based on IAEA calibration standards. Precision is 0.06‰ and 0.20‰, respectively (one sigma).

All statistical analyses were performed with MINITAB for Windows, version 12.2 except for the repeated measures ANOVA with subsequent Scheffe's test that were performed with STATISTICA for Macintosh, version 4.1 after testing the data for assumptions of normality and homogeneity of variance.

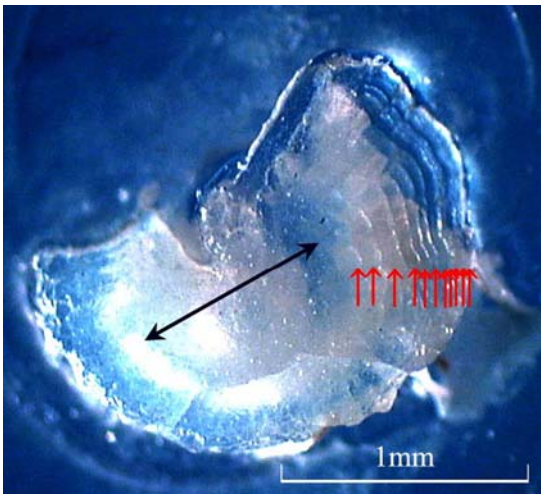

Fig. 1 A *Panaque nigrolineatus* otolith under the light microscope (×80). The long oblique arrow at the center of the otolith shows the loss of ring structure after a polishing step. The transparent plate overlap can be seen on the right side of the otolith. Short vertical arrows show the edge of each growth plate (eleven arrows in the image)

Results

General

Although there were several wood species submerged on the river bottom, *P. nigrolineatus* were found exclusively on a species of coconut tree, *Scheelea phalerata* ($\delta^{13}C_{(wood)}$ = –26.0‰ VPDB). A systematic assessment of gut contents was not made, but during the dissection of *P. nigrolineatus* gastrointestinal tracts, the only apparent stomach contents were wood shavings. The guts of all *H. regani* were filled with uniform green slurry, presumably comprised primarily of algae.

Field-collected otoliths

Otoliths of both species of loricariid were very delicate and small regardless of fish body size (Fig. 1). The average number of growth rings from the field collected *P. nigrolineatus* was 15.2 ± 3.76 SD and that of *H. regani* was 8.3 ± 2.3 (Table 1). Wet and dry seasons alternate once per year at the field sites, so that each otolith ring is thought to represent an annual growth ring. Accordingly, this suggests that growth rates for these two loricariid catfish species are low. The reported maximum size for each species is 43 cm for *P. nigrolineatus* (Fisch-Muller 2003) and 30 cm for *H. regani* (Baensch and Riehl 1991). Thus, these loricariid catfish are presumed to be long-lived to achieve such large body sizes despite low growth rates. A total of 12 *P. nigrolineatus* and 7 *H. regani* had complete data for age and $\delta^{13}C_{(otolith)}$ values. These data were used to test the relationship between $\delta^{13}C_{(otolith)}$ and other parameters. There were positive correlations between body length and weight ($P < 0.05$) but no correlations between the size of the fish (length or weight) and the number of growth rings ($P > 0.05$) in both species (Table 2). The size of the fish was not a good predictor of fish age, suggestive of large variation in growth rates. Mean $\delta^{13}C_{(otolith)}$ values of fish did not correlate with body length, weight, or age rings for either species ($P > 0.05$) (Table 2).

