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PRIMARY RESEARCH PAPER

Trophic niche segregation among herbivorous serrasalmids from rapids of the lower Xingu River, Brazilian Amazon

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Abstract In the Amazon Basin, several species of herbivorous serrasalmid fishes inhabit rapids, but it is unknown if they partition food resources during the annual low-water period when fish densities are high within greatly reduced volume of aquatic habitat. We investigated the trophic ecology of juveniles and adults of three species, *Myleus setiger*, *Ossubtus xinguense*, and *Tometes kranponhah*, common in rapids of the Xingu River during the low-water period. Diets, stable isotope ratios of muscle tissue, and functional traits were analyzed for 59 specimens of *M. setiger*, 175 of *O. xinguense* and 215 of *T. kranponhah*. The three species overlapped in dietary and isotopic space, with adult *O. xinguense* being most divergent. Juvenile and adult *T. kranponhah* and juvenile *O. xinguense*, two groups with broad diets, had lowest trophic positions estimated from isotopic data. Adult *O. xinguense* had the highest trophic position despite having large amounts of Podostemaceae in the diet. High trophic overlap during the low-water period suggests that either food resources are not limiting, or niches are partitioned by other means. Differences in functional traits of the three serrasalmids could be associated with differential efficiencies of swimming and feeding within microhabitats that vary according to water velocity and/or structural complexity.

Keywords Dietary analysis · Herbivory · Niche overlap · Niche partitioning · Ontogenetic niche shift

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Introduction

Species specializing in swift water, often referred to as rheophilic (Lincoln et al., 1985), have special morphological, physiological, and behavioral adaptations to extreme environmental conditions, and rapids-adapted fishes worldwide possess convergent suits of phenotypes (Stewart & Roberts, 1976; Lujan & Conway, 2015). For example, fishes from widely divergent evolutionary lineages have reduced swimbladders and elongate bodies that reduce buoyancy and drag, respectively, when resting on substrates in fast-flowing water (Conway et al., 2012; Birindelli & Britski, 2013). Many benthic fishes possess broad pectoral and pelvic fins that function like hydrofoils, as well as mouths capable of suctioning against solid substrates, to assist in holding position in fast water (Conway et al., 2012; Leitão et al., 2015). Given the strong selective pressure for convergent morphologies and behavior in these habitats, rapids in tropical rivers provide a unique opportunity to understand mechanisms promoting the stable coexistence of diverse fish assemblages.

Evidence that fishes use resource-partitioning to limit interspecific competition is widespread (Gatz, 1981; Ross, 1986; Lujan et al., 2012; Montaña et al., 2014), and several features of rapids may increase the need for coexisting species to segregate trophic niches. Rapids create physical barriers to longitudinal dispersal for many aquatic organisms (Junk & Soares, 2001). Rheophilic species inhabiting isolated rapids also can have limited dispersal among local populations when these are separated by long stretches of slower moving water (Carvalho et al., 2007). This dispersal limitation may increase competition for food resources, as well as the importance of niche partitioning in facilitating coexistence of rheophilic fishes. Competition may be further intensified during low-water periods if high-quality food resources become less available (Lowe-McConnell, 1987). Because many rapids-adapted fishes have limited dispersal and exploit food resources associated with swift-water habitats, niche partitioning of food resources or morphological features that impact foraging behavior may be necessary to promote stable coexistence (Vitorino Júnior et al., 2016).

