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Trophic ecology of speckled peacock bass *Cichla temensis* Humboldt 1821 in the middle Negro River, Amazon, Brazil

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Abstract

The aim of this study was to analyse the trophic ecology of speckled peacock bass *Cichla temensis* inhabiting two tributaries of the middle Negro River, the Aracá River and the Demeni River. Using an analysis of stomach contents and stable isotope composition ($\delta^{15}N$, $\delta^{13}C$) of scales, we describe the diet and evaluate the trophic position of subadult and adult individuals. We then test whether diet shifts and trophic positions occurred among successive size classes and among sample locations. The stomach content analysis confirmed the piscivorous feeding habit of the species and showed that the speckled peacock bass preyed on a variety species belonging to different trophic guilds. The length of the ingested prey increased with the size of the speckled peacock bass. Diet composition and trophic position were not different among size classes. $\delta^{13}C$ values yielded significant shifts among the size classes: larger individuals displayed higher $\delta^{13}C$ values than smaller individuals. Trophic position varied between locations, with lowest values observed in fish from the Aracá River. This study demonstrated that diet of *C. temensis* may vary according to the size of the fish, even at the subadult or adult stages, and according to the river/locality, even within a same basin. We then suggest that further studies take into account local availability of food resources to better explore *C. temensis* diet and tropic ecology.

KEYWORDS
carbon sources, diet shift, niche width, piscivory, stable isotopes, trophic position

1 | INTRODUCTION

The speckled peacock bass *Cichla temensis* Humboldt, 1821 is distributed in the Negro and Orinoco River drainages in Brazil, Colombia and Venezuela. In addition, it also colonises the black-water tributaries of the Solimões-Amazonas River (Kullander & Ferreira, 2006). It is one of the most important fishery resources of Negro River basin (Inomata & Freitas, 2015). The speckled peacock bass *C. temensis* is a large (reaching 99 cm; IGFA, 2001) diurnal piscivore (Jepsen, Winemiller, & Taphorn, 1997), occupying the deeper littoral areas in lakes and along sandy and rocky banks in the main channel of

rivers (Winemiller, Taphorn, & Barbarino-Duque, 1997). Adult *C. temensis* feed preferentially on small fish that colonise these shallow areas, looking for feeding and refuge. Due to its protruding jaws, it can swallow large whole prey (Winemiller et al., 1997). The peacock bass possesses an advanced swimming capacity that allows them to conduct sudden swimming starts from a resting state, making it efficient hunter. Prey is usually captured by ambush or stalking (Brejão, Gerhard, & Zuanon, 2013).

In the Negro River basin, *C. temensis* is exploited by subsistence, commercial and recreational fisheries (Freitas & Rivas, 2006). Given the low human population density, subsistence and commercial

fisheries are of low intensity. The dominant type of capture is the recreational fishery following a catch-and-release attitude producing a low mortality rate (Barroco, Freitas, & Lima, 2017). However, even modest exploitation rates can reduce the abundance of large speckled peacock bass in this region (Holley, Maceina, Thomé-Souza, & Forsberg, 2008). Due to the important role of these predatory fishes in the maintenance and regulation of fish diversity (Freitas, Siqueira-Souza, Prado, Yamamoto, & Hurd, 2010; Petry, Gomes, Piana, & Agostinho, 2010), speckled peacock bass reduction may produce deleterious effects on the fish assemblage.

The feeding habits of *C. temensis* in the Negro River basin in Brazil are still weakly described. In the llanos Venezuelans, this species consumes mainly Characiformes fishes and adapt their diet to prey abundance (Jepsen et al., 1997; Winemiller et al., 1997). Some studies carried out on other species of the Genus *Cichla* in natural environments of the Amazon basin (Mérona & Rankin-de-Mérona, 2004; Oliveira, Soares, Martinelli, & Moreira, 2006; Rabelo & Araújo-Lima, 2002) provided evidence for ontogenetic and seasonal shifts in diet. In Brazil, most of the studies on the trophic ecology of the peacock bass species have been conducted in dammed environments, where speckled peacock bass are known to be important and prolific predators (Gomiero & Braga, 2004; Novaes, Caramaschi, & Winemiller, 2004).

Studies on the trophic ecology of fish can help to understand the trophic organisation of the aquatic ecosystem, providing information about the energy flow, availability of food items, diet and feeding behaviour of the species (Costa & Freitas, 2013; Prudente, Carneiro-Marinho, Valente, & Montag, 2016). Stomach content analysis (SCA) is a common method for dietary analysis. It provides information on food item that has recently been ingested, requiring continuous and standardised sampling protocols over time to capture seasonal, spatial or ontogenetic shifts in the diet (Baker, Buckland, & Sheaves, 2014). SCA could be usefully completed by stable isotope analysis (SIA) in order to describe and interpret fish feeding ecology (Carreon-Martinez, Johnson, Ludsin, & Heath, 2011). SIA has proven to be an efficient tool for the identification of energy sources and the estimation of trophic position. It also presents the advantage to characterise food items that are assimilated (and not only ingested) by the consumers during a longer period of time (Post, 2002).