$\delta^{13}C_{(otolith)}$ values of *P. nigrolineatus* were normally distributed, while those of *H. regani* were not. Because of the significant heterogeneity of variance, a Kruskal–Wallis test was used to ascertain that mean $\delta^{13}C_{(otolith)}$ values for *P. nigrolineatus* and *H. regani* were significantly

Table 1 (i) Summary of measurements on field-caught *Panaque nigrolineatus* (*[1] otoliths of Fish# 2 and 16 were not successfully removed in the field dissection; *[2] $\delta^{13}C_{(otolith)}$ values of eight additional fish were not obtained. ''St Dev'' is standard deviation). (ii) Summary of measurements on field-caught *Hypostomus regani*. (*[3] otoliths of Fish# 1, 2, 3, 4, 11, and 14 did not show the growth ring structure due to poor crystallization or damage; *[4]; $\delta^{13}C_{(otolith)}$ values of Fish# 1, 2, and 5 were not obtained. ''St Dev'' is standard deviation)

	Min	Max	Mean	St Dev	*n*
(i)					
Length (cm)	20.50	31.2	27.14	±2.50	22
Weight (g)	250.0	570.0	406.5	±89.9	22
Growth rings	10.0	22.0	15.15	±3.76	20*[1]
$\delta^{13}C_{(otolith)}$ (‰)	–15.1	–13.8	–14.4	±0.35	12*[2]
(ii)					
Length (cm)	13.0	23.0	18.49	±2.4	14
Weight (g)	46.0	275.0	144.0	±58.2	14
Growth rings	4.0	11.0	8.25	±2.3	8*[3]
$\delta^{13}C_{(otolith)}$ (‰)	–12.5	–8.8	–10.7	±1.3	11*[4]

Table 2 (i) Summary of correlations between measurements of *Panaque nigrolineatus* (probability is in parentheses). (ii) Summary of correlations between measurements of *Hypostomus regani* (probability is in parentheses)

	Weight	# of growth rings	$\delta^{13}C_{(Otolith)}$
(i)	*Panaque nigrolineatus*		
Length	0.84 (0.00)	0.37 (0.17)	–0.34 (0.22)
Weight	NA	0.30 (0.29)	–0.14 (0.61)
# of growth rings	NA	NA	–0.37 (0.18)
(ii)	*Hypostomus regani*		
Length	0.97 (0.00)	0.34 (0.45)	–0.15 (0.74)
Weight	NA	0.37 (0.41)	–0.08 (0.87)
# of growth rings	NA	NA	0.15 (0.75)

different ($H_{[1,47]} = 35.28$; $P < 0.001$). Since the micromilling technique gives serial samples going back in time, another way to examine these data is to treat them as repetitive samples of individuals. Repeated measures ANOVA demonstrated no significant correlation between fish age and $\delta^{13}C_{(otolith)}$ values in either species (Fig. 2; $F_{[2,28]} = 0.045$; $P = 0.956$), yet the otolith $\delta^{13}C$ values differed again between species by this analysis ($F_{[1,14]} = 8.19$; $P < 0.05$). *Panaque nigrolineatus* displayed $\delta^{13}C_{(otolith)}$ values of approximately –14 to –15‰ that were relatively uniform throughout life, whereas $\delta^{13}C_{(otolith)}$ values of *H. regani* ranged from –9 to –12‰ and showed varied patterns of change with age (Fig. 2).

Laboratory diet experiment

There were no significant differences among the three tanks within each dietary treatment so data from the three replicate tanks were pooled for each treatment. Feeding *Hypostomus* sp. different diets generated $\delta^{13}C_{(otolith)}$ values that mirrored the differences in $\delta^{13}C_{(diet)}$ values (Fig. 3). A plot of $\delta^{13}C_{(otolith)}$ as a function of $\delta^{13}C_{(diet)}$ for the laboratory experiment produced a linear relationship (one-way ANOVA showed significant differences between treatments ($F_{[3,34]} = 695.02$; $P < 0.001$))(Fig. 4).