In the Neotropics, many rapids-adapted fishes exploit autochthonous food resources, including crabs, snails, aquatic insect larvae, sponges, and aquatic plants (Horeau et al., 1998; Moreira & Zuanon, 2002; Pagezy & Jégu, 2002; Zuanon & Sazima, 2002). Anostomid and loricariid fishes inhabiting rapids of Neotropical rivers encompass diverse phenotypes (Zuluaga-Gómez et al., 2016), but many species within these families seem to have relatively narrow trophic niches reflecting herbivory or detritivory. The family Serrasalmidae is well known for carnivorous piranhas (Chakrabarty & Fink, 2011), but several species of the family are herbivorous, some of which inhabit rapids nearly exclusively. The family, as a whole, encompasses diverse feeding strategies, including carnivory, insectivory, omnivory, herbivory, frugivory, and lepidophagy (Goulding, 1980; Sazima, 1983; Leite & Jégu, 1990; Sazima & Machado, 1990; Loubens & Panfili, 1997; Santos et al., 1997; Correa et al., 2007; Loubens & Panfili, 2001; Trindade & Juca-Chagas, 2008; Correa & Winemiller, 2014; Correa et al., 2014, 2016). Some serrasalmid species are habitat generalists, but others are restricted to specific habitat types. For example, the tambaqui [*Colossoma macropomum* (Cuvier 1816)] and pacu (*Piaractus* spp.) inhabit lowland river and floodplain habitats where they feed on fruits and seeds (Goulding, 1980; Jégu & Keith, 1999) while species of the genera *Myleus*, *Mylesinus*, *Tometes*, *Ossubtus*, and some species of *Myloplus* are restricted to rapids habitats (Jégu et al., 1989; Jégu & Santos, 2002; Pagezy & Jégu, 2002; Jégu et al., 2002, 2003; Jégu & Zuanon, 2005; Andrade et al., 2016a, b).

Both juveniles and adults of the rheophilic species *Myleus setiger* Müller & Troschel, 1844, *Tometes kranponhah* Andrade, Jégu & Giarrizzo, 2016, and *Ossubtus xinguense* Jégu, 1992 are commonly found in fast-flowing waters of rapids, often in close association with rocks covered by aquatic macrophytes of the family Podostemaceae (Andrade et al., 2013). *Myleus setiger* is the most widely distributed of the three species, occurring in several major Amazon tributaries that drain the Guiana and Brazilian shields, whereas *T. kranponhah* and *O. xinguense* are endemic to the Xingu Basin (Andrade et al., 2016a). *Myleus setiger* and *T. kranponhah* are not listed as species of conservation concern due their wide distribution and local abundance within the Xingu Basin (Andrade et al., 2016a). *Ossubtus xinguense* is considered highly threatened (Jégu & Zuanon, 2005; Andrade et al., 2016c) and is listed as vulnerable in Brazil (National Red List, 2016). None of these species have been assessed by the International Union for the Conservation of Nature (IUCN, 2018). Despite differences in their listing within Brazil, both *T. kranponhah* and *O. xinguense* may suffer harmful and irreversible effects due to recent hydrologic alterations in the Xingu River caused by the construction of Belo Monte hydropower dam, the third largest hydroelectric dam complex in the world (Andrade et al., 2016a, c). Changes in seasonal flow dynamics combined with anticipated impacts of gold mining in the region (Tófoli et al., 2017) will likely impact niche relationships of the Xingu's endemic diversity, making it necessary to understand the mechanisms facilitating coexistence of rapids-adapted species for conservation efforts in the region.

Here we test whether three sympatric serrasalmids (*M. setiger*, *T. kranponhah*, and *O. xinguense*) from the lower Xingu River partition trophic niche space during the annual low-water period. All three species are known to be herbivorous, with diets dominated by aquatic macrophytes (Jégu & Santos, 2002; Jégu & Zuanon, 2005; Andrade et al., 2015, 2016a). Coexistence of these three rapids-adapted serrasalmids might be facilitated by differences in how they exploit microhabitats, food resources, or both (e.g., Dias & Fialho, 2011; Mouchet et al., 2013; Burress, 2014; Gracan et al., 2016). We evaluated both inter- and intraspecific niche variation in diet, stable isotope ratios, and functional morphology related to feeding. We predicted that intraspecific variation would be most strongly associated with ontogenetic diet shifts, with some species exploiting mostly macroinvertebrates during the juvenile stage and shifting to a diet comprised mostly of aquatic macrophytes during the adult stage. We further predict that interspecific trophic niche overlap would be lower for adults feeding on different plants or plant parts, and that morphological traits reflecting how these fishes use microhabitats and acquire food would differ according to species and life stage.

Materials and methods

Study site

The study was conducted in the lower portion of Xingu River Basin, which is characterized by numerous rapids within the network of channels comprising the Xingu, Iriri, and Bacajá rivers (Fig. 1). Collections were made along a 270-km stretch of river from Cachoeira Grande (Big Falls) on the Iriri River (3°51′10″S 52°43′40″W) to the downstream end of the Volta Grande (Big Bend reach of the lower Xingu) near Belo Monte (3°03′57″S 51°49′35″W) where, in 2016, the third largest hydroelectric power plant complex in the world was completed (Belo Monte Dam). In addition, the Bacajá River was sampled from its confluence with the Xingu River (3°45′26″S 51°34′57″W) to a location approximately 40-km upstream (Fig. 1).

