GEOSCIENCES

Carbon and oxygen stable isotopes of freshwater fish otoliths from the São Francisco River, northeastern Brazil

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Abstract: Fish otoliths store geochemical and isotopic signatures that can be used as proxies for environmental conditions in ecological investigations. We investigated the $\delta^{13}C$ and $\delta^{18}O$ of otoliths of four freshwater fish species (*Astronotus ocellatus*, *Serrasalmus brandtii*, *Plagioscion squamosissimus,* and *Cichla ocellaris*) from the Moxotó Reservoir in the São Francisco River, Brazil. The enriched $\delta^{13}C$ signatures that distinguish *A. ocellatus* from other species could be caused by dissolved inorganic carbon ($\delta^{13}C_{DIC}$) in reservoir waters. Macrophytes growing along the reservoir margins would favor photosynthetic uptake of ^{12}C, leading to enriched $\delta^{13}C_{DIC}$ incorporated into the otoliths of those fish. Otolith $\delta^{18}O$ signatures appear to reflect water column preferences, in which species preferentially inhabiting surface waters (such as *S. Brandtii*) show more enriched values (due to high surface evaporation ratios), while bottom water species show depleted $\delta^{18}O$ values. Our results represent the first investigation of the isotopic compositions of fish otoliths in freshwater environments in the São Francisco River, and shed light on the interpretation of isotopic information stored in otoliths and how they can be used to infer the ecological strategies of freshwater fish.

Key words: Biogeochemistry, freshwater ecology, isotopic ecology, otolith chemistry.

INTRODUCTION

Stable isotopes have been used in recent decades to examine interactions between living organism and their surrounding environments, to identify and elucidate gaps in our ecological knowledge, and to track environmental changes in ecosystems (*e.g.*, Rundel et al. 1989, Thompson et al. 2005). The application of stable isotopes in ecological studies has helped to increase our understanding of complex processes in many different ecosystems (e.g., carbon flux and food chain relationships) and highlight the importance of this approach as a tool for ecological investigations (Thompson et al. 2005, Dawson & Siegwolf 2007).

Stable isotope information can be stored in many different substrates, with isotopic compositions being largely influenced by a mix of environmental and physiological conditions (*e.g.* Radtke et al. 1996, Pruell et al. 2010, Zeigler & Whitledge 2011). Tree rings, coral skeletons, animal and vegetal tissues, bird feathers, fish scales, and fish otoliths are examples of materials that can be used in ecological investigations.

Teleost fish otoliths are hard, long-lived structures composed of calcium carbonate (mostly aragonite and calcite) with well-defined growth bands that can serve as natural archives of past environmental conditions and the life histories of those fish (Radtke et al. 1996). During a fish's life, calcium carbonate is deposited or

immersed in the otolith protein matrix that continuously grows in accreting layers. That $CaCO_3$ accretion incorporates trace elements and isotopic ratio signals in seasonal records that can be used to track past environmental conditions of the water, the fish's dietary history, and migration patterns (Kalish 1990, Radtke et al. 1996, Hart et al. 2015).

The carbon and oxygen stable isotopes of otoliths have been suggested as potential tools for deciphering the temperature and dietary histories of fish respectively (Radtke et al. 1996). $\delta^{18}O$ signals in carbonates are influenced by local temperatures and by the oxygen isotopic composition of water at the moment of carbonate biomineralization (Urey 1947, Epstein et al. 1953), and researchers have proposed the use of the $\delta^{18}O$ signals recorded in fish otoliths as proxies for local temperature histories (Devereux 1967, Hart et al. 2015, Radtke et al. 1996 among others). The use of $\delta^{18}O$ signals to establish local temperatures assumes that otoliths are accreted in isotopic equilibrium with the surrounding water – which may represent an issue when dealing with biogenic carbonates in light of metabolic effects (McConnaughey 1989a, b), as during otolith accretion, the precipitated $CaCO_3$ may incorporate metabolic CO_2 instead of bicarbonate ions (HCO_3^-) from local waters (Radtke 2004).

