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FEATURE ARTICLE

Reconstructing lifetime nitrogen baselines and trophic position of *Cynoscion acoupa* from $\delta^{15}N$ values of amino acids in otoliths

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ABSTRACT: Habitat connectivity and trophic shifts during the lifetime of an individual fish are important determinants of fish population growth and persistence, yet remain little understood for many species. We investigated whether insights into individual lifetime migration, trophic position (TP) and environmental nitrogen dynamics could be achieved using compound-specific nitrogen isotope analysis of otolith proteinogenic amino acids (AAs). By comparing acoupa weakfish *Cynoscion acoupa* otoliths and muscle tissue from the monsoonal Amazon area in Pará with otoliths from semi-arid Rio Grande do Norte (RGN), Brazil, this study illustrates estuarine to coastal shelf habitat use and trophic ecology during juvenile and adult stage growth. Muscle tissue and otoliths gave comparable TPs for both life stages, while weighted mean $\delta^{15}N$ values of all source AAs differed between tissues. These differences reflected large seasonal and spatial changes in nitrogen biogeochemical cycles and anthropogenic nitrogen influences from the Amazon River onto the coastal shelf of Pará. AA $\delta^{15}N$ values of fish otoliths from the Pará region indicated changes in TP and sources of nitrogen between life stages, whereas analysis of fish otoliths from the RGN region indicated similarities in individual TP and sources of nitrogen through ontogeny. However, in both areas, individual adult TP ranged between 3 and 4, whereas juvenile TP remained around 2.8 to 3.0 in Pará and RGN, respectively. Since otoliths preserve a record of baseline $\delta^{15}N$ values over the lifetime of individual fish it may be possible to infer migration and TP across prehistoric ecosystems from AA isotopic analysis of ancient otoliths.

KEY WORDS: Otolith chemistry · Organic matrix · Brazil · Migration · Amazon · Trophic level

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In the juvenile stage *Cynoscion acoupa* individuals have similar trophic ecologies, while in the adult stage different feeding strategies are employed.

Photo: Kim Vane

INTRODUCTION

Ontogenetic shifts in habitat use and trophic positions (TPs) are a common trait in many fish species due to changing resource needs and the need to minimize predation risk during different life stages (Kimirei et al. 2013, Nagelkerken et al. 2015). The habitats and food webs upon which different life stages rely are being increasingly altered by anthropogenic influences, i.e. climate change, habitat degradation and overfishing (Levin et al. 2015). Yet for some species there is insufficient insight into lifetime habitat utilization and connectivity due to limited approaches for elucidating individual fish life histories. This makes it difficult to predict the reaction of a fish population to environmental distur-

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bances, and also impedes effective implementation of conservation measures (Olds et al. 2016).

Chemical analysis of fish otoliths can be used to gain information about movement and environmental conditions, such as salinity and temperature, at different life stages of an individual (Thorrold et al. 1997, Elsdon & Gillanders 2002, Elsdon et al. 2008). This is possible because growth of otoliths often results in visible (sub-)annual increments and incorporation of environmental inorganic chemistry (Campana 1999). Carbon isotope analysis of essential amino acids (AAs) in otolith protein (Campana & Neilson 1985, Nagasawa 2013) has recently been used to identify fish residency and habitat connectivity (McMahon et al. 2011a,b, 2012). Yet to understand exact fish movement, the geographic variation in $\delta^{13}C$ values of essential AAs in primary producers must be known. With $\delta^{15}N$ values of AAs, lifetime ecosystem biogeochemistry, TP and migration can be inferred directly from the otolith.

Proteinogenic AAs have consistent differences in $\delta^{15}N$ values, which lead to their classification as source or trophic AAs (Popp et al. 2007). Source AAs directly indicate the nitrogen baseline, as they remain largely unaffected by metabolic processes in consumers (McClelland & Montoya 2002, Chikaraishi et al. 2007) and therefore reflect nitrogen biogeochemical processes of the ecosystems in which the fish resided (Choy et al. 2012, Lorrain et al. 2015, Hetherington et al. 2016). Since nitrogen biogeochemical processes can vary across geographical landscapes, the $\delta^{15}N$ values of source AAs, like that of $\delta^{13}C$ values of essential AAs, can be used to reconstruct fish migrations (Madigan et al. 2014, 2016). Trophic AAs are enriched in ^{15}N relative to source AAs due to complex nitrogen cycling in organisms (Chikaraishi et al. 2007, O'Connell 2017), which occurs with each trophic step when trophic AAs undergo transamination, transfer of an amino group to a ketoacid or deamination by removing amine functional groups (McClelland & Montoya 2002, Chikaraishi et al. 2007, Braun et al. 2014). Importantly, the difference in $\delta^{15}N$ values between trophic and source AAs provides an estimate of the TP of the fish that is normalized to baseline isotopic compositions (McClelland & Montoya 2002, Chikaraishi et al. 2009, Bradley et al. 2015, Nielsen et al. 2015).

The size and low protein content of otoliths limit isotopic analyses of individual AAs. This limitation is particularly problematic for nitrogen isotope analysis of AAs since $\delta^{15}N$-AA analysis typically requires ~9× the amount of protein compared to $\delta^{13}C$-AA analysis. With their typically small sizes and low protein content of 1 to 4% (Degens et al. 1969), most otoliths do not contain enough material for nitrogen isotopic analysis of individual AAs. However, some fish species possess relatively large otoliths, such as *Cynoscion acoupa*, a large commercial fish with adults possessing 8 g otoliths up to 5 cm in length. This species occurs along the entire coast of Brazil; their early life stages are generally associated with mangrove areas, while adults are exclusively caught in offshore coastal shelf waters (Barletta et al. 2003). The present study, therefore, aimed to identify whether $\delta^{15}N$ values of AAs from *C. acoupa* otoliths can be used to infer habitat use, migration and TP at juvenile and adult life stages. Otoliths and muscle tissue of *C. acoupa* from the Amazon state Pará, Brazil, were collected simultaneously in the dry season to determine whether source AA $\delta^{15}N$ values and TP of 2 life stages extracted from both tissues were similar. To compare these life history parameters of *C. acoupa* in 2 distinct Brazilian ecosystems, adult otoliths were collected in the monsoonal Amazon area of Pará and the semi-arid area of Rio Grande do Norte (RGN), Brazil.