Species characteristics

Myleus setiger and *O. xinguense* (hereafter '*Myleus*' and '*Ossubtus*,' respectively) reach standard lengths of 27 cm and 23 cm, respectively. *Tometes kranponhah* (hereafter '*Tometes*') is generally larger, reaching standard lengths of 37 cm. All three species have functional traits specialized for herbivory and feeding in rapids, such as incisiform teeth that effectively cut leaves, and laterally compressed bodies that reduce hydraulic drag in fast water (Meunier et al., 2004). All three species undergo ontogenetic transitions in the length of the gastrointestinal tract, with adults having relatively longer guts (Jégu et al., 1989, 2002). This

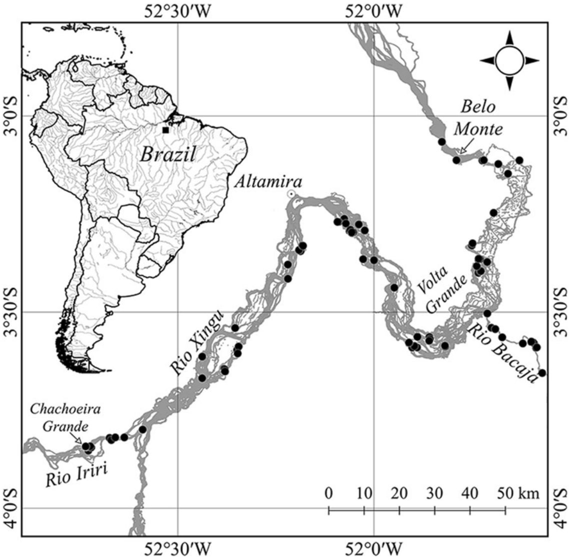

Fig. 1 Xingu River Basin showing the study area prior to flow modification made in 2016 due to the construction of Belo Monte Dam and the sampling sites

size allometry is assumed to be associated with a shift from generalist feeding to a diet dominated by epilithic macrophytes, including riverweeds (Podostemaceae) and bryophytes (mosses and liverworts).

Sample collection

Fishes were collected during low-water periods when the three serrasalmid species are restricted to patchily distributed rapids. Sampling was performed during June–July 2012, September–October 2013, October 2014, and October 2015, prior to flow modification by the Belo Monte Hydroelectric Complex. Except *Myleus*, which was not sampled in 2012, *Ossubtus* and *Tometes* were sampled every year. Fishes were captured from rapids using gillnets placed in relatively deep, slow-flowing areas, and castnets thrown in shallower, faster-flowing sections of rapids in or near epilithic macrophyte beds. To test whether species partition niches between different ontogenetic stages, we first classified individuals as juveniles (< 100 mm SL for *Myleus* and *Ossubtus*; < 130 mm SL for *Tometes*) or adults (≥ 100 mm SL for *Myleus* and *Ossubtus*; ≥ 130 mm SL for *Tometes*; Table 1), based on size at first maturation (Jégu & Santos, 2002; Andrade et al., 2016a, c). For stable isotopes analysis, a sample of muscle tissue was removed following euthanasia and prior to preservation of the specimen in 10% formalin. Morphological and dietary data were obtained from specimens after storage in 70% ethanol.

Morphological traits

Sixteen morphological features were measured according to methods in Gatz (1979) and Winemiller (1991): standard length (SL), body depth, body width, head depth, eye position, head length, caudal-peduncle length, caudal-peduncle depth, caudal-peduncle width, pectoral-fin length, pectoral-fin width, eye diameter, mouth width, mouth orientation, gastrointestinal length, and swimbladder length (Table 2). We also measured the length of the nasal chamber of the skull, i.e., the space that houses the olfactory bulb, in the longitudinal dimension (Andrade et al., 2016c). Linear measures were made on preserved specimens using digital callipers (precision 0.1 mm). Morphological traits were selected based on their functional roles in feeding or swimming performance and use of microhabitat (Table 1). For example, body size (indexed by SL) influences feeding ecology (Verwaijen et al., 2002; Montaña & Winemiller, 2013), relative body depth influences lateral turning (Zuluaga-Gómez et al., 2016), and relative intestine length is associated with diet (Wagner et al., 2009).