The use of stable isotopes is based on the assumption that the consumer isotopically reflects its diet (Fry, 2006). Carbon isotope values (δ^{13}C) are generally used to identify energy sources due to the small fractionation rate (~ +0.5‰−1.0‰) of carbon isotopes between consumers and their diets, conducting to a relative stability of δ^{13}C along the food chain (McCutchan, Lewis, Kendall, & McGrath, 2003). Nitrogen isotope values (δ^{15}N) are used as a proxy of trophic position because, due to a higher fractionation process, they varied from one trophic level to another (~ +2.5‰−3.5‰ between a consumer and its prey) all along the trophic chain (Vanderklift & Ponsard, 2003). Thus, the understanding of the trophic relationships is reinforced through the complementary use of SCA and SIA. By combining the use of both these methods, it is possible to obtain a comprehensive view of the trophic ecology and to perceive shifts in the diet of the species according to different spatial, temporal or physiological parameters.

Fishes often present ontogenetic shifts in diet because they undergo modifications in the morphological structures in relation to the growth of individuals (Bozza & Hahn, 2010). Also, these changes in diet could be a result of changes in body size during subadult or adult phases. Prey switching behaviour is known to occur in many species and could be related to ontogenetic or individual size changes, but also could be related to the abundance of prey species, which may be opportunistically used by predators exploiting the diversity of resources as they are available in the environment (Winemiller, 1989). Therefore, we designed a study to assess the trophic ecology of speckled peacock bass *C. temensis* inhabiting two tributaries of the middle Negro River. Our intent was to test (a) whether there are distinct dietary shifts among *C. temensis* size classes through an assessment of diet composition and stable isotopic ratios of carbon and nitrogen according to fish size class and (b) whether there is clear variation of diet composition and trophic positions among sample locations (i.e. different rivers of the same subbasin) which could be related to their unique environmental characteristics and type of available prey.

2 | MATERIALS AND METHODS

2.1 | Study area

This study was conducted in two tributaries on the left bank of the middle Negro River: Demeni River and the Aracá River. Both these rivers are located near the city of Barcelos (Amazonas, Brazilian Amazon) (Figure 1). The Demeni River is a clear water river with low sediment load, which can turn muddy during the rainy season due to the increase in suspended material (Sioli, 1984). It has low conductivity (~10.0 μS/cm^2) and pH values ranging among slightly acid to alkaline (~6.0). The Aracá River is a black-water river with a low concentration of dissolved salts and suspended material (conductivity ~15.0 μS/cm^2), low pH (<5.0) and high concentrations of humic and organic acids suspended into the water (Küchler, Miekeley, & Forsberg, 2000).

2.2 | Sampling

Sampling was performed over a five-day period in April 2016, during the rising water season (License number 22121-1 SISBIO/IBAMA/MMA). The samplings were performed in the main river channel and adjacent bays (Figure 1), and *C. temensis* specimens were caught with fishing rod and artificial bait. After capture, the fish were euthanised through hypothermic treatment in an isothermal box with water and ice. Once the fish died, it was measured (standard length, cm), employing an ictiometer, and stomach and scales from the area below and above the lateral line were extracted. Stomach samples were numbered, fixed in 10% formalin and stored in plastic bags. Scale samples were stored in glass
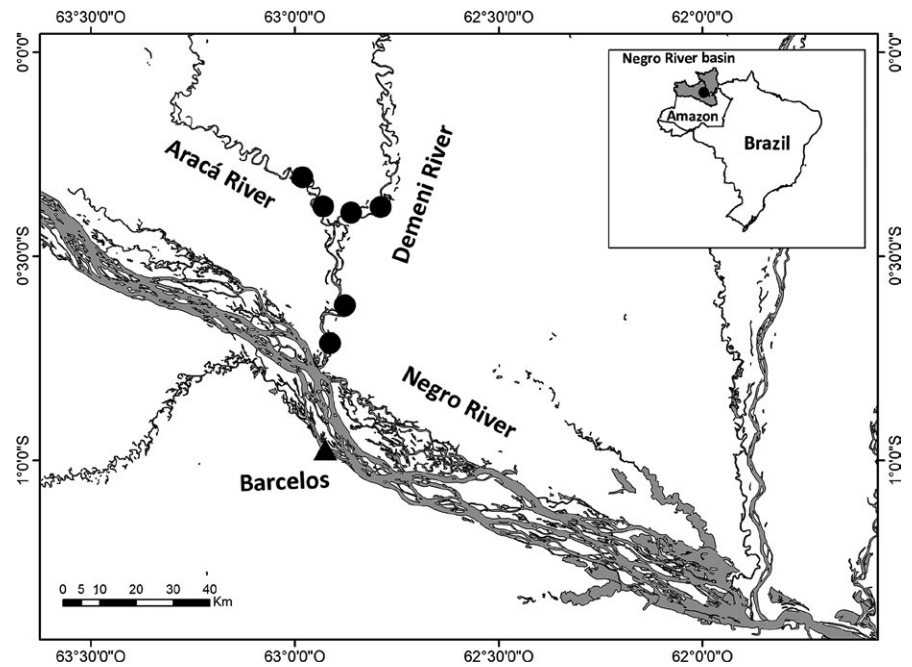
FIGURE 1 Location of the sampled sites (dots) in the region of middle Negro River, Amazon, Brazil

scintillation vials and placed in a freezer where they were kept until processing. Scales, rather than muscle, were utilised for our sampling due to the established close relationship between the isotopic composition of scale and muscle tissue (Satterfield & Finney, 2002), their ease of collection and storage and long-term isotopic stability in preservation.