MATERIALS AND METHODS

Species and study areas

The acoupa weakfish *Cynoscion acoupa* is a marine demersal sciaenid species. Juvenile *C. acoupa* utilize mangrove ecosystems in estuaries, whereas adults are strictly found in coastal shelf habitats and have a carnivorous diet that consists mainly of shrimp and fish (Barletta et al. 2003, Ferreira et al. 2016). Adult *C. acoupa* are caught up to ~125 cm in size and typically weigh ~16 kg (de Matos & Lucena 2017). In northern Brazil this species matures around a length of 40 cm and displays biannual offshore spawning at the onset and during the rainy season (Almeida et al. 2016). The *C. acoupa* fishery at Pará and RGN is artisanal and fish are sold at local fish markets (Barletta et al. 1998, K. Vane pers. obs.).

The coastal ecosystem at Pará is influenced by high annual precipitation of over 2000 mm that results in a river outflow with a high nutrient load in the wet season (Smith & Demaster 1996). The coast is strongly macro-tidal with amplitudes of ~4 m, harbours large estuarine mangrove deltas and has an extensive coastal shelf. In contrast, the coast of RGN is semi-arid with an annual precipitation of 1250 mm, has very low estuarine mangrove coverage and a narrow coastal shelf. Mean tidal amplitude is ~2 m and the

coastal areas receive very low freshwater runoff (Schaeffer-Novelli et al. 1990). A strong North Brazil Current, as a branch from the Southern Equatorial Current, runs along this northeast coast of Brazil (Medeiros et al. 1999).

Sample collection

Four otoliths of *C. acoupa* with a body length of 92 to 114 cm standard length (SL) and muscle tissue of 2 similarly sized individuals (91 and 100 cm SL) were collected at the fish market in Bragança (1° 3′ S, 46° 46′ W), Pará, Brazil. Simultaneously, muscle tissue of 4 juveniles (26 to 36 cm SL) were sampled in mangrove areas near Bragança in November and December 2014 (dry season). An additional 4 *C. acoupa* otoliths were collected at the fish market of Natal (5° 46′ S, 35° 12′ W), Rio Grande do Norte, Brazil; the original body sizes of these fish were unknown. However, with our own collection (n = 66) of *C. acoupa* otoliths of which otolith weight (mg) and fish SL (mm) was known, the SL could be estimated with the exponential relationship 24.649 × otolith weight$^{0.4379}$.

All otoliths were embedded in resin, sectioned to 2 mm thickness and mounted on a glass slide. Otolith surfaces were not polished after sectioning and were only wiped with acetone. Material of the outer edge and inner part of all otoliths were subsampled with a mounted hand-microdrill with a 0.5 mm drill bit to a calcium carbonate sample mass of 45 mg (average protein content 0.7%).

Stable isotope analysis

Otolith powder (~45 mg) and homogenized muscle tissue (5 mg) were hydrolyzed with 0.1 ml 6 N HCl mg^{-1} of material at 150°C in a heating block for 70 min. The resulting 5.8 N HCl was evaporated at 110°C in a heating block under a gentle stream of N_2 for approximately ~3 to 4 h. AAs were isolated using cation exchange resin (DOWEX 50WX8, 100 to 200 mesh, hydrogen form; Metges et al. 1996, Takano et al. 2010). The sample was again dried under a stream of N_2 at 80°C in a heating block and after addition of 0.2 N HCl again heated to 110°C for 5 min and dried at 55°C under N_2. AAs were derivatized with an acetyl chloride and isopropanol mixture (4:1, v/v), heated to 110°C for 60 min and dried at 60°C under a N_2 stream. Subsequently, AAs were derivatized with 600 µl methylene chloride and 200 µl trifluoro-acetic anhydride by heating at 100°C for 15 min and dried under a N_2 stream at room temperature. The trifluoroacetyl and isopropyl ester derivatives were further purified with a liquid/liquid extraction of 2 ml 1 M P-buffer and 1 ml chloroform as described by Hannides et al. (2009). The AAs in chloroform were evaporated under N_2 at room temperature, methylated again and stored at −20°C. Before analysis, the derivatization agents were evaporated under N_2 at room temperature and samples were dissolved in 15 µl (otolith) and 125 µl (muscle tissue) ethyl acetate.

The $\delta^{15}N$ values of AAs in the otolith and muscle tissue samples were measured using a Delta V Plus mass spectrometer interfaced to Trace GC gas chromatograph through a GC-C III combustion furnace (980°C), reduction furnace (650°C) and liquid nitrogen cold trap via a GC-C III interface. All samples were injected onto a forte BPx5 capillary column (60 m × 0.32 mm × 1.0 µm film thickness) with a split/splitless injector in splitless mode. The injector temperature was 180°C and had a constant helium flow rate of 1.4 ml min^{-1}. The column was initially held at 50°C for 2 min and increased to 120°C at 15°C min^{-1}. Subsequently, temperatures were increased to 195°C at 4°C min^{-1}, then to 255°C at 5°C min^{-1} and to 300°C at 15°C min^{-1}, where it was held for 8 min. All samples were analyzed in triplicate, and measured $\delta^{15}N$ values were normalized to the known nitrogen composition of internal reference compounds (L-2-aminodipic acid and L-(+)-Norleucine) co-injected with each sample. When the co-injected reference compounds were not useable due to co-elution effects, a linear correction was applied to measured isotope values. The linear correction was derived from a suite of 14 AAs of known isotopic composition that was analyzed between each triplicate sample analysis. The average standard deviation of $\delta^{15}N$ values derived from multiple AA analyses was 0.4‰ and ranged from 0.0 to 1.3‰. AA molar percentages were determined from individual AA peak areas relative to the total AA peak area and using the external standard approach.