Diet

Each specimen examined for dietary analysis was measured (SL mm) before dissection and removal of the stomach. Empty stomachs were recorded but did not contribute to sample sizes reported for dietary analyses. Food items from stomachs were classified according to eight categories: 1—leaves and flowers of terrestrial plants; 2—seeds (mainly from riparian plants); 3—Podostemaceae (mainly leaves of this aquatic macrophyte); 4—periphyton (benthic algae and associated microfauna and biofilm); 5—detritus (particulate organic material); 6—sediments (mainly silt and sand); 7—fish fins and scales; and 8—aquatic macroinvertebrates (mainly Chironomidae and

Table 1 Number of individuals from multiple sites of the Xingu River basin analyzed for each method in this study, and classified according to the ontogeny pre-established into juveniles (J) and adults (A)

	Life stage	Ecomorphology	Diet	Stable isotopes	Mean ± SD of SL (mm)
Myleus setiger	J	8	7	7	85.5 ± 6.7
	A	11	10	14	130.3 ± 24.8
Ossubtus xinguense	J	23	23	9	58.4 ± 11.6
	A	14	70	36	176.5 ± 22.0
Tometes kranponhah	J	30	109	26	82.4 ± 21.2
	A	5	18	27	179.4 ± 45.9
Total		91	237	119	

Table 2 Morphological traits with their respective formula and ecological explanation

Morphological trait	Formula	Explanation
Habitat use		
Relative depth of body	$\mathrm{RBD} = \frac{\text{Body depth}}{\mathrm{SL}}$	Inversely proportional to the water velocity and directly proportional to the ability to make manoeuvres
Relative length of caudal peduncle	$\mathrm{CPL} = \frac{\text{length of caudal peduncle}}{\mathrm{SL}}$	Directly related to the swimming ability
Relative width of caudal peduncle	$\mathrm{WCP} = \frac{\text{width of caudal peduncle}}{\mathrm{SL}}$	Inversely proportional to the amplitude of the swimming movements, trend to be higher in swimmers less active
Aspect ratio of pectoral fin	$\mathrm{ARP} = \frac{\text{width of pectoral fin}}{\text{length of pectoral fin}}$	Directly related swimming ability and with benthic habits, function to brake, maintain the position, accelerating the braking
Eye position	$\mathrm{EPO} = \frac{\text{height of centre eye}}{\text{head length}}$	Directly related to the water column position preference
Relative length of swimbladder	$\mathrm{SWB} = \frac{\text{swimbladder length}}{\mathrm{SL}}$	Inversely related to the deeper on the water column
Mouth orientation	MOR = coded as: 1 to upturned; 2 to terminal; and 3 to inferior	Indicates in which position the fish acquire food
Trophic strategy		
Standard length	SL = measure of standard length	Directly proportional to the ability to acquire bigger preys
Relative length of head	$\mathrm{HL} = \frac{\text{length of head}}{\mathrm{SL}}$	Directly proportional to the ability to acquire bigger preys
Relative height of head	$\mathrm{HH} = \frac{\text{height of head}}{\text{head length}}$	Directly proportional to the ability to acquire bigger preys
Relative size of eye	$\mathrm{SIE} = \frac{\text{eye diameter}}{\text{head length}}$	Directly related to the importance of the vision on feeding
Relative width of mouth	$\mathrm{WMO} = \frac{\text{width of mouth}}{\text{head length}}$	Directly proportional prey size or amount of prey per bite.
Relative length of gastrointestinal tract	$\mathrm{GIT} = \frac{\text{gastrointestinal length}}{\mathrm{SL}}$	Directly related to herbivory
Relative opening of olfactory fossae	$\mathrm{OFO} = \frac{\text{width of olfactory fossae}}{\text{head length}}$	Assumed here as directly related to find food using the sense of smell

Traits are subdivided into those mainly related to habitat use or trophic strategy

Simuliidae). The wet weight of each prey item from each stomach was determined with a digital electronic balance and recorded to the nearest 0.0001 g.