Potential prey of *C. temensis* was captured with gillnets (2 m height × 15 m long, mesh sizes of 30–120 mm). Gillnets were left fishing for 4 hr in the morning (05:00–09:00 hr) and 4 hr in the evening (17:00–21:00 hr). We collected fishes of four trophic groups to represent the fish assemblage. Detritivore: *Curimata vittata*, *Curimata roseni*, *Psectrogaster amazonica* and *Cyphocharax leucostictus*; Herbivore: *Hemiodus unimaculatus*; Omnivore: *Hemiodus immaculatus*, *Argonectes longiceps*, *Serrasalmus serrulatus* and *Triportheus albus*; Carnivore: *Tetragonopterus argenteus*, *Serrasalmus robertsoni* and *Serrasalmus eigenmanni* (Goulding, Carvalho, & Ferreira, 1988; Mérona & Rankin-de-Mérona, 2004). The fish were rapidly stunned and sacrificed immediately after capture to minimise suffering. Scales of the prey fish were sampled as described above.

Individuals of *C. temensis* were separated into three size classes for analysis: <31 (23.5–30.5 cm), 31–39.9 and >40 cm SL (40–50.5 cm). The class of fish at <31 cm are assumed to be fast-growing young individuals, the class of fish 31–39.9 cm are intermediate-size individuals with a decelerating growth rate, and the class of fish at >40 cm are the largest individuals with smallest growth rate. These class divisions were determined according to the length–growth curve for *C. temensis* estimated for the middle Negro River region (Campos, Freitas, & Amadio, 2015). The first division that roughly corresponds to the literature, established length at first maturation, is 34.0 cm with captivity data (Fontenele, 1950) and 31.1 cm with wild fish data (Campos et al., 2015).

2.3 | Data analysis

2.3.1 | Stomach content analysis

The diet of individuals was determined through stomach content analysis. The content present in each stomach was placed in a Petri dish and analysed under stereomicroscope to identify the food items to the lowest possible taxonomic level. Any fully or partially digested prey was excluded from analyses. Whenever possible, we obtained the standard length of prey. The Alimentary Index (IAi), derived from volumetric and frequency of occurrence indices (Hynes, 1950; Hyslop, 1980), was employed to identify the importance of each prey item on the *C. temensis* diet (Kawakami & Vazzoler, 1980) through the formula: $IAi = Fi * Vi / \sum (Fi * Vi)$; where IAi = Alimentary Index, i = food item, Fi = frequency of occurrence (%) of i food item, and Vi = volume relative (%) of i food item.

Trophic niche breadth of each size class was estimated using volume data through Levin's Standardised Index (Hurlbert, 1978; Krebs, 1999): $B_A = (B - 1)/(n - 1)$, where B_A is the Levin's Standardised Index by the number items (n) and $B = 1/(\sum_{i=1}^{n} pi^2)$, where B = trophic niche breadth, pi is the proportion of the item i in the diet, and n is the number of food items. Food items, which were not identifiable, were not included in analysis (Pouilly, Yunoki, Rosales, & Torres, 2004). This index ranges from 0 to 1, that is, the closer the value is to 1, the individuals consume equally on all the resources available, and the closer to 0, the individuals consume only on one type of resource (Krebs, 1999). Trophic niche breadth was considered accord to the following value ranges: low (0–0.39), intermediate (0.4–0.6) or high (0.61–1) (Corrêa, Albrecht, & Hahn, 2011).

The relationship between *C. temensis* size and the size of its prey was evaluated using the Pearson's correlation, utilizing standard length (cm) of prey and standard length (cm) of *C. temensis*.

To test the null hypothesis of similarity of diet composition (absence–presence data) between size classes was tested through of a permutational multivariate analysis of variance (PERMANOVA) on an array of dissimilarity of Jaccard (Anderson, 2001).

All values were evaluated using a significance level of $\alpha < 0.05$, and all statistical analyses were performed using the software R (R Core Team, 2017) with SPecies Association Analysis (spaa) package (Zhang, 2016) for Levin's Standardised Index and vegan package (Oksanen et al., 2017) for PERMANOVA.

2.3.2 | Stable isotopes analysis

The scales of all the individuals captured were dried in an oven at 50°C for 24 hr. Following desiccation, all scales were stored in 3.55-ml glass vials for transport to the Unity College Stable Isotope Ecology Laboratory (Unity, USA) for isotopic analysis. In the laboratory, all samples were cleaned using double-distilled water and freeze-dried prior to subsampling procedure. Each sample was then ground and homogenised using a glass mortar and pestle. In order to obtain values for $\delta^{13}C$ and $\delta^{15}N$, 1.0 ± 0.2 mg of this material was then subsampled into tin capsules (Costech 5×9 mm) using a Sartorius CPA2P microbalance.

All capsules were filled using clean microspatulas or forceps, folded and sealed using clean forceps and stored in a desiccator cabinet to ensure sample stability for transport and later isotope analysis. C and N isotopic composition were analysed at the Central Appalachians Stable Isotope Facility in Frostburg, Maryland (USA), using a Carlo Erba NC2500 elemental analyser interfaced with a Thermo Delta V+ isotope ratio mass spectrometer.

Stable isotope ratios were expressed as $\delta^{13}C$ or $\delta^{15}N = ((R_{sample}/R_{standard}) - 1) \times 1,000$, where $R_{sample}/R_{standard}$ are the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$. Data were expressed using delta notation (δ) in parts per thousand (‰) with the reference material for $\delta^{13}C$ being Vienna PeeDee Belemnite and as atmospheric air as reference for $\delta^{15}N$. Measurement precision was estimated at ±0.11‰ and ± 0.12‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Lipid corrections of $\delta^{13}C$ values were not considered appropriate because all fish samples displayed C/N ratios <3.5 (Post et al., 2007).