Discussion

Use of $\delta^{13}C$ values to uncover trophic information from wild animals has a productive history and a bright future (e.g. Kelly 2000). In combination with other sources of information, stable isotope values can provide information on the food web in the community, intraspecific differences between population diets or thermal history, metabolism, or

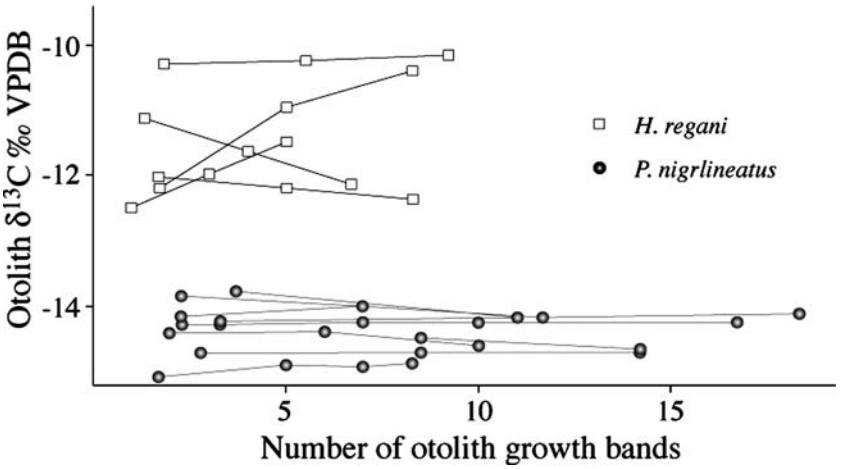

Fig. 2 Stable carbon isotope values of *Panaque nigrolineatus* (12 fish) ◎ and *Hypostomus regani* (7 fish) □ plotted against the number of their otolith rings

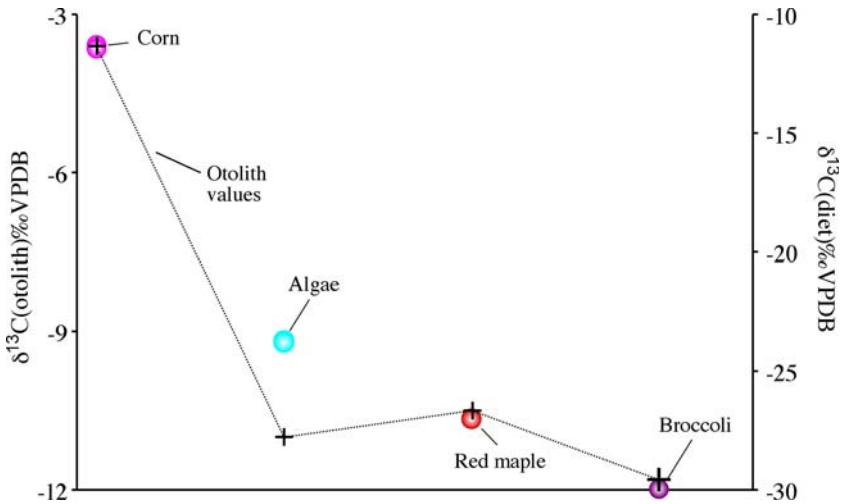

Fig. 3 Relationship between $\delta^{13}C_{(diet)}$ (circled symbols (◎), right ordinate. Corn = –11.4‰ ± 0.09 (n = 2), algae = –23.8‰ ± 0.04 (n = 2), red maple wood = –27.0‰ ± 0.02 (n = 2), and broccoli = –30.0‰ ± 0.09 (n = 2)) and $\delta^{13}C_{(otolith)}$ (cross symbols (+), left ordinate) in laboratory reared juvenile *Hypostomus* sp. (23 fish; corn = –3.6‰ ± 0.46 (n = 8), algae = –11.0‰ ± 0.55 (n = 7), red maple wood = –10.5‰ ± 0.48 (n = 4), broccoli = –11.8‰ ± 0.48 (n = 4)). Means and standard deviations are plotted (standard deviations are smaller than the symbols)

even paleoclimate details for certain sites (e.g. Radtke et al. 1996; Patterson et al. 1993; Hobson et al. 1999; Zanden et al. 1999; Weidman and Millner 2000; Carpenter et al. 2003; Wurster and Patterson 2003; Wurster et al. 2005). Thus, the use of carbon stable isotope information holds tremendous potential for numerous applications in biology, many of which are already being realized.