Stable isotopes

For isotopic analysis, 2 g of muscle tissue was taken from the dorsal flank region of fish specimens using a clean scalpel. Most tissue samples were stored frozen (*N* = 108), but a few were preserved in salt (*N* = 11), which has been shown to have negligible influence on carbon and nitrogen isotope ratios of fish muscle tissue (Arrington & Winemiller, 2002). Tissue samples were dried to constant weight in an air-circulating oven at 60°C, pulverized to a fine homogeneous powder, weighed, and packed into tin capsules according to methods described in Zeug & Winemiller (2008). The samples were analyzed for carbon and nitrogen stable isotope ratios at the Center for Stable Isotopes, University of New Mexico, using a Costech ECS 4010 Elemental Analyzer coupled to a ThermoFisher Scientific Delta V Advantage mass spectrometer via a CONFLO IV interface. Isotope ratios are reported using the delta (δ) notation relative to carbon from Pee Dee Belemnite as the standard for carbon and atmospheric molecular nitrogen as a standard for nitrogen. Routine analysis of laboratory standards indicated measurement error was less than 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Data analysis

Morphological functional space

To describe ecomorphological trends and to ordinate species and life stages, Principal Components Analysis (PCA) was performed using the correlation matrix calculated from log-transformed morphological data for species and ontogenetic stages (juveniles, adults). PCA was performed using the 'factoextra' package (Kassambara & Mundt, 2016) in R version 3.2.3 (R Development Core Team, 2017). Significance of morphological functional groups (i.e., species and life stages) was tested using Permutational Multivariate Analysis of Variance (PERMANOVA) made with 9999 permutations based on a Bray–Curtis distance matrix using the package 'vegan' 2.4-5 (Oksanen et al., 2017) in R.

Trophic niche

The dietary importance of food categories was estimated using the alimentary index: $\mathrm{Ai} = \mathrm{Fi} \times \mathrm{Wi} \big/ \sum_{i=1}^{n} (Fi \times Wi)$, where Fi is the relative frequency of occurrence of prey category *i* and Wi is the relative weight of prey category *i* (Kawakami & Vazzoler, 1980). Dietary similarity was calculated as Bray–Curtis similarity based on Ai values; similarity of food category consumption was based on Whittaker's index of association (Clarke & Gorley, 2015). Both similarity indexes consider relative abundances, but Whittaker's index is more sensitive to differences in distributions of abundances than Bray–Curtis (Legendre & Legendre, 2012). The level of specialization between life stages was inferred using Levin's measure of niche breadth (Krebs, 1999): $B = 1/\sum p_j^2$, where p_j is the proportion of individuals found using resource *j*. Niche partitioning among species and between life stages were assessed using Pianka's niche overlap index: $O_{\mathrm{jk}} = \sum_1^n p_{\mathrm{ij}} p_{\mathrm{ik}} \Big/ \sqrt{\sum_1^n p_{\mathrm{ij}^2} \sum_1^n p_{\mathrm{ik}^2}}$, which measures the niche overlap between species *j* and *k*, where p_{ij} is the proportion of the *i*th resource to the species *j*, p_{ik} is the proportion of *i*th resource to the species *k*, and *n* is the number of resource categories (Pianka, 1973). This metric ranges from zero (no overlap) to 1 (perfect overlap). The significance of niche overlap among groups (species and life stages) was tested by comparison with a null model based on the RA3 algorithm in the package EcoSimR v0.1.0 of Gotelli & Ellison (2013) with 9999 Monte Carlo randomizations. The RA3 algorithm maintains the niche breadth of each group and randomizes entries in the resource matrix by assuming all resources are used equally by all groups. To reveal dietary patterns among life stages and species, we performed Principal Coordinate Analysis (PCoA) using Ai values; significance of between-group dietary variation was tested using Permutational Multivariate Analysis of Variance (PERMANOVA), using 9999 permutations based on a Bray–Curtis dissimilarity matrix and the package 'vegan' 2.4-5 (Oksanen et al., 2017) in R.