Aiming to test the hypothesis that differences in the isotopic signatures by sampling sites, two analyses of covariance (ANCOVAs) (Zar, 2010) were performed using $\delta^{15}N$ and $\delta^{13}C$ as response variables. The factor was the sampling site with three levels: Aracá River, Demeni River and below the confluence of the Aracá and Demeni River stretches, and the standard length was employed as a covariate, as body size could be related to trophic position (represented by $\delta^{15}N$) and dominant autotrophic source represented by $\delta^{13}C$.

Nitrogen isotopic signatures were used to estimate trophic positions (TP) of all individuals of *C. temensis* according to the following equation: TP = [2 + ($\delta^{15}N_{consumer}$ − $\delta^{15}N_{reference}$)/2.3‰], where 2 is the trophic level of the organism used to estimate $\delta^{15}N_{reference}$. The

$\delta^{15}N_{reference}$ was estimated using the mean $\delta^{15}N$ of the detritivore species *C. vittata* and applying a trophic fractionation value of 2.3‰ (Molina et al., 2011; Post, 2002). *C. vittata* was used as a baseline because it is a primary consumer (Pauly & Palomares, 2005) that fulfils the conditions proposed by Post, 2002. A two-way analysis of variance was performed to compare the TP of *C. temensis* among size classes and sampling sites. Assumptions of normality and homoscedasticity were tested using the Shapiro–Wilk and Levene's test, respectively.

The isotopic niche of each size class was quantified based on standard ellipse areas estimation (SEA) expressed in ‰^{2}. This approach has been proven to be a useful measure of niche width (Bearhop, Adams, Waldron, Fuller, & Macleod, 2004). The standard ellipse areas were corrected for small sample size (SEAc) for estimating the isotopic niche of the young and adult individuals (Jackson et al., 2012). The Bayesian standard ellipse areas (SEA$_b$) were generated with bootstrapping data ($n = 10,000$) to test for significant differences among size classes isotopic niche breadth by comparing their confidence intervals, using the R-package Stable Isotope Bayesian Ellipses (Jackson, Inger, Parnell, & Bearhop, 2011).

3 | RESULTS

3.1 | Diet composition

In total, we sampled 51 specimens of *C. temensis* (Table 1). Standard length and weight of speckled peacock bass varied from 23.5 to 50.5 cm and from 244 to 2,890 g, respectively. Seven of the analysed stomachs were empty, resulting in a total of 15, 19 and 10

TABLE 1 Sampling data of size class of *C. temensis* examined for stomach content (SCA) and stable isotope analysis (SIA), from middle Negro River

Cichla temensis	<31 cm		31–39.9 cm		>40 cm	
Type of Analysis	**SCA**	**SIA**	**SCA**	**SIA**	**SCA**	**SIA**
No. stomachs empty	3	–	2	–	2	–
No. stomachs examined	15	–	19	–	10	–
No. stable isotope samples	–	19	–	20	–	12
Region						
Aracá River	6	9	7	7	1	1
Demeni River	3	3	3	3	5	5
Confluence Region	6	7	9	10	4	6

Sections of SCA and SIA indicate the number of stomach and scale samples, respectively, used in the analyses.

TABLE 2 Alimentary Index (%) of individuals of *C. temensis* from region of middle Negro River

TAXON	Guild	Size class		
		<31 cm	31–39.9 cm	>40 cm
Characiformes				
Curimatidae	Detritivorous	0.8	–	–
Cyphocharax spp.	Detritivorous	–	–	1
Curimata vittata (Kner, 1858)	Detritivorous	–	0.5	1.9
Hemiodontidae				
Argonectes spp.	Omnivorous	–	0.7	–
Clupeiformes				
Engraulidae				
Lycengraulis spp.	Carnivorous	0.8	–	–
Osteoglossiformes				
Osteoglossidae				
Osteoglossum spp.	Carnivorous	–	–	1
Siluriformes				
Pimelodidae				
Pimelodus albofasciatus Mees, 1974	Carnivorous	–	–	1.9
Loricariidae				
Ancistrus spp.	Detritivorous	–	0.7	–
Loricariinae	Detritivorous	0.4	–	–
Gymnotiformes				
Sternopygidae				
Rhabdolichops nigrimans Correa, Crampton & Albert, 2006	Carnivorous	–	0.7	–
Cichliformes				
Cichlidae				
Cichlasomatinae	–	0.8	–	–
Geophaginae	–	0.4	0.7	–
Unidentified fish		96.6	93.2	94.2
Digested items		–	0.7	–
Vegetable fragments		0.4	2.8	–

stomachs used for the analyses of size classes <31, 31–39.9 and >40 cm, respectively.

Due to the advanced stage of digestion, some items could not be identified to a specific taxon. Overall, it was possible to identify 12 different food items in the diet of *C. temensis*, represented mainly for fishes, and a little amount of vegetal fragments. The prey fishes were organised in six orders, eight families and eight species of fishes. One item was only able to be identified to the family level, while three other items were only able to be identified to the subfamily level.

Alimentary Index (IAi) indicated that *C. temensis* present a highly specialised diet based on prey fish consumption (>98%, Table 2). Other food items (IAi < 2%–3%) only correspond to vegetal fragments. No detritus or invertebrates rests were found. Among the fish prey identified in the stomach, *Lycengraulis* spp., Cichlasomatinae and Geophaginae were exclusive in the diet of smaller individuals and

Osteoglossum spp. and *Pimelodus albofasciatus* were exclusive to the diet of larger individuals (Table 2). Prey size analysis showed a positive correlation with speckled peacock bass length ($n = 9$, $p < 0.05$; Figure 2), indicating that prey size increased with predator size.