Trophic position estimations

The nitrogen isotopic composition of 6 trophic AAs (alanine, Ala; leucine, Leu; isoleucine, Iso; proline, Pro; aspartic acid, Asp; glutamic acid, Glu) and 4 source AAs (glycine, Gly; serine, Ser; phenylalanine, Phe; lysine, Lys) could be measured consistently. Weighted mean values ($\overline{x}_w$) for groups of trophic and source AAs were calculated as:

$$\delta^{15}N_{\bar{x}_w} = \frac{\sum \frac{\delta^{15}N_x}{\sigma_x^2}}{\sum \frac{1}{\sigma_x^2}} \quad (1)$$

where $\delta^{15}N_x$ is the nitrogen isotopic composition of a specified AA within the grouping and σ_x is the standard deviation of the specific AA based on triplicate analysis of each sample (Hayes et al. 1990).

TP was calculated with the equation:

$$TP = [(\delta^{15}N_{Trp} - \delta^{15}N_{Src} - \beta) / TDF_{AA}] + 1 \quad (2)$$

where $\delta^{15}N_{Trp}$ and $\delta^{15}N_{Src}$ are the nitrogen isotopic composition of selected trophic and source AAs, respectively. In this study, a combination of source AAs (Gly, Lys, Phe) and trophic AAs (Ala, Glu, Leu) was used, which has been shown to provide reliable TP calculations for teleosts (Bradley et al. 2015). The symbol β is the difference between the $\delta^{15}N$ values of trophic and source AAs in primary producers, and was calculated to be 3.4 ± 0.9‰ for the aforementioned combination of AAs (see Bradley et al. 2015). The trophic discrimination fractionation factor (TDF_{AA}) is the weighted mean average ^{15}N enrichment in trophic AAs (Ala, Glu, Leu) relative to source AAs (Gly, Lys, Phe) per trophic level, and was found by Bradley et al. (2015) to be 5.7 ± 0.3‰ for this combination of trophic and source AAs.

Uncertainty in TP was calculated by propagation of errors:

$$\sigma_{TP}^2 = \left(\frac{\partial TP}{\partial \delta^{15}N_{Trp}}\right)^2 \sigma^2_{\delta^{15}N_{Trp}} + \left(\frac{\partial TP}{\partial \delta^{15}N_{Src}}\right)^2 \sigma^2_{\delta^{15}N_{Src}} + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma^2_{\beta} + \left(\frac{\partial TP}{\partial TDF}\right)^2 \sigma^2_{TDF} \quad (3)$$

where σ is the standard deviation of TP, β and TDF (see Bradley et al. 2015, Jarman et al. 2017). The σ for weighted mean $\delta^{15}N$ values for trophic and source AAs was calculated as:

$$\left[\sum (1/\sigma_x^2)\right]^{-0.5} \quad (4)$$

(Hayes et al. 1990). Indicated uncertainties are reported here as 2 standard deviations.

RESULTS

Reconstructed individual *Cynoscion acoupa* body lengths from the RGN otoliths (92 to 107 cm SL) were in the same size range as those collected in Pará (92 to 114 cm SL). Samples obtained from the outer edge of Pará and RGN otoliths were estimated to cover a life period of ~2 yr based on the drill holes covering 2 opaque and translucent areas (Fig. 1). A wider surface was micro-drilled in the inner part of the otoliths compared to the edges (Fig. 1). However, otolith growth is known to be generally higher in the early life stages of the fish, and therefore the wider surface of the inner part was also estimated to cover a ~2 yr life span in the juvenile stage.

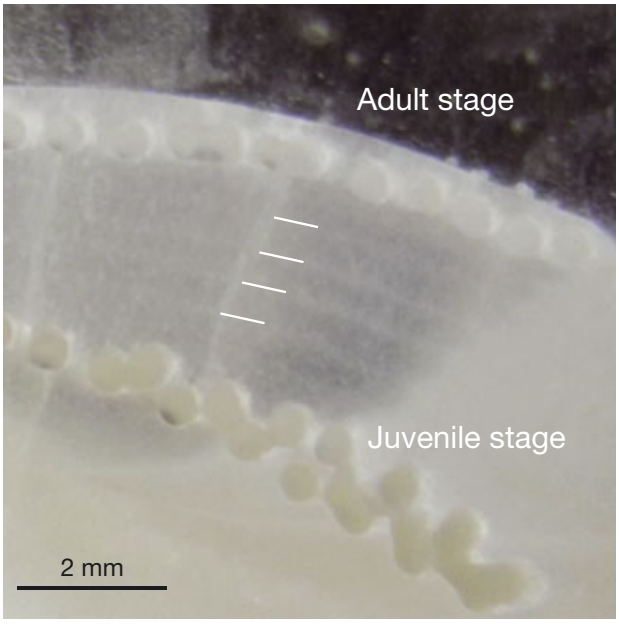

Fig. 1. Example of drill holes (0.5 mm diameter) in the inner part (juvenile stage) and outer edge (adult stage) of a Rio Grande do Norte *Cynoscion acoupa* otolith of 2 mm section thickness. Displayed are also the incremental rings indicated with white lines, which are only detected in the otoliths collected in Rio Grande do Norte