While otolith $\delta^{18}O$ have been proposed as proxies for water temperature, otolith $\delta^{13}C$ signatures have been used as proxies for inferring nutritional sources (Radtke et al. 1996). The interpretation of $\delta^{13}C$ signals is challenging, however, due to many influencing factors. The carbon used for otolith biomineralization can be incorporated from two different sources: endogenous (derived from catabolic processes), and exogenous (derived from dissolved inorganic carbon – DIC, mostly in its bicarbonate form) (Høie et al. 2004, Solomon et

al. 2006). The proportional contributions of each carbon source has been estimated at ~20% for endogenous sources, and approximately 80% for DIC sources (Høie et al. 2004, Solomon et al. 2006, Nelson et al. 2011).

Numerous reports have suggested that herbivores and detritivores tend to reflect the $\delta^{13}C$ signal of their dietary sources (Black & Bender 1976, DeNiro & Epstein 1977). Thus, the $\delta^{13}C$ signals of different trophic levels may reflect original carbon-fixing pathways as well as subsequent fractionations through the trophic chain.

The São Francisco River is one of the most important hydrographic basins in Brazil, and has great social and economic importance for the northeastern region of that country. The main course of the river has been altered in the last 50 years due to the construction of a hydroelectric plant, which has had ecological consequences for all of its freshwater communities. Additionally, many species have been introduced into the São Francisco River, altering its original biotic composition.

Studied species

Native to the Amazon basin, the species *Astronotus ocellatus* (Agassiz 1831) is a cichlid classified as omnivorous that performs parental care; its reproduction is preferentially in lentic waters (Melo et al. 2018 and references therein). The species *C. ocellaris* (Bloch & Schneider 1801) is a predatory cichlid native to the Amazon basin; it can be found in lotic or lentic water and presents a piscivorous habit (Brandão & Silva 2009, Januário et al. 2019). Also native to the Amazon basin, *Plagioscion squamosissimus* (Heckel 1840) was introduced into rivers in northeastern Brazil by 1940. It has a piscivorous feeding habit, inhabits multiple biotopes, and completes its biological cycle in freshwater and/or in brackish water (Stefani & Rocha

2009). The species Serrasalmus brandtii (Lütken 1875) is native to the São Francisco basin, has piscivorous habit, and preferentially inhabits lentic environments (Britski et al. 1988). It is known as a mutilating predator because it tears off body parts of its prey such as fins and scales (e.g., Sazima & Machado 1990).

We investigated the carbon and oxygen isotopic compositions of the otoliths of the species *Astronotus ocellatus*, *Serrasalmus brandtii*, *Plagioscion squamosissimus,* and *Cichla ocellaris* and their stomach contents, and correlated those data with the known ecological behaviors of each species. We hypothesized that the C and O isotopic signatures of otoliths derived from fish from distinct trophic categories in the Moxotó Reservoir in the São Francisco River would be influenced by their life histories, especially their diets and habitats, and that those isotopic signatures could be used to distinguish the ecological strategies of each species.