The diet composition of *C. temensis* did not differ significantly by size class (PERMANOVA: Pseudo-$F = 0.727$; $df = 2, 10$; $p = 0.76$). Thus, it was not possible to reject the null hypothesis that the diet compositions of individuals of the size classes of *C. temensis* are the same, despite the existence of exclusive prey species within each size class.

In general, niche breadth values were considered low and dependent of the size class of *C. temensis* (Table 2). In this way, the largest individuals presented the lowest values ($B_A = 0.2$), while individuals in the <31 cm and 31–39 cm size ranges showed closest values of B_A at 0.34 and 0.36, respectively.
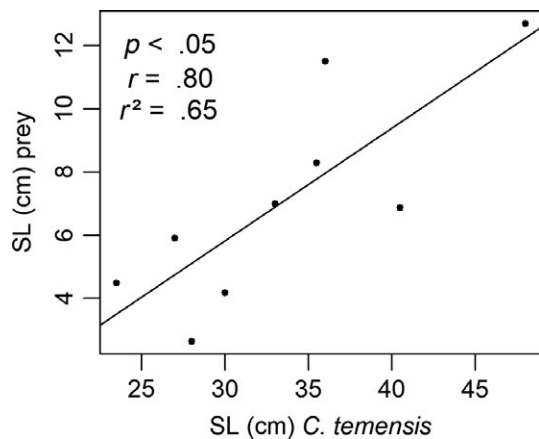
FIGURE 2 Relationship between standard length (cm) of prey and standard length (cm) of *C. temensis* from the middle Negro River. Nine peacock bass and the means of prey SLs by stomach contents were used in the analysis

3.2 | Stable isotopes analysis

Cichla temensis $\delta^{13}C$ values ranged from −31.31‰ to −26.9‰, and the $\delta^{15}N$ values ranged from 10.44‰ to 12.64‰ (Table 3). Individuals of the size class >40 cm appeared more related to prey more enriched in ^{13}C than individuals of small and intermediate size.

The isotope signatures of trophic groups ranged from −33.44‰ to −23.02‰ for $\delta^{13}C$ and 7.36‰ to 8.68‰ for $\delta^{15}N$ for herbivore fish; from −36.21‰ to −29.29‰ for $\delta^{13}C$ and 7.17‰ to 10.44‰ for $\delta^{15}N$ for detritivore fish; from −35.59‰ to −25.08‰ for $\delta^{13}C$ and 8.39‰ to 10.92‰ for $\delta^{15}N$ for omnivore fish; and from −30.65‰ to −25.82‰ for $\delta^{13}C$ and 9.25‰ to 14.18‰ for $\delta^{15}N$ for carnivore fish (Table 4).

Individuals of the size class <31 and 31–39.9 cm seem to consume detritivore fish, while larger individuals are preying on carnivore fish and herbivore fish. Omnivorous fish are being prey on by all specked peacock bass. It is possible that *C. temensis* consumes a variety of prey fish from different trophic groups. *C. temensis* seems to be dependent on a food chain a mixture of basal energy sources, with the exception of submerged herbaceous plants (Figure 3).

There was a significant positive relationship between $\delta^{13}C$ and standard length for all environments (ANCOVA: $F = 17.388$; $df = 1$, 47; $p < 0.05$, Figure 4a), but there are no differences in the slope and intercept of the regressions by sampling site (ANCOVA: $F = 1.58$; $df = 2$, 47; $p > 0.05$; Figure 4a). Standard length and $\delta^{15}N$ were not

related (ANCOVA: $F = 1.444$; $df = 1, 47$; $p > 0.05$), but there were differences in the intercept by locality (ANCOVA: $F = 10.857$; $df = 1, 47$; $p < 0.05$, Figure 4b).

The data follow a normal distribution (Shapiro–Wilk: $W = 0.959$; $p = 0.076$), and the variances are homogeneous for the factors of size class (Levene: $F = 0.6977$; $df = 2, 48$; $p = 0.5027$) and sampling sites (LEVENE: $F = 3.0811$; $df = 2, 48$; $p = 0.055$). A two-way analysis of variance yielded no significant differences in the trophic position between size class (ANOVA: $F = 3.066$; $df = 2, 42$; $p = 0.057$) or for the interaction between ontogeny and locality (ANOVA: $F = 1.249$; $df = 4, 42$; $p = 0.305$). Differences were observed in the trophic position by locality (ANOVA: $F = 9.374$; $df = 2, 42$; $p = 0.0004$) with *C. temensis* from Aracá River displaying a lower trophic position than *C. temensis* from the Demeni River and Confluence Region (Figure 5).

The analysis of the isotopic niche breadth based on SEA_b showed that there is no significant difference in the area of the standard ellipse size classes of speckled peacock bass ($p > 0.05$; Figure 6). The isotopic niche breadth, according to the SEAc estimations, was $1.83‰^2$, $2.11‰^2$ and $0.72‰^2$ for <31, 31–39.9 and >40 cm individuals, respectively. The Bayesian approach used in SIAR indicated an overlap of 100% between the ellipse areas of <31 and 31–39.9 cm individuals, an overlap of just 6.5% between <31 and >40 cm individuals and an overlap of 34% between 31–39.9 and >40 cm individuals.