Comparable TPs were derived from adult muscle tissue and otolith adult stages from Pará, with TP averages of 3.8 and 3.4 ± 0.3, respectively. However, TPs obtained from the adult stage in RGN otoliths (3.6 ± 0.4) were similar to those in adult Pará otoliths (3.5 ± 0.3) (Wilcoxon rank-sum, W = 9, p = 0.885; Fig. 2A, Table 1). TPs acquired from juvenile muscle tissue from Pará were similar to the juvenile stage of Pará otoliths (averages 3.0 ± 0.2 and 2.8 ± 0.3, respectively; Wilcoxon rank-sum, W = 15, p = 0.057). Muscle tissue from RGN was not available for analysis. TPs from the juvenile and adult stage in Pará otoliths were different (Wilcoxon rank-sum, W = 0, p = 0.028) in contrast to the similar juvenile and adult stage TPs from RGN otoliths (Wilcoxon rank-sum, W = 2, p = 0.114; Fig. 2A, Table 1). Furthermore, a comparison between otoliths from Pará and RGN indicated similar juvenile (Wilcoxon rank-sum, W = 15, p = 0.057) and adult stages (Wilcoxon rank-sum, W = 9, p = 0.885; Fig. 2B, Table 1).

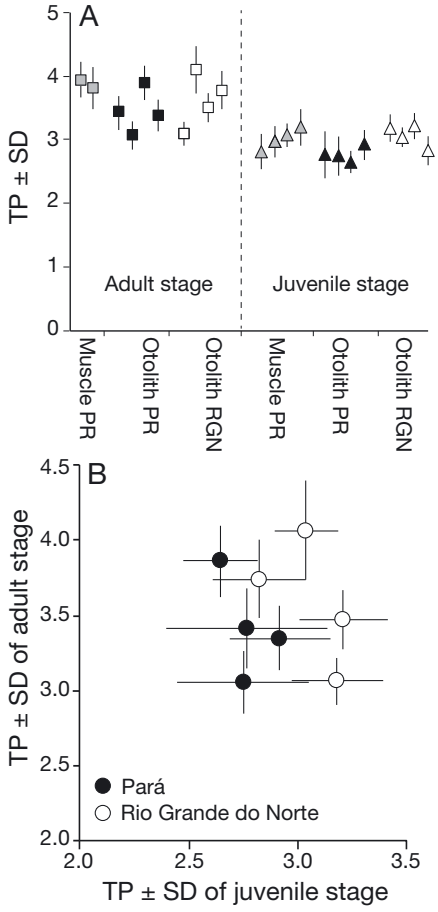

Fig. 2. (A) Overview of the trophic position (TP) and its uncertainty calculated from adult and juvenile muscle tissue from Pará (PR) and adult and juvenile stages within the adult otoliths from Pará and Rio Grande do Norte (RGN). (B) TP of juvenile and adult stage measured in each individual otolith from PR and RGN

In Pará, significant differences were observed between otolith juvenile and adult stages for both source (Wilcoxon rank-sum, $W = 16$, $p = 0.028$) and trophic AA $\delta^{15}N$ values (Wilcoxon rank-sum, $W = 0$, $p = 0.028$). Source AA weighted mean $\delta^{15}N$ values of juvenile and adult *C. acoupa* muscle tissue collected during the dry season in Pará were similar (mean values 5.9 ± 0.2 and 5.7‰, respectively), although different in trophic AA $\delta^{15}N$ values (19.5 ± 1.8 and 24.5‰, respectively). However, source AA $\delta^{15}N$ values of adult muscle tissue were ~1.4‰ lower than those in otolith edges, but similar in trophic AA $\delta^{15}N$ values (averages 24.5 ± 0.5 and 22.8‰, respectively). Juvenile stage otolith and muscle tissue from Pará also displayed distinct source AA $\delta^{15}N$ values (Wilcoxon rank-sum, $W = 0$, $p = 0.028$), although trophic AA $\delta^{15}N$ values were comparable (Wilcoxon rank-sum, $W = 0$, $p = 0.114$; Fig. 3, Table 1).

In RGN, no differences were found between otolith juvenile and adult stages for either source (Wilcoxon rank-sum, $W = 3$, $p = 0.2$) or trophic AA $\delta^{15}N$ values (Wilcoxon rank-sum, $W = 2$, $p = 0.114$). Source and trophic AA $\delta^{15}N$ values in RGN otoliths were also not different from Pará otolith juvenile (Wilcoxon rank-sum, $W = 8$, $p = 1$ and $W = 0$, $p = 0.114$) or adult stages (Wilcoxon rank-sum, $W = 2$, $p = 0.114$ and $W = 1$, $p = 0.057$; Fig. 3, Table 1).

Most difference in source AA $\delta^{15}N$ values was displayed by Gly with lower $\delta^{15}N$ values in muscle tissue than otoliths, while Lys had similar $\delta^{15}N$ values across sample groups. Trophic AA $\delta^{15}N$ values of both otolith and muscle tissue were generally higher in adult stage than in juvenile stages (Fig. 4). Individual AA abundances were similar between juvenile and adult stage of otoliths, although juvenile muscle tissue had a lower abundance of Lys and Gly than adult muscle tissue. Overall, Asp, Glu and Ser were the most abundant AAs in otoliths, while Lys, Ala and Leu were more abundant in muscle tissue (Table 2).