One of the advantages of measuring $\delta^{13}C_{(otolith)}$ in fish biology is that the information may be useful in reconstructing the trophic history of the fish throughout its life. Traditional methods such as stomach contents analysis can only provide dietary information at the time of capture and $\delta^{13}C_{(tissue)}$ may only provide information about recent dietary or metabolic history due to the faster turnover of soft tissues (Tieszen et al. 1983; Jardine et al. 2005). Thus, soft tissue represents an ephemeral recent record whereas otolith carbonate preserves details throughout the life of the organism.

The $\delta^{13}C_{(otolith)}$ values of *P. nigrolineatus* and *H. regani* ranged from –15.1‰ to –8.8‰, relatively

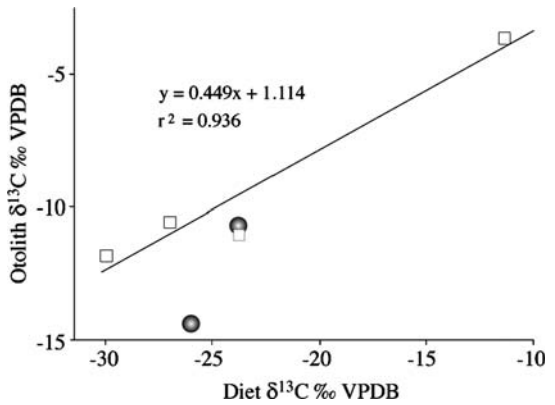

Fig. 4 Relationship between $\delta^{13}C_{(diet)}$ and $\delta^{13}C_{(otolith)}$ values (means) in laboratory reared juvenile *Hypostomus* sp. (n = 23; □ (from left; broccoli, wood, algae, and corn)) and field collected *Panaque nigrolineatus* and *Hypostomus regani* (n = 12 and 7 respectively; ◉). The least-squares regression line and equation developed from the laboratory fish are also depicted. Palm wood was used as the $\delta^{13}C_{(diet)}$ for *Panaque nigrolineatus* (left symbol) whereas *Spirulina* sp. was used as the $\delta^{13}C_{(diet)}$ for *Hypostomus regani* (right symbol)

higher than the $\delta^{13}C_{(tissue)}$ values reported for other loricariid catfish (–30‰ to –23.5‰; Forsberg et al. 1993). It is known that $\delta^{13}C$ of fish otoliths can be more enriched in ^{13}C than that of tissue (Radtke et al. 1996). Radtke et al. (1996) found that Atlantic cod, *Gadus morhua*, showed approximately 15–17‰ more positive $\delta^{13}C$ values in otoliths than in muscle tissue, but the $\delta^{13}C$ relationships between different diets and tissue or those diets and otoliths were clearly related to each other. Our study extends these results to include herbivorous fishes foraging on plant material. Although the magnitude of positive shifts in $\delta^{13}C_{(otolith)}$ can be smaller in freshwater species (Degens et al. 1969), herbivorous loricariid catfishes have a variety of microbial and fungal species in their gastrointestinal tracts that degrade cellulose (Nelson et al. 1999) that may also contribute to the relatively high $\delta^{13}C_{(otolith)}$ values for the loricariids reported here.

The $\delta^{13}C_{(otolith)}$ values of field collected *P. nigrolineatus* and *H. regani* differed significantly by 3.7‰ (Table 1 & Fig. 2). Because there were no significant relationships between body size and age and the environments were similar, the most likely cause of this offset were differences in metabolic status or diet. Since these are ectothermic species, captured in the relatively stable climate between 16° and 22° S. latitude, differences in metabolic rate due to temperature are expected to be minimal. Furthermore, the fact that *H. regani* captured from higher latitude (more southerly locale) than *P. nigrolineatus* had a higher $\delta^{13}C_{(otolith)}$ value is opposite to the usual latitudinal trends found in other animals (Kelly 2000). The higher values are expected given the lower temperatures and therefore lower metabolic rate (e.g. Wurster et al. 2005) expected at higher latitudes. Additionally, $\delta^{13}C_{(otolith)}$ values of all field collected *P. nigrolineatus* were invariant around –14 to –15 ‰ throughout their life, while *H. regani* displayed more variation between individuals during ontogeny (Fig. 2). These differences are suggestive of distinctive ecologies involving either dietary shifts, migration to new habitats, or seasonal temperature variability in *H. regani* only. The more narrow range of *P. nigrolineatus* $\delta^{13}C_{(otolith)}$ values (from –15.1‰ to –13.8‰) may reflect a relatively invariant metabolism and/or diet throughout life.