4 | DISCUSSION

Although the stocks of peacock bass are exploited by commercial, subsistence and recreational fisheries in the Amazon basin, few studies have been carried out on the trophic ecology of these species in natural environments of this region. Our study, using stomach content and stable isotope, analysed the trophic ecology of speckled peacock bass *C. temensis* of two tributaries in the middle Negro River. The results confirmed the highly specialised piscivorous diet of this species, independent of its size (for the studied range 23–50 cm). The piscivorous habits of species in the genus *Cichla* have been recorded in other studies in the Amazon Basin (Oliveira et al., 2006; Rabelo & Araújo-Lima, 2002).

Stomach content analysis did not detect any clear pattern of diet composition shifts related to size class. The diet of *C. temensis* has been shown to be composed of a variety of prey fish belonging to different fish families, indicating little selectivity in prey type (Bozza & Hahn, 2010). Low selectivity is common among predatory fish.

TABLE 3 Isotopic composition of *Cichla temensis* for each river and size class. $\delta^{13}C$ and $\delta^{15}N$ average and standard deviation values for *Cichla temensis* are given when *N* (number) >1

| Local | n | *Cichla temensis* (<31 cm) | | n | *Cichla temensis* (31.1–39.9 cm) | | n | *Cichla temensis* (>40 cm) | |
		$\delta^{13}C‰$	$\delta^{15}N‰$		$\delta^{13}C‰$	$\delta^{15}N‰$		$\delta^{13}C‰$	$\delta^{15}N‰$
Aracá River	9	−29.33 ± 0.5	11 ± 0.3	7	−29.3 ± 1.2	11.49 ± 0.7	1	−28.17	11.62
Demeni River	3	−29.99 ± 1.3	12.2 ± 0.2	3	30.28 ± 1	11.99 ± 0.1	5	−28.53 ± 0.3	12.21 ± 0.2
Confluence Region	7	−29.88 ± 1	12 ± 0.3	10	−29.52 ± 1.1	11.72 ± 0.6	6	−28.47 ± 0.6	11.94 ± 0.5

TABLE 4 Isotopic composition of prey fish species for the Demeni River and the Confluence Region. $\delta^{13}C$ and $\delta^{15}N$ average and standard deviation values for prey are given when *N* (number) >1

| Group	n	Demeni River $\delta^{13}C‰$	$\delta^{15}N‰$	n	Confluence Region $\delta^{13}C‰$	$\delta^{15}N‰$
Detritivore						
Curimata vittata	6	−31.80 ± 2.3	9.24 ± 1	3	−34.29 ± 1.7	8.45 ± 0.8
Curimata roseni	2	−33.64 ± 1.1	8.96 ± 0.4	–	–	–
Psectrogaster amazonica	5	−32.14 ± 1.4	9.31 ± 0.3	–	–	–
Cyphocharax leucostictus	5	−32.7 ± 1.6	8.3 ± 1	–	–	–
Herbivore						
Hemiodus unimaculatus	10	−26.46 ± 3.4	8 ± 0.5	–	–	–
Omnivore						
Hemiodus immaculatus	–	–	–	2	−32.25 ± 4.1	9.65 ± 0.6
Argonectes longiceps	–	–	–	4	−31.51 ± 2.8	9.68 ± 0.4
Serrasalmus serrulatus	3	−27.59 ± 0.4	10 ± 0.8	–	–	–
Triportheus albus	5	−26.63 ± 1	9 ± 0.7	–	–	–
Carnivore						
Tetragonopterus argenteus	1	−28.87	9.98	2	−27.31 ± 0.1	9.78 ± 0.7
Serrasalmus robertsoni	1	28.59	11.36	–	–	–
Serrasalmus eigenmanni	7	−28.13 ± 10	11.28 ± 4.2	–	–	–
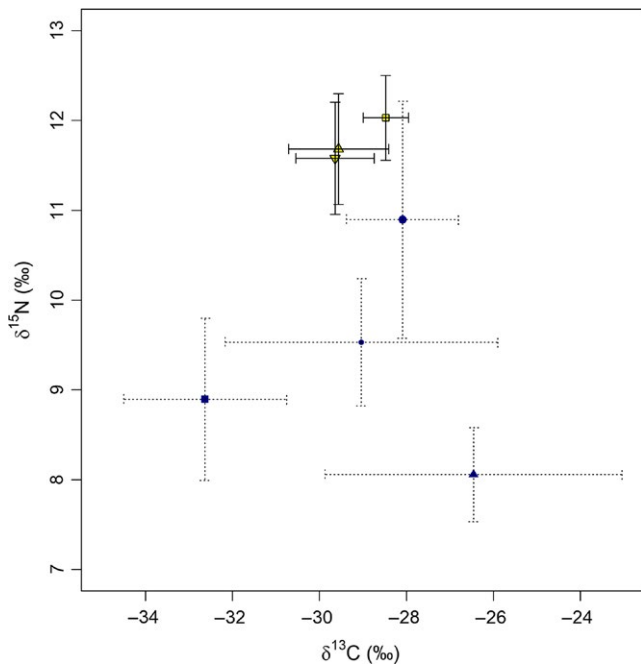
FIGURE 3 $\delta^{13}C$ and $\delta^{15}N$ biplot of peacock bass (*C. temensis*) by size classes: the size class <31 cm (yellow inverted triangle), the size class 31–39.9 cm (yellow triangle) and the size class >40 cm (yellow square) of *C. temensis*; prey fish: detritivorous (blue square), herbivorous (blue triangle), omnivorous (small circle) and carnivorous (large circle). For all, each point represents the mean value and standard deviation