MATERIALS AND METHODS

Study area

The São Francisco River Basin (Fig. 1) encompasses an area of approximately 645,000 km², between the coordinates 13° to 21° S latitude and 36° to 48° W longitude. It is the third most import hydrographic basin in Brazil, draining five states and the Federal District, under the influence of
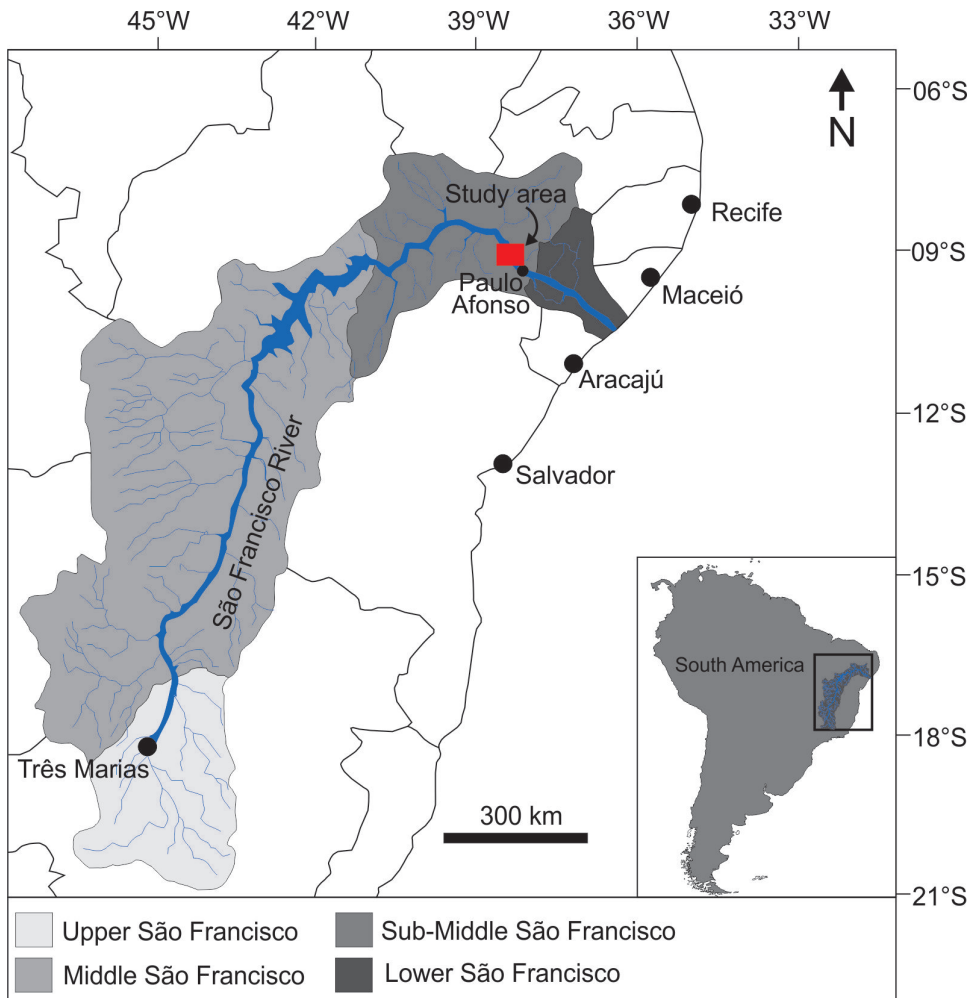

Figure 1. The São Francisco River basin and its sub-basin with the study area location in the Sub-Middle São Francisco.

three Brazilian biomes: Cerrado, Caatinga, and the Atlantic Forest. The hydrographic basin is divided into four sectors: Upper, Middle, Sub-Middle, and Lower (Godinho & Godinho 2003).

The São Francisco Basin has been crucial for the economic development of northeastern Brazil, providing freshwater, electricity, and food resources for riverside cities, as well as tourism (Godinho & Godinho 2003), although economic development in recent decades has led to increased anthropogenic impacts throughout the area. Our study area was situated in the Sub-Middle sector of the São Francisco River, at the Moxotó Reservoir (Fig. 1).

Sample collection

The specimens were captured with help of the artisanal fishermen using gillnets of varying sizes, during the period between (March/2016 to February/2017). The fishing equipment was set up at dusk and removed at dawn, approximately 12 hours later. The collected specimens were transferred to laboratory, identified, and their otoliths extracted; the otoliths were subsequently washed with distilled water and stored in Eppendorf tubes.

Isotopic analysis

We manually crushed 47 otoliths of the species *A. ocellatus* (12), *S. brandtii* (14), *P. squamosissimus* (8) and *C. ocellaris* (13) using an agate mortar and pestle and again stored in Eppendorf tubes. Subsamples (ca. 20 mg) were used for carbon and oxygen determinations at the Federal University of Pernambuco. CO_2 was extracted from powdered carbonates under a high vacuum, after reacting with anhydrous orthophosphoric acid for 12 h at 25° C (Craig 1957). The CO_2 released was analyzed for its carbon and oxygen isotopes using a Delta V advantage mass spectrometer, with BSC (Borborema Skarn Calcite) as the reference gas. We calibrated the BSC against

NBS (National Bureau of Standards)-18 and NBS-19. The analysis precision was better than 0.1‰ (2σ), based on multiple analyses of the internal standard.

The C and O isotopic data of the four species was submitted to one-way ANOVA and the means were compared by the Tukey test at a 5% level of significance.

Diet composition

We assessed stomach content data from an earlier study (March/2016 to February/2017) at the Moxotó Reservoir that evaluated the food preference of the species *A. ocellatus*, *S. brandtii,* and *P. squamosissimus* to help interpret the C and O isotopic signals from the otoliths.