DISCUSSION

Fish otoliths can provide individual lifetime information about migration, TP and environmental nitrogen dynamics based on AA $\delta^{15}N$ values. This study showed that the average TP acquired from AA $\delta^{15}N$ values for *Cynoscion acoupa* muscle tissue and otoliths agreed well for each life stage in fish collected from the Pará region. On the contrary, these tissues often recorded different source AA $\delta^{15}N$ values. These differences could be due to dissimilar rates of AA incorporation into muscle tissue compared to the otolith matrix. The turnover time of specific AAs in muscle tissue can range from months to a year (Madigan et al. 2012, Bradley et al. 2014), and thus some AAs can record (inter-)seasonal variations of $\delta^{15}N$ values in the environment. Due to the low protein content in otoliths, samples here average isotopic values over approximately 2 yr. Otolith proteins are produced de novo daily by the saccular epithelium (Payan et al. 1999, Takagi et al. 2005), of which only ~1% is deposited within the calcium carbonate structure without turnover (Edeyer et al. 2000, Borelli et al. 2001). A strong relationship between essential AA $\delta^{13}C$ values of muscle and otolith edge of *Lutjanus* spp. indicated that both tissues derive AAs from the bloodstream with minor fractionation (McMahon et al. 2011b). Subtle $\delta^{15}N$ value differences of particular AAs can be expected between tissues depending on the individual's metabolism, although

Table 1. Individual amino acid (AA; see 'Trophic position estimations' for definitions) measurements with standard deviations and calculated weighted means of all source and trophic AAs. Trophic position (TP) estimations and its uncertainty are based on source (Src) AAs (Gly, Phe, Lys) and trophic position (Trp) AAs (Ala, Leu, Glu). This is displayed for muscle tissue from different sized *Cynoscion acoupa* individuals from Pará (PR) and the edge (adult stage) and inner (juvenile stage) otoliths from both PR and Rio Grande do Norte (RGN). SL: standard length

SL (cm)	Stage	Src AAs				Trp AAs					Weighted mean		TP
		Gly	Ser	Phe	Lys	Ala	Leu	Pro	Asp	Glu	Trp AAs	Src AAs	
Pará													
Muscle tissue													
91	Adult	5.4 ± 0.1	3.1 ± 0.4	7.4 ± 0.9	6.2 ± 0.0	26.5 ± 0.0	23.8 ± 0.2	20.1 ± 0.2	20.4 ± 0.1	24.8 ± 0.2	6.0	24.8	3.9 ± 0.2
100	Adult	4.9 ± 0.2	5.5 ± 0.1	5.3 ± 0.2	7.2 ± 0.2	26.9 ± 0.3	23.6 ± 0.2	22.4 ± 0.3	20.9 ± 0.4	25.3 ± 0.4	5.8	24.1	3.8 ± 0.3
36.5	Juvenile	8.1 ± 0.3	6.3 ± 0.3	5.6 ± 0.1	6.1 ± 0.3	22.6 ± 0.5	19.4 ± 0.2	15.2 ± 0.2	17.5 ± 0.1	19.2 ± 0.2	6.1	18.7	2.8 ± 0.3
34.5	Juvenile	7.3 ± 0.2	4.4 ± 0.1	6.6 ± 0.5	5.7 ± 0.2	23.4 ± 0.2	20.0 ± 0.0	17.6 ± 0.1	16.8 ± 0.4	21.1 ± 0.1	5.6	19.9	3.0 ± 0.3
25.5	Juvenile	4.7 ± 0.2	4.9 ± 0.3	6.2 ± 0.4	5.7 ± 0.1	21.7 ± 0.2	19.3 ± 0.4	17.7 ± 0.2	17.8 ± 0.1	20.6 ± 0.2	5.3	18.9	3.1 ± 0.2
26.5	Juvenile	4.2 ± 0.1	4.9 ± 0.7	7.1 ± 0.1	7.2 ± 0.2	22.5 ± 0.1	19.0 ± 0.2	15.7 ± 0.3	18.1 ± 0.1	20.7 ± 0.1	5.6	20.4	3.2 ± 0.3
Otolith													
92	PR-1 Adult	10.0 ± 0.8	6.9 ± 0.9	7.9 ± 0.1	7.1 ± 0.5	26.8 ± 0.4	23.2 ± 0.8	20.6 ± 0.2	19.9 ± 0.1	24.8 ± 0.2	7.9	22.5	3.4 ± 0.3
103	PR-2 Adult	8.7 ± 0.3	3.6 ± 0.2	11.3 ± 0.6	7.0 ± 0.8	26.2 ± 0.3	23.4 ± 0.1	21.0 ± 0.1	19.3 ± 0.1	25.3 ± 0.3	6.3	22.2	3.1 ± 0.2
114	PR-3 Adult	8.7 ± 0.9	7.1 ± 0.3	8.8 ± 0.4	5.5 ± 0.1	26.7 ± 0.5	26.2 ± 0.4	22.2 ± 0.1	19.7 ± 0.3	26.3 ± 0.2	6.7	24.0	3.9 ± 0.2
100	PR-4 Adult	9.4 ± 0.3	6.8 ± 0.6	9.3 ± 0.7	5.4 ± 0.2	25.1 ± 0.5	23.3 ± 0.0	19.2 ± 0.3	18.4 ± 0.1	24.8 ± 0.0	7.2	22.6	3.4 ± 0.2
92	PR-1 Juvenile	11.6 ± 0.3	7.3 ± 0.5	9.3 ± 0.0	5.6 ± 0.6	24.9 ± 0.2	19.2 ± 0.3	16.1 ± 0.5	18.8 ± 0.3	23.5 ± 0.3	9.3	21.3	2.8 ± 0.4
103	PR-2 Juvenile	11.8 ± 0.4	7.2 ± 1.0	11.0 ± 0.8	5.8 ± 0.4	24.9 ± 0.2	20.2 ± 0.2	16.0 ± 0.1	18.5 ± 0.2	23.2 ± 0.1	9.1	19.7	2.8 ± 0.3
114	PR-3 Juvenile	11.9 ± 0.3	9.4 ± 0.7	7.3 ± 1.0	5.7 ± 0.6	23.4 ± 0.8	21.0 ± 0.5	17.5 ± 0.1	17.2 ± 0.1	22.7 ± 0.7	9.4	18.8	2.7 ± 0.2
100	PR-4 Juvenile	11.9 ± 0.4	9.1 ± 0.5	8.9 ± 0.3	5.8 ± 0.4	24.2 ± 0.2	21.2 ± 0.3	19.0 ± 0.2	17.6 ± 0.1	23.7 ± 0.1	9.0	21.5	2.9 ± 0.2
Rio Grande do Norte													
Otolith													
100	RGN-1 Adult	11.3 ± 0.1	7.7 ± 0.4	6.1 ± 0.9	6.0 ± 0.5	25.8 ± 0.2	24.5 ± 0.1	21.6 ± 0.4	19.0 ± 0.4	24.4 ± 0.3	9.3	23.7	3.1 ± 0.2
92	RGN-2 Adult	6.9 ± 0.7	3.8 ± 0.9	11.7 ± 0.8	7.5 ± 0.1	28.5 ± 0.4	27.9 ± 0.4	25.5 ± 0.3	20.3 ± 0.3	29.2 ± 0.3	7.5	26.0	4.1 ± 0.3
96	RGN-3 Adult	10.2 ± 0.9	7.5 ± 0.3	9.4 ± 0.3	5.2 ± 0.8	26.1 ± 1.1	26.5 ± 0.5	23.0 ± 0.3	18.8 ± 0.3	25.8 ± 0.3	8.1	23.5	3.5 ± 0.2
107	RGN-4 Adult	11.1 ± 0.4	7.7 ± 0.3	12.5 ± 0.5	6.8 ± 0.1	28.1 ± 0.4	27.1 ± 0.2	22.8 ± 0.1	20.4 ± 0.3	28.2 ± 0.1	8.5	26.2	3.7 ± 0.3
100	RGN-1 Juvenile	10.9 ± 0.4	8.6 ± 0.1	5.3 ± 0.8	6.0 ± 0.6	25.3 ± 0.5	23.4 ± 0.2	19.9 ± 0.1	17.9 ± 0.2	23.8 ± 0.5	8.4	21.0	3.2 ± 0.2
92	RGN-2 Juvenile	10.8 ± 0.0	8.7 ± 0.7	7.2 ± 0.1	6.4 ± 0.5	25.4 ± 0.3	23.6 ± 0.4	19.6 ± 0.2	18.1 ± 0.2	24.2 ± 0.4	9.4	22.1	3.0 ± 0.2
96	RGN-3 Juvenile	13.7 ± 0.2	9.3 ± 1.3	8.9 ± 0.5	8.5 ± 0.4	28.7 ± 0.4	25.8 ± 0.7	23.6 ± 0.3	21.9 ± 0.2	27.6 ± 0.4	11.4	25.0	3.2 ± 0.2
107	RGN-4 Juvenile	11.2 ± 0.4	7.8 ± 0.3	11.6 ± 1.3	6.2 ± 0.8	25.0 ± 0.4	22.0 ± 0.2	21.2 ± 0.2	19.4 ± 0.1	24.5 ± 0.2	8.9	21.5	2.8 ± 0.2