The group of *Cichla* showed low selectivity in the rivers of Venezuela (Jepsen et al., 1997). However, in the black-water rivers of Venezuela, characif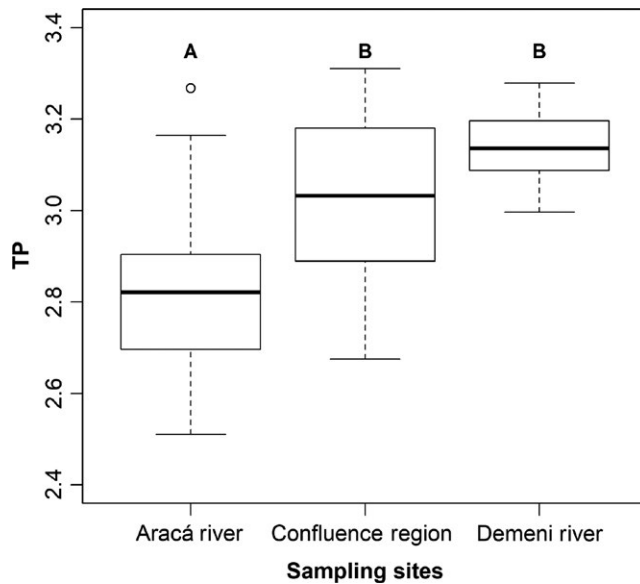ormes were the main components of the peacock bass diet (Jepsen et al., 1997; Winemiller et al., 1997) with *Semaprochilodus kneri* accounting for about 45% of the diet of *C. temensis* during the receding water period (Winemiller & Jepsen, 2002). This discordance could be explained due to the fact that piscivorous fish usually consumed different prey species according to their availability in the system (Popova, 1978). Predatory fish that consume preys indiscriminately are usually opportunistic predators that adjust their feeding habit to coincide with the relative abundance of prey (Juanes, Buckel, & Scharf, 2002). Most species of Amazonian fish are opportunistic feeders, but exhibit some degree of prey preference (Lowe-McConnell, 1999). In our study, *C. temensis* appears as highly specialised on fish prey, but without a clear preference for a species or a group of species, then they could be presented as opportunist strict piscivore.

The size of the prey consumed increased with the size of the speckled peacock bass. For most piscivorous fish, the size of the prey consumed usually increases with the size of the predator (French, Platell, Clarke, & Potter, 2017; Juanes et al., 2002). This affirmation is verified in our study and could be attributed to the ability of *Cichla* of detecting and capturing prey to according to its development, which can be stimulated by an increase in visual acuity with increasing body size (Caves, Sutton, & Johnsen, 2017). In addition, when the predator is young, its morphological limitations, such as small mouth (restriction of large prey) and swimming capacity (impossibility of catching agile prey) (Jo, Gim, Jeong, Kim, & Joo, 2014), limit the possibility to feed on larger prey and so instead focus on smaller size prey which are more vulnerable to predation (Scharf, Juanes, & Rountree, 2000). Despite this, *C. temensis* showed preference in consuming the smallest individuals of *S. kneri* in the Cinaruco
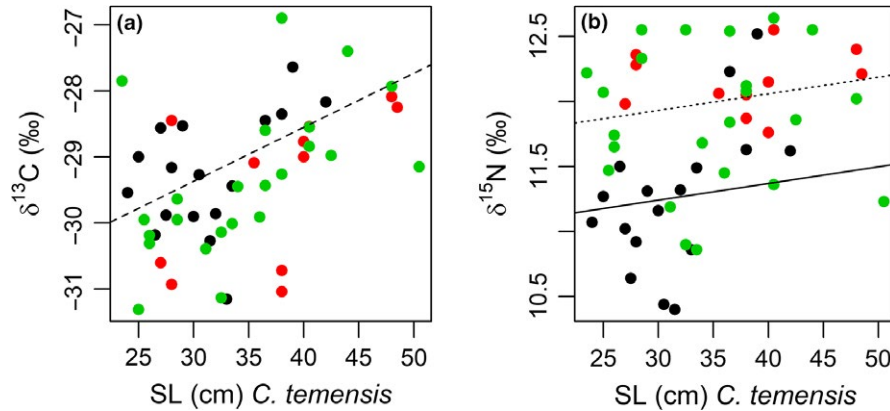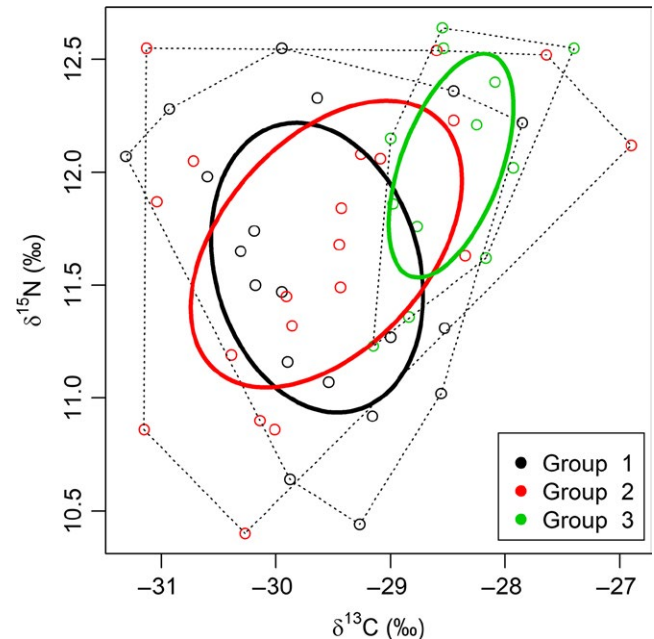
FIGURE 4 Relationship between $\delta^{13}C$ values and standard length (cm) (a) and relationship between $\delta^{15}N$‰ values and standard length (cm) (b). Each point represents an individual *C. temensis* from Demeni River (red circle), Confluence Region (green circle) and Aracá River (black circle). In a, the dashed line represents the significant relationship between standard length and $\delta^{13}C$‰ for all individuals ($r^2 = 0.46$, $p < 0.05$). In b, the dotted line represents the relationship between standard length and $\delta^{15}N$ for individuals from Demeni River and Confluence Region, and solid line represents the relationship between standard length and $\delta^{15}N$‰ for individuals from Aracá River. Both relationships were not statistically significant, but the difference between the intercepts shows higher $\delta^{15}N$‰ for individuals from Demeni River and Confluence Region than for individuals from the Aracá River