The fish stomachs were collected and then weighed (using a precision balance). We subsequently examined their contents using a stereomicroscope and classified the food items to the lowest possible trophic level. The frequency of occurrence analyses followed the methodology proposed by (Bowen 1992) using the equation: FO = 100 x (ni / n), where: "FO"= Frequency of Occurrence of item "i" in the diets of the individuals in the sample; "Ni"= the numbers of stomachs in the sample containing item "i"; and "n"= P total number of stomachs with any contents in the sample. The percentage volume values of each food item in the total contents of each stomach were visually estimated, and used for the food composition analysis of the Food Importance Index (IAi), following Kawakami & Vazzoler (1980). That analysis assigns the relative importance of each item (whatever its condition) as its frequency of occurrence and volume**:** AIi = FO x Vi / Σ (FO x Vi)**,** where: IAi = Food Importance Index of item "i" in the diet of the individuals in the sample; "FO": Frequency of Occurrence of item "i" in the sample; and "Vi" = the Volumetric Analysis Index of food item "i" in the sample.

The data used for *C. ocellaris* was that available in the literature (Zaret 1980, Santos et al. 2001). The food preferences of those species were used to evaluate their trophic ecologies and their consequences for carbon isotopic composition.

This research was authorized by the Animal Care and Use Committee of the State University of Bahia (N° 0603170027856).

RESULTS

C and O stable isotopes

The carbon isotopic compositions of the species analyzed in this study (Fig. 2) varied from –8.48 to –3.89‰ (mean = –6.59 ± 1.28‰) for *S. brandtii*, which was similar to the values observed for *C. ocellaris*, with its $\delta^{13}C$ values varying from –8.58 to –4.88‰ (mean = –6.36 ± 1.28‰). The species *A. ocellatus* showed a $\delta^{13}C$ varying from –5.74 to –1.25‰ (mean = –2.92 ± 1.51‰), while the carbon isotopic composition of *P. squamosissimus* ranged from –6.70 to –3.70‰ (mean –4.99 ± 1.14‰).

The oxygen isotopic composition of the fish otoliths (Fig. 2) ranged from –3.57 to –2.05‰ (mean = –2.98 ± 0.52‰) for *S. brandtii;* from –4.47 to –3.24‰ (mean = –3.83 ± 0.43‰) for *C. ocellaris;* from –4.52 to –2.41‰ (mean = –3.46 ± 0.72‰) for *A. ocellatus;* and from –5.09 to –3.35‰ (mean = –4.39 ± 0.57‰) for *P. squamosissimus.*

The $\delta^{13}C$ signature of the species *A. ocellatus* was statistically distinct from the others, whereas *P. squamosissimus* was similar to *C. ocellaris,* but statistically distinct from *S. brandtii*; the latter was more similar to *C. ocellaris* (Table I).

Regarding the $\delta^{18}O$ signatures, the species *S. brandtii* was similar to *A. ocellatus* but distinct from *C. ocellaris* and *P. squamosissimus. C. ocellaris* was similar to *A. ocellatus,* while *P. squamosissimus* was similar to *C. ocellaris* (Table I).

The cross plot of carbon against oxygen stable isotopes allowed us to group the species according to their isotopic signals, which are believed to be ultimately driven by ecological factors (e.g., Radtke et al. 1996). Our results showed that *A. ocellatus* is very well discriminated by its more positive $\delta^{13}C$ values as compared to the other species (Fig. 3).

Dietary preferences

Food items found in the stomach contents of *A. ocellatus* were grouped into four categories: mollusks, shrimps, plant structures, and others, with mollusks being the most prominent food resource used by that species (IAi – 0.804) (Table II). *A. ocellatus* was therefore classified as a carnivore, with a malacophagic tendency, which diverges from its diet recorded in a more natural environment, possibly due to the abundance of mollusks in the Moxotó Reservoir.

P. squamosissimus showed a dietary preference for fish (IAi = 0.280) and Crustaceans (IAi = 0.148), indicating that the species is essentially carnivorous, but with a tendency to piscivory (yet with opportunistic feeding on other items, when fish availability might decrease [such as shrimps], which are abundant in the environment and made up an important item in its diet). That feeding flexibility may help explain the abundance of *P. squamosissimus* in the reservoir, even with declining populations of its preferred prey.