Isotopic niche

Prior to numerical analysis, isotopic ratios of samples from different years, sites and preservation methods (i.e., frozen vs. salt) for each species and life stage were tested for potential isotopic differences using the Kruskal–Wallis test due to heteroscedasticity of the data. Given no significant differences, samples were retained in a merged dataset. Interspecific and ontogenetic partitioning of isotopic space was evaluated using the package Stable Isotope Bayesian Ellipses in R (SIBER) version 2.0.2, which estimates isotopic spaces and their overlap for groups (Jackson & Britton, 2014). The isotopic space occupied by each life stage of each species was estimated using sample-size-corrected standard ellipse areas ($\mathrm{SEA_C}$ ‰^{2}) (Jackson et al., 2011). This analysis infers that relationships based on isotopic space reflect trophic niche relationships (Layman et al., 2007). Lipid concentrations were lower than 5% (C:N ratios < 3.5 for aquatic animals); therefore, samples were analyzed without lipid correction (Skinner et al., 2016).

Trophic positions were estimated using the method proposed by Vanderklift & Ponsard (2003). Trophic position was calculated as $\mathrm{TP} = [(\delta^{15}\mathrm{N}_{fish} - \delta^{15}\mathrm{N}_{snail})/2.54] + 2$, where $\delta^{15}\mathrm{N}_{fish}$ was the average δ^{15}N for a particular life stage and species; $\delta^{15}\mathrm{N}_{snail}$ was the average δ^{15}N of a common primary consumer; 2.54 is the average enrichment in δ^{15}N per trophic level (Vanderklift & Ponsard, 2003), and 2 corresponds to the trophic level of the primary consumer. For the primary consumer, we used the soft tissues of *Doryssa starksi* (Baker, 1913), a freshwater snail common in the rapids. To avoid bias in TP outcomes inherent to different places or periods (Jepsen & Winemiller, 2007; Zaia Alves et al., 2017), *Doryssa starksi* were collected from the same rapids during the fish surveys, such that spatial or temporal variation in isotopic ratios should not bias TP estimates (Jepsen & Winemiller, 2007; Zaia Alves et al., 2017),

Results

Morphological traits

PCA showed that the six groups (three species, each with two life stages) occupied separate regions of morphological trait space (PERMANOVA, *Pseudo-F* = 190.2; $P < 0.001$). Juvenile and adult *Ossubtus* showed the greatest separation from other groups (Fig. 2). The first two principal components together modeled 57.6% of total morphological variation among life stages and species (Table 3). PC1 (37.6% of variance) identified a gradient contrasting fishes with relatively deep bodies, narrow caudal peduncles, small eyes, wide and subterminal mouths, small olfactory chambers and long gastrointestinal tracts versus fishes with the opposite suite of attributes. PC1 therefore involved both habitat-use and trophic associated traits, and separated adult *Ossubtus* from remaining groups, especially juvenile and adult *Myleus* that both have long gastrointestinal tracts and wide mouths. PC2 (20.0% of variance) identified a different gradient contrasting fishes with relatively small and shallow bodies, high pectoral fin aspect ratios, supraterminal mouth orientation, and long heads versus fishes with the opposite combination of traits (Table 3). PC2 separated juvenile *Ossubtus*, a group with a relatively high aspect ratio of the pectoral fin and a longer head, from the remaining serrasalmids (Fig. 2).

Diet

According to the alimentary index, juvenile and adult *Myleus* fed mostly on allochthonous plants (Ai = 51.8%, and Ai = 38.9%, respectively). Juvenile *Ossubtus* fed primarily on aquatic macroinvertebrates (Ai = 29.8%), whereas adult *Ossubtus* consumed Podostomaceae (Ai = 76.9%). Juvenile *Tometes* fed mostly on allochthonous plants (Ai = 26.5%), whereas the diet of adult *Tometes* was dominated by Podostomaceae (Ai = 62.2%; Supplementary Table S1). Whittaker's index of association revealed that the diet of adult *Ossubtus*, which was dominated by Podostemaceae (Appendix 1), was most differentiated from diets of other groups (Fig. 3). Juvenile *Ossubtus* fed mostly on macroinvertebrates (Appendix 1), but grouped with juvenile *Tometes* that fed mostly on allochthonous plant material (Fig. 3).