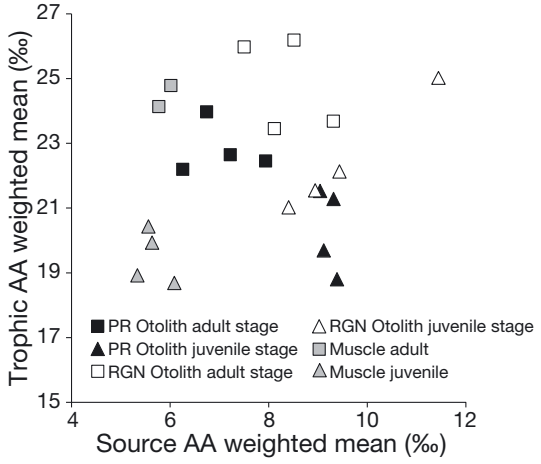

Fig. 3. Weighted mean of all source amino acids (AAs) versus weighted mean of all trophic AAs in otolith and muscle tissue of *Cynoscion acoupa* from Pará (PR) and Rio Grande do Norte (RGN)

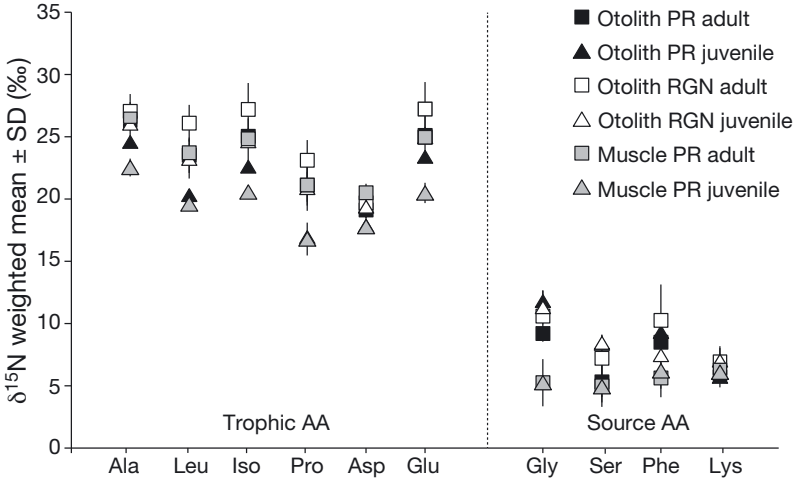

Fig. 4. Individual amino acid (AA; see 'Trophic position estimations' for definitions) $\delta^{15}N$ values of muscle tissue of juvenile and adult *Cynoscion acoupa* from Pará (PR) and otoliths from Pará and Rio Grande do Norte (RGN). Error bars: ±SD of all *C. acoupa* individuals collected within each category