The food items identified for *S. brandtii* were divided into four categories: Teleostei, Mollusca, Insecta, and others. The Teleostei group showed the highest IAi (0.529), followed by Mollusca (0.214). Although *S. brandtii* has a carnivorous food habit, with a piscivorous tendency, it was classified as a generalist species because of its high consumption of different food items.
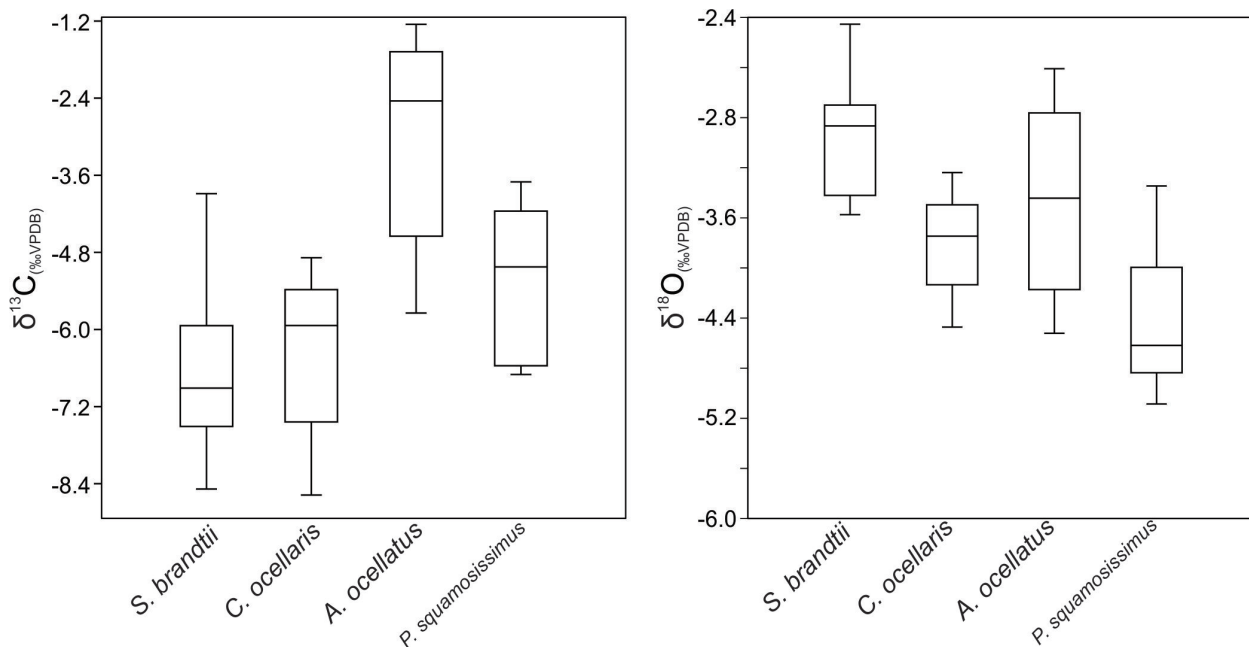
Figure 2. Boxplot of the δ¹³C and δ¹⁸O values of *S. brandtii, P. squamosissimus, C. ocellaris,* and *A. ocellatus.*

Table I. Mean values of δ¹³C and δ¹⁸O for the species *S. brandtii, P. squamosissimus, C. ocellaris,* and *A. ocellatus.*

Species	δ¹³C	δ¹⁸O
S. brandtii	-6.59 c*	-2.98 a
C. ocellaris	-6.36 bc	-3.83 bc
A. ocellatus	-2.92 a	-3.46 ab
P. squamosissimus	-4.99 b	-4.39 c

***Means followed by the same letter in each column do not differ significantly (Tukey test, 5% probability level).**

DISCUSSION

δ¹³C variability and diet preference

To understand if diet preference is a key factor influencing the δ¹³C signals in the otoliths of each species, and why the otoliths of *A. ocellatus* are comparatively more enriched in ¹³C compared than the other species, we evaluated the dietary components of each species.

The stomach content data for the species studied, together with data available in the literature, show that *A. ocellatus* has a distinct feed strategy, with a malacophagic tendency,

while the other species are predominantly piscivorous (Table I).