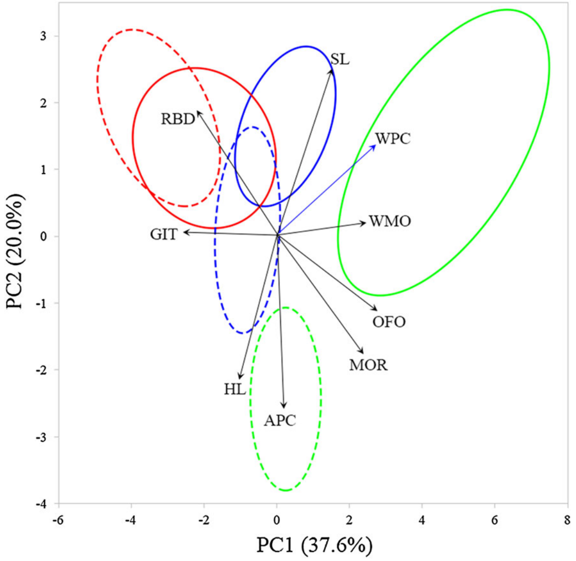

Fig. 2 Plot of principal components analysis axes 1 and 2 derived from analysis of 14 ecomorphological traits of juvenile (dashed lines) and adult (solid lines) *Myleus* (red ellipses), *Ossubtus* (green ellipses), and *Tometes* (blue ellipses) collected during the low-water period in lower Xingu. Ellipses represent 95% confidence intervals (individual data points not shown for clarity). High variable loadings on axes are indicated by vectors: *APC* aspect ratio of pectoral fin, *GIT* relative gastrointestinal tract length, *HL* relative head length, *MOR* mouth orientation, *OFO* relative olfactory fossae opening, *RBD* relative body depth, *SL* standard length, *WCP* relative width of caudal peduncle, *WMO* relative width of the mouth

The first two PCoA axes explained 66.7% of the total variance in diet (Fig. 4). High scores on PCo1 were associated with consumption of terrestrial plants, aquatic macroinvertebrates, periphyton, sediments, and seeds. High scores on PCo2 were associated with greater consumption of Podostemaceae, fins and scales (Table 4). PCoA revealed two groups, one corresponding to adult *Ossubtus*, and another formed by juveniles and adults of the other two species plus juvenile *Ossubtus* (Fig. 4). Juvenile *Ossubtus* overlapped with adults of *Myleus* and *Tometes*, whereas

Table 3 Principal components analysis dominant axis (PC 1, 2) scores derived from 14 functional morphological traits of three rapids-adapted

	PC1	PC2
Eigenvalue	5.26	2.79
% of variance	37.6	20.0
Morphological traits		
Habitat use		
RBD—relative body depth	0.29	− **0.34**
CPL—relative caudal-peduncle length	0.21	0.10
WCP—relative width of caudal peduncle	− **0.36**	− 0.25
APC—aspect ratio of pectoral fin	− 0.03	**0.47**
EPO—relative position of the eyes	− 0.23	0.04
SWB—relative swimbladder length	< 0.01	− 0.17
MOR—mouth orientation	− **0.31**	**0.33**
Trophic strategy		
SL—standard length	− 0.20	− **0.46**
HL—relative head length	0.14	**0.40**
HH—relative head height	− 0.11	0.14
SIE—relative eye size	− **0.40**	− 0.12
WMO—relative width of the mouth	**0.33**	− 0.03
GIT—relative gastrointestinal tract length	**0.34**	< 0.01
OFO—relative olfactory fossae opening	− **0.37**	0.21