FIGURE 5 Trophic position (TP) of *C. temensis* from Aracá River, Confluence Region and Demeni River

FIGURE 6 Standard ellipse areas estimated from the carbon and nitrogen stable isotopes (‰) for <31 cm (Group 1), 31–39.9 cm (Group 2) and >40 cm (Group 3) individuals of *C. temensis*

River (in the Venezuelan llanos) even when their gape size allowed for the consumption of larger individuals, but this was due to the great abundance of the prey, especially during its migratory period, (Hoeinghaus, Winemiller, Layman, Arrington, & Jepsen, 2006), which may confirm the opportunistic nature of this species.

The observed differences in *C. temensis* $\delta^{15}N$ values may be due to site-related effects. Individuals from the Demeni River exhibited higher values of $\delta^{15}N$ compared to those from the Aracá River, whereas individuals from the Confluence Region had a very large range of values. The spatial differences in ^{15}N isotopic values are also reflected in the trophic position. Individuals from the Demeni River had high values, and individuals from the Aracá River had low

trophic position values. These values reflect the available prey in each environment. We suggest that these results indicate an environmental distinctiveness that demands further investigation to determine the key limnological variables driving the trophic differences among top predator fish from different but connected areas. In tropical rivers, variation in body size of primary consumers influences the trophic position of predatory fish (Layman, Winemiller, Arrington, & Jepsen, 2005). This indicates that *C. temensis* eats a variety of prey fish of different size and trophic levels in each river, regardless of its size.

We hypothesise that the smaller individuals consume of a wider range of feeding items than did larger fish. These results contrast with patterns observed in other predatory fish in the Amazon region where adult fish focus on a higher variety of food resources (Carvalho et al., 2017). This difference may be due to the fact that adult/larger of *C. temensis* exhibit a variety of movement patterns, including localised/restricted movements, river interchanges and possible natal philopatry (Sousa, Humston, & Freitas, 2016). Thus, it could be expected that larger individuals had greater trophic niche breadth, but we observed that this does not occur with *C. temensis*.

Both SCA and SIA results underscored that speckled peacock bass fed on a variety of prey fish species among size classes. We therefore hypothesized that the speckled peacock bass consumes same the species of fish among size classes, but with different prey sizes. Similar such patterns have been observed elsewhere, as well. In the Cinaruco River, a black-water river in Venezuela, small individuals of *C. orinocensis* (<8 cm) fed predominantly on copepods and cladocerans, and the fish consumption was recorded only in individuals larger than 20 cm (Jepsen et al., 1997). On the other hand, individuals of *C. temensis* (5–60 cm) fed predominantly on prey fish (Jepsen et al., 1997). Davis, Blanchette, Pusey, Jardine, and Pearson (2012) observed piscivorous fish species become more specialised and enriched in ^{13}C as they grow. This increasing specialisation meant that larger peacock bass displayed a narrower isotopic niche width when compared to smaller individuals consuming a wide range of prey including crustaceans and small fish (Oliveira et al., 2006).

The niche of a species can be differentiated in many ways, one of which is through the differential use of resources (Begon, Townsend, & Harper, 2007). Thus, despite the great overlap of isotopic niche among *C. temensis* individuals as observed through Bayesian ellipses, we suggest that *C. temensis* showed a partition of food resource available in their immediate area according to their size and then capacity to feed on larger fish prey.

Dietary shifts related to size could be a competitive life strategy, as it permits a species to exploit a broad range of available prey items. This study demonstrated that, in addition to size linked variability in the diet of *C. temensis*, there are also spatial variations, even within a same basin. Thus, we suggest that future studies consider the spatial variation in the availability of food resources for *C. temensis* and make effort to identify the mechanisms involved in the trophic relationships within different subbasins. Conducting such examinations of fish diet in the natural environment is essential for successful management of this region and to reach the objectives of conservation, preservation and sustainability of these invaluable fishery resources.

CONFLICT OF INTEREST

The authors do not report any conflict of interest.

AUTHOR'S CONTRIBUTION

J. A. S and C. E. C. F. conceived and designed the study. J. A. S. and F. K. S. S. collected the data. J. A. S., P. A. P. D., C. E. C. F. and F. K. S. S. analysed and interpreted the data. J. A. S. drafted the manuscript. P. A. P. D., M. P. and C. E. C. F. reviewed the manuscript. All authors discussed the results and contributed to the final manuscript.ORCID

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