The diet of *S. brandtii* was evaluated by Pompeu (2010) in the floodplain of the São Francisco River, and was characterized by a predominance of fish, fish components (scales, fins), and insects (Diptera, Odonata, Hemiptera, Trichoptera, and Ephemeroptera), with lower contributions of mollusks (Gastropods and bivalves), zooplankton, and plant material (macrophytes and filamentous algae). That same author observed that *S. brandtii* had a well-defined trophic ontogeny, in which its juveniles are characterized principally by

consuming insects, which are later substituted by fish components (scales and fins) to fish fragments and small fish. The variability of the dietary resources of *S. brandtii* seems to be associated with seasonal resource availability in floodplains and lakes (mainly due to the rainy and dry seasons).

C. ocellaris also showed a piscivorous feeding habit. (Santos et al. 2001) analyzed the stomach contents of *Cichla monoculus* and found that up to 87% of its prey was composed of fish. Zaret (1980) reported that juvenile *C. ocellaris* individuals feed on freshwater shrimp and insects, whereas adults primarily consume small fish.

Stefani & Rocha (2009) analyzed the stomach contents of *P. squamosissimus* in the Tietê River, and reported that the most important food items were fish (71% volume), followed by insects (27.2%).

ocellatus was introduced into the São Francisco River from the Amazon basin. It is characterized as carnivorous, with an insectivorous-piscivorous tendency (Trindade & Queiroz 2012). Trindade & Queiroz (2012) recently reported that the diet of *A. ocellatus* in the central Amazon was mainly composed of insects and fish, although the proportion of fish was small concerning the constant and high proportions of insects. The stomach contents of *A. ocellatus* as analyzed here, however, showed that mollusks were its most important food source (IAi = 0.804), distinct from its diet in its native environment (reflecting the predominance of mollusks in the Moxotó Reservoir, in the São Francisco River).

Despite the clear dietary differences between *A. ocellatus* and the other three species, its δ^{13}C signature was not what would be expected for a species that largely feeds on mollusks – as mollusks usually feed on rock biofilm (which has a very depleted δ^{13}C signature, which can

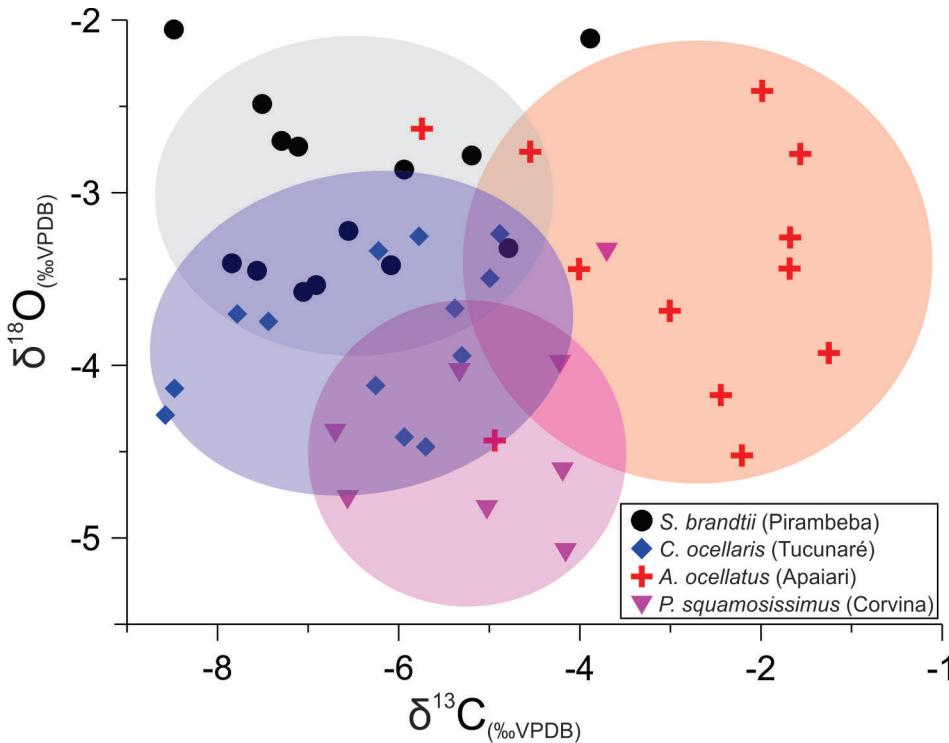**Figure 3.** Cross plot of the δ^{13}C and δ^{18}O values of *S. brandtii*, *P. squamosissimus*, *C. ocellaris,* and *A. ocellatus.*

vary from -14 to -27‰, according to Hadwen et al. (2010). As *A. ocellatus* feeds at lower levels of the trophic structure in the Moxotó Reservoir, why are its otolith $\delta^{13}C$ signatures comparably enriched?