The 9 months of controlled diet in *Hypostomus* sp. generated significant differences in $\delta^{13}C_{(otolith)}$ values (Figs. 3, 4). The corn diet, which had the highest carbon isotope value ($\delta^{13}C_{(corn)}$ = –11.4‰), resulted in highest $\delta^{13}C_{(otolith)}$ values. The other diets likewise generated otolith carbon values that reflect $\delta^{13}C_{(diet)}$ values (Figs. 3, 4). Although fish used in the maple wood treatment only lived for 5 months or less it was sufficient to generate distinctive $\delta^{13}C_{(otolith)}$ values (Fig. 3). Despite the fact that the pre-experiment diet would have contributed some of the carbon to our analysis, 9 months on a controlled diet was enough to produce significant differences in fish otolith $\delta^{13}C$ values. Similarly, Australian salmon, *Arripis trutta*, showed a 3.0‰ shift in the $\delta^{13}C_{(otolith)}$ value if the $\delta^{13}C_{(diet)}$ changed by 10.0‰ (Kalish 1991b).

The relationship between $\delta^{13}C_{(otolith)}$ and $\delta^{13}C_{(diet)}$ values in laboratory-reared fish suggests that differences between wild *P. nigrolineatus* and *H. regani* $\delta^{13}C_{(otolith)}$ values resulted from species-specific diets. *Panaque nigrolineatus* had only wood shavings in its gut while *H. regani* had consumed primarily algae at the time of dissection. Although the $\delta^{13}C$ of algae from the Rio Mogi-Guaçu is unknown, the fact that the mean $\delta^{13}C_{(otolith)}$ for the algivorous *H. regani* was almost identical to the $\delta^{13}C_{(otolith)}$ of *Hypostomus sp.*

raised on *Spirulina* sp. algae in the laboratory is intriguing (Fig. 4). Our conclusion is that $\delta^{13}C_{(otolith)}$ analyses were successful in confirming that these two loricariid species from a similar geographic region occupy different trophic niches.

Because a majority of research on Amazonian fish biology has been dependent on sampling during the dry seasons (Schaefer and Stewart 1993; Tejerina-Garro et al. 1998; Flecker et al. 2002), stable isotope techniques could improve the study of trophic dynamics in the Neotropics by providing year-round data on dietary and/or metabolic histories. By distinguishing between two loricariid species of known presumed dietary differences, this study provides a simple example of the potential use of these techniques in Neotropical aquatic ecosystems. Indeed, the potential for employing these methods to learn more about the trophic ecology of the many loricariid species is significant. Forsberg et al. (1993) estimated that 12.7–42.9% of total carbon in the diet of Amazonian *Hypostomus plecostomus* was derived from C_4 plants. Since macrophytes in the Amazon flood plains are predominantly C_4 grasses ($\delta^{13}C = -12.8‰$) with a minor component of C_3 plants ($\delta^{13}C = -27.6‰$; Forsberg et al. 1993), the potential for identifying fish specializing on aquatic macrophytes exists. Similarly, because Amazonian algae $\delta^{13}C$ values can be so low (Araujo-Lima et al. 1986), monophagous fishes that are algivorous may be readily distinguished. The results of the current study encourage the use of otolith stable isotopes to study trophic ecology of Neotropical fishes.

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