this 'biological noise' can be overcome by using multiple AAs (O'Connell 2017). The similarities in AA abundances between muscle tissues and otoliths of this study also suggested a comparable AA incorporation into both tissues. Although muscle tissue and otolith AA $\delta^{15}N$ measurements from the same individual would confirm the comparability of AA incorporation, the large temporal differences represented by these tissues (months vs. ~2 yr) and individual fish behavior might account for the observed differences in source AA $\delta^{15}N$ values found in this study. Thus, we attribute the differences in source AA $\delta^{15}N$ values found between the tissues as a likely result of the temporal differences represented by the otolith sampling and muscle turnover time.

Seasonal nitrogen isotopic variations in the Amazon

The estuarine and coastal shelf habitats in the Amazon region are influenced by strong seasonal hydrodynamics that can create distinct baseline $\delta^{15}N$ values. During the wet season, most of the dissolved inorganic nitrogen is discharged by the Amazon River into coastal areas and dispersed several kilometres offshore (Demaster & Pope 1996, Subramaniam et al. 2008). Much of this nitrogen originates from organic nitrogen fertilization in the increasing agricultural use of the Amazon rainforests and untreated sewage from adjacent cities (Martinelli et al. 2012, Bustamante et al. 2015). Anthropogenic organic nitrogen input often leads to high $\delta^{15}N$ values (>10‰) in dissolved inorganic nitrogen as well as in the tissues of local organisms (Montoya 2007, van de Merwe et al. 2016). This is reflected in bulk $\delta^{15}N$ values of surface zooplankton collected in the wet season at an Amazon estuary with values of 8 to 11‰ (Giarrizzo et al. 2011), which decreased to between 3 and 5‰ in oceanic areas (Loick-Wilde et al. 2012). No bulk $\delta^{15}N$ values have been published for the dry season in the Amazon. However, diminished river discharge and lower offshore dissolved nitrate concentrations during this season (23 µmol kg^{-1} with rising river discharge and 12 µmol kg^{-1} with falling river discharge; Demaster & Pope 1996) might imply that anthropogenic nitrogen is contained in the estuaries and leads to lower offshore $\delta^{15}N$ values.

Source AA $\delta^{15}N$ values in adult and juvenile muscle tissue samples collected in the Pará dry season were similar; however, they were lower than those measured in otoliths. Due to the turnover time of muscle tissue AAs of on average several months, we posit that muscle tissue likely recorded the dry season baseline $\delta^{15}N$ values. Otolith AA measurements, which represent 2 life years, probably average the changing baseline $\delta^{15}N$ values of the wet and dry season and result in overall elevated source AA $\delta^{15}N$ values. On the contrary, otolith AA measurements, which represent 2 life years, probably average the changing baseline $\delta^{15}N$ values of the wet and dry season and result in overall elevated source AA $\delta^{15}N$ values. Higher source AA $\delta^{15}N$ values in otolith juve-

Table 2. Molar percentages with standard deviation of 12 individual amino acids in adult and juvenile stage muscle tissue and otoliths of *Cynoscion acoupa* from Pará

	Muscle		Otolith	
	Adult (n = 2)	Juvenile (n = 4)	Adult (n = 4)	Juvenile (n = 4)
Source				
Gly	11.7	8.6 ± 0.8	7.8 ± 0.4	7.9 ± 0.6
Thr	3.5	4.4 ± 0.9	6.5 ± 0.8	5.8 ± 0.7
Ser	3.7	3.4 ± 0.3	7.3 ± 0.8	7.2 ± 0.7
Val	2.6	4.3 ± 0.9	7.3 ± 1.4	7.8 ± 1.1
Phe	4.3	3.8 ± 1.0	1.6 ± 0.1	1.3 ± 0.3
Lys	25.0	16.9 ± 0.6	6.3 ± 0.7	5.5 ± 0.7
Trophic				
Ala	9.7	10.1 ± 0.8	5.9 ± 0.9	6.7 ± 1.4
Leu	10.1	9.8 ± 0.5	4.9 ± 0.2	4.4 ± 0.3
Ile	3.7	4.7 ± 0.3	3.1 ± 0.9	3.1 ± 0.5
Pro	3.9	4.8 ± 0.9	8.5 ± 0.6	8.9 ± 0.3
Asp	6.8	12.2 ± 1.6	19.8 ± 1.0	19.5 ± 1.2
Glu	15.1	16.9 ± 0.8	21.1 ± 1.1	21.8 ± 0.8

nile stages compared to otolith adult stages could indicate habitat separation. Generally, mangrove estuaries are important feeding areas for *C. acoupa* juveniles; these fishes can be found there before and after major rainfall events, following the growth cycles of zooplankton and shrimp populations (Barletta-Bergan et al. 2002, Krumme et al. 2004, Nóbrega et al. 2013, Lima et al. 2015). Adults remain in offshore coastal shelf areas during the entire year, and thus, the similar juvenile and adult muscle tissue source AA $\delta^{15}N$ values might suggest seasonal movement of juveniles towards the coastal shelf during the dry season. However, it cannot be excluded that the $\delta^{15}N$ baseline differences between estuaries and coastal shelf areas are negligent during the dry season.