Nelson et al. (2011) ran an experiment involving *Sciaenops ocellatus*, in which specimens were submitted to controlled feeding and DIC conditions; their results revealed that about 85 to 92% of the otolith $\delta^{13}C$ was derived from local DIC. That value was similar to previous studies that investigated the percentages of different carbon sources composing fish otoliths, in which the $\delta^{13}C$ values of the otoliths were found to reflect approximately 80% DIC and 20% food (Weidman & Millner 2000, Solomon et al. 2006, Tohse & Mugiya 2008). Those results raised another question: if otolith $\delta^{13}C$ is mainly governed by DIC signatures, and a reservoir like Moxotó can be isotopically stratified – how can we use those signatures to understand fish ecology?

DIC and life strategy

The $\delta^{13}C_{DIC}$ signatures of freshwater reservoirs can be complex and influenced by different ^{13}C sources, including atmospheric CO_2, carbon from runoff and groundwater inflows, and carbon compounds derived from microbial mineralization of organic matter (Myrbo & Shapley 2006).

Photosynthesis activities in surface waters preferentially take up ^{12}C, increasing the $\delta^{13}C_{DIC}$, whereas buried organic matter (isotopically depleted) decreases the $\delta^{13}C_{DIC}$ of deeper waters. Thus, an isotopic gradient of DIC can be established in freshwater reservoirs, as was observed by Myrbo & Shapley (2006) in different lakes in Minnesota and Montana (USA), which presented a general trend of more enriched $\delta^{13}C_{DIC}$ values in surface waters and comparatively depleted $\delta^{13}C_{DIC}$ in deeper waters.

If that type of isotopic gradient was present in the Moxotó Reservoir, how could it affect the carbon isotopic compositions of otoliths concerning fish life strategies? Would marginal and superficial species show more enriched $\delta^{13}C$ values as compared to species living deeper in the water column?

ocellatus commonly inhabits marginal shallow waters in the Moxotó Reservoir, while the other three species are more active and constantly move throughout the reservoir. Another possibility is that fish otoliths record

Table II. List of food items found in the stomachs of *A. Ocellatus, P. squamosissimus*, and *S. brandtii* captured in the Moxotó Reservoir. Frequency of occurrence (F.O.); Volume (Vol%); Food importance index (IAi = Fi x Vi / Σ (Fi x Vi), where: IAi= Importance index of item "i" in the diet of the sample; "Fi" = Frequency of Occurrence of item "i" in the sample; and "Vi" - Volumetric Analysis Index of the food item "i" in the sample.

	Food items	F.O.	Vol%	IAi
A. Ocellatus	Mollusca	74.71	170.25	0.804
	Crustacean	14.65	23.14	0.136
P. squamosissimus	Teleostei	67.85	58.92	0.280
	Crustacean	53.57	39.57	0.148
	Insecta	17.85	1.35	0.001
S. brandtii	Teleostei	49.3	209.015	0.529
	Mollusca	59.6	32.160	0.214
	Insecta	30.4	30.620	0.147
	Crustacean	10.7	13.771	0.022

the environmental histories of different habitat uses in the reservoir (such as the juvenile phases of the fish, as the isotopic compositions of different larval nursery areas might be distinct).

Although we do not have $\delta^{13}C_{DIC}$ data for the Moxotó Reservoir, it seems plausible that the mechanisms responsible for the observed differences in otolith $\delta^{13}C$ might be related to the local DIC where the species occur during most of their life histories. Other possible explanations may be related to physiological differences between the species.

ocellatus is originally from the Amazon basin (which experiences periodic inundation pulses that form flooded areas ["varzeas"] occupied by macrophytes) and the Moxotó Reservoir may therefore represent an oxygen-suppressed environment to them. Some Cichlids, such as *A. ocellatus*, have developed physiological and biochemical adaptations to tolerate hypoxic environments and can decrease their basal metabolic rates (Muusze et al. 1998). The consequences of a low metabolism on carbon stable isotope signatures in fish otoliths are difficult to quantify. But it would be expected that less metabolic CO_2 would be incorporated into the overall isotopic carbon signatures of the otoliths of species with low metabolic rates.