Higher scores highlighted in bold

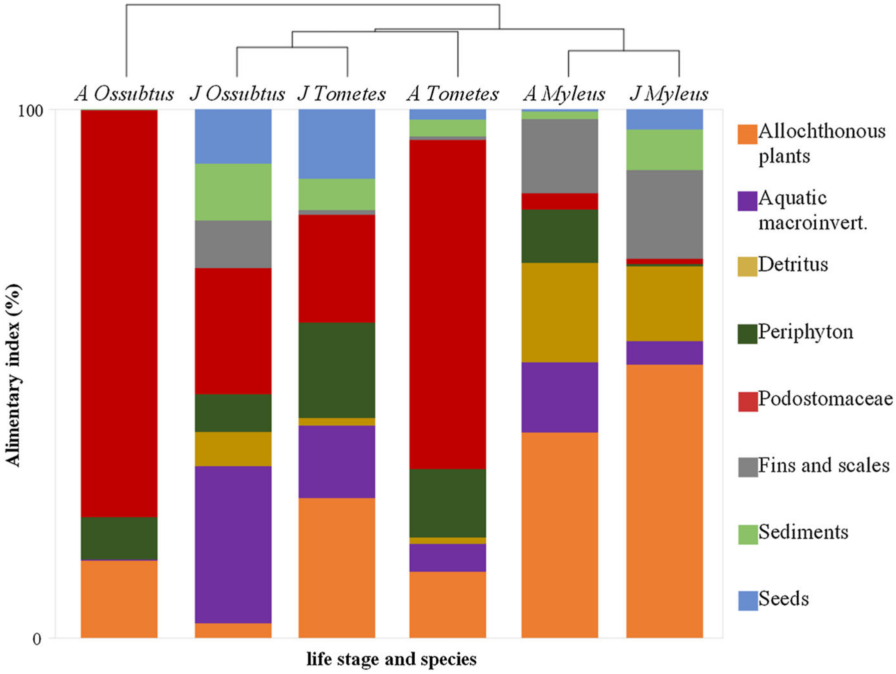

Fig. 3 Alimentary index (Ai%) of juvenile (J) and adult (A) *Myleus*, *Ossubtus*, and *Tometes* collected during the low-water period in lower Xingu. Cluster diagram shows the similarity of food category consumption based on Whittaker's index of association

juveniles of *Myleus* and *Tometes* were different (Fig. 4). PERMANOVA analysis confirmed significant dietary differentiation among the three species when both stages were combined (*Pseudo-F* = 35.09; $P < 0.001$), between the two ontogenetic stages within species (*Pseudo-F* = 13.21; $P < 0.001$), and

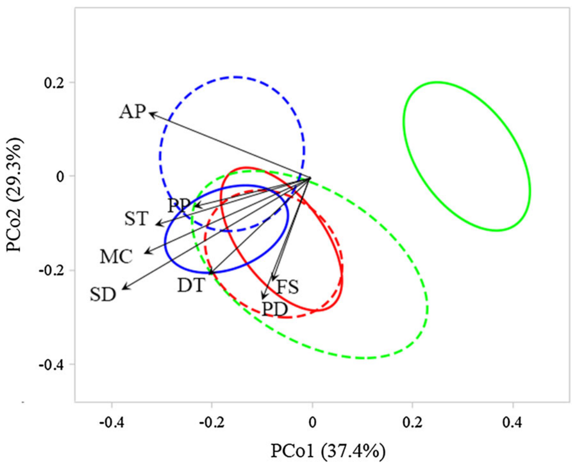

Fig. 4 Dietary niche of juvenile (dashed lines) and adult (solid lines) *Myleus* (red ellipses), *Ossubtus* (green ellipses), and *Tometes* (blue ellipses) collected in low-water period in lower Xingu River basin. Principal coordinate analysis, axis 1 and 2 derived from analysis of diet composition, *AP* allochthonous plants, *DT* detritus, *FS* fins and scales, *MC* macroinvertebrates, *PD* Podostemaceae, *PP* Periphyton, *SD* seeds, *ST* sediments. Ellipses represent 95% confidence intervals of juveniles (individual data points not shown for clarity)

Table 4 Principal coordinates (PCo) scores derived from eight prey items consumed by the three rapids-dwelling serrasalmids from the lower Xingu during the dry season

	PCo1	PCo2	r^2
Allochthonous plants	− **0.93**	0.37	0.22
Aquatic macroinvertebrates	− **0.90**	− 0.44	0.25
Detritus	− 0.70	− 0.71	0.15
Periphyton	− **0.97**	− 0.25	0.10
Podostemaceae	− 0.38	− **0.93**	0.12
Fins and scales	− 0.36	− **0.93**	0.10
Sediments	− **0.96**	− 0.29	0.18
Seeds	− **0.85**	− 0.53	0.33

Scores with highest loadings highlighted in bold, based on PERMANOVA test, both axes had $P < 0.0001$

among the six combinations of species and ontogenetic stages (*Pseudo-F* = 14.69; $P < 0.001$).

The largest ontogenetic shift