C. acoupa in distinct Brazilian ecosystems

In contrast to Pará otoliths, the source AA $\delta^{15}N$ values and average TP estimations of juvenile and adult stage from RGN otoliths were similar. This may indicate a habitat overlap during both life stages in RGN or that the fish lived in different habitats with indistinguishable baseline $\delta^{15}N$ values. RGN mangrove estuaries, as those in Pará, also receive anthropogenic nitrogen due to intensive shrimp farming, agriculture and bovine husbandry (de Lacerda et al. 2006, Bustamante et al. 2015). These similar organic nitrogen inputs likely underlie the comparable source AA $\delta^{15}N$ values in otolith juvenile stages from Pará and RGN. Nevertheless, low river runoff at the RGN coast prevents high deposition of anthropogenic nitrogen onto the coastal shelf (Schaeffer-Novelli et al. 1990) and thus will have a negligible effect on offshore baseline $\delta^{15}N$ values. Yet similarly high source AA $\delta^{15}N$ values were measured in the adult and juvenile stage of individual otoliths. As *C. acoupa* adults are exclusively caught offshore from RGN, the high source AA $\delta^{15}N$ values in otolith adult stages from RGN are likely not of anthropogenic origin. The coastal shelf of RGN is an oligotrophic environment (Medeiros et al. 1999), which is distinct from the eutrophic Pará coast due to input of nutrients from the Amazon River (Nittrouer & Demaster 1996, Smith & Demaster 1996). Nevertheless, there is a lack of knowledge on biogeochemical processes at the RGN coastal shelf. It is thus possible that estuarine anthropogenic nitrogen and offshore nitrogen cycling in RGN led to similar source AA $\delta^{15}N$ values. This seems to be supported by similar bulk $\delta^{15}N$ values of zooplankton of 6 to 8‰ during the dry season in RGN estuaries and the coastal shelf 10 km offshore (Schwamborn et al. 1999). However, a habitat overlap of juvenile and adult *C. acoupa* at offshore RGN habitats is also conceivable, and is consistent with the experience of local fishermen who reported catching both life stages at the same offshore locations (K. Vane pers. obs.). Through microbial transformations of nutrients in mangrove ecosystems, estuarine mangrove habitats play a large role in the productivity of aquatic food webs (Holguin et al. 2001). With smaller mangrove coverage, the primary productivity of RGN estuaries and offshore habitats is lower than at the coast of Pará (Schaeffer-Novelli et al. 1990, Ekau & Knoppers 1999). We speculate that Pará juveniles might benefit from larger estuarine productivity on lower trophic levels, inducing offshore migration at a slightly later life stage than in RGN.

Variations in TP

While juvenile stage TPs of both Pará and RGN otoliths were highly consistent between individuals with 0.2 to 0.4 TP variation, individual TPs from otolith adult stages in both areas varied by 0.8 to 1 TP. Such high TP variation in adult *C. acoupa* may be an indication of individual diet variation or could relate to how we calculated TP. The uncertainties surrounding TDF values due to different turnover times of AAs can complicate TP estimations of high trophic level organisms (Chikaraishi et al. 2015,

Nielsen et al. 2015, McMahon & McCarthy 2016). This can lead to TP calculations with up to 1 TP variation, although can be avoided with multiple source and trophic AAs (Bradley et al. 2014, 2015). Controversy exists as to whether Gly should be designated a source AA (Chikaraishi et al. 2009, McMahon et al. 2015), which could introduce variation in TP calculations. However, Bradley et al. (2015) found patterns of Gly $\delta^{15}N$ values consistent with other source AAs in over 200 marine teleosts. In addition, Fuller & Petzke (2017) speculated that distinct enzymatic activities and pathways in different organisms and tissues, such as muscle tissue, can also make Gly behave like a source AA. We suggest that in *C. acoupa*, which has a high protein diet, Gly is routed with minimal isotopic modification to proteins (Webb et al. 2017).

The variations in adult TPs are more likely an indication of individual specialization within the *C. acoupa* population and are similar to that found for *Caranx ignobilis* by Papastamatiou et al. (2015). Noteworthy are the low source AA $\delta^{15}N$ values that often coincided with high adult otolith TP, indicating less productive marine environments based on the bulk isotope trends at both RGN and Pará. Thus, individual adult *C. acoupa* diet can change according to coastal distance; this has also been observed in freshwater fish, which displayed dietary variations with lower TP in littoral areas than in pelagic areas (Beaudoin et al. 1999, Svanbäck et al. 2015). Such individual behavior can be a complex result of resource availability, food–predation risk trade-offs, spatial overlap of food webs as well as phenotypic variations among individuals (Bolnick et al. 2003, Matich et al. 2011) and can play an important role in the persistence and adaptability of a population to environmental disturbances (Bolnick et al. 2011, Levin et al. 2015).

Future perspectives

This study showed that $\delta^{15}N$ values of AAs in otoliths indicate significant differences between life stages and various ecosystems that can be explained relatively well with known nitrogen processes in the Amazon area. However, changes in nitrogen cycling and indicative $\delta^{15}N$ values of AAs over various connected habitats and time scales are not yet fully understood, as was illustrated with RGN otoliths. With a better understanding of such nitrogen cycling changes and variations, $\delta^{15}N$ values of AAs in otoliths could also be used to derive migration patterns from archaeological otoliths. Isotopic measurements of AAs in ~100 000 yr old shells and bones indicate stable preservation in biogenic carbonate structures (Serban et al. 1988, Edgar Hare et al. 1991) with low probability of contamination. Thus, archaeological fish otoliths could also potentially provide unique insight into how trophic ecology and habitat connectivity of fish populations differed during periods before anthropogenic influences, and large environmental and climate changes.

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