Kalish (1991) hypothesized that the $\delta^{13}C$ values of otoliths are related to fish metabolic rates, and found that fish with higher metabolic rates tend to be more ^{13}C depleted, and also provided insights into the relationships between otolith $\delta^{13}C$ and oxygen consumption in different marine fish species.

Thus, the lower metabolic rate of *A. ocellatus* would be expected to result in lower CO_2 contributions to otoliths, and species inhabiting marginal shallow waters in the Moxotó Reservoir should show enriched $\delta^{13}C$ values as compared to the other species.

$\delta^{18}O$ and environmental interpretation

There were very distinct differences between the $\delta^{18}O$ values of the species *S. brandtii*, *C. ocellaris,* and *P. squamosissimus*, whereas *A. ocellatus* specimens demonstrated a wider spread of those values (Fig. 3). Many researchers have attributed otolith $\delta^{18}O$ signatures to local $\delta^{18}O_{water}$, temperature, and geographical differences, which might serve as promising proxies for defining the nursery environments of fishes (Kalish 1991, Radtke et al. 1996, Zeigler & Whitledge 2011).

Considering that the species analyzed here were collected in a semiarid reservoir, the effects of evaporation on $\delta^{18}O$ of water must be considered. According to Kohli & Frenken (2015), evaporation rates in artificial lakes and reservoirs are higher than in natural surface water systems, mainly because dams and reservoirs usually have increased surface areas, leading to more exposure to air and direct sunlight (which increase evaporation). Those effects would lead to more enriched $\delta^{18}O_{water}$ values in the lake epilimnion, with its consequent incorporation into fish otoliths.

Perini et al. (2009) reported a direct relationship between lake evaporation and $\delta^{18}O_{water}$ enrichment in epilimnion water during the summer in different lakes in the Italian Alps, resulting in an isotopic stratification in their water columns. Considering all of the above-mentioned observations, the $\delta^{18}O$ isotopic gradient observed in the otoliths of the species analyzed here might indicate fish preferences for certain habitats in the Moxotó Reservoir. The species *P. squamosissimus*, for example, is known to inhabit and feed in the hypolimnion, whereas *S. brandtii* is more commonly encountered in the epilimnion.

Our results appear to correlate the different $\delta^{18}O_{water}$ sources that compose the otoliths of each species with their preferences for

inhabiting distinct environments in the reservoir (i.e., the water column), although to confirm that hypothesis, water samples from epilimnion and hypolimnion will need to be analyzed to determine their oxygen stable isotope contents.

CONCLUSIONS

Differences in the otolith carbon and oxygen isotopic signatures of different freshwater fish species in the Moxotó Reservoir, São Francisco Basin, Brazil, were observed and related to their preferred habitats. $\delta^{13}C$ signatures can distinguish *A. ocellatus* from the other fish species examined. Although the dietary preference of *A. ocellatus* (malacophagic tendency) was different from the other species studied (with piscivorous tendencies), the enriched $\delta^{13}C$ values observed were not expected for a species that feeds at basal levels in the trophic food chain. Thus, its enriched values of $\delta^{13}C$ appear to reflect the DIC of marginal shallow water at the Moxotó Reservoir, where the intense photosynthesis by macrophytes preferentially takes up ^{12}C, leading to DIC enriched in ^{13}C. The $\delta^{18}O$ signatures in the otoliths also showed distinct differences between the species, indicating that oxygen isotopic compositions reflect the local $\delta^{18}O_{water}$, with species preferentially inhabiting surface waters (epilimnion) having otoliths with enriched $\delta^{18}O$ values, while species associated with deeper waters have otoliths with low $\delta^{18}O$ values. We therefore present here the first data concerning otolith $\delta^{13}C$ and $\delta^{18}O$ values in freshwater fishes of the São Francisco Basin, Brazil, and provide insight into how the isotopic signatures of otoliths can reflect fish habitat use by different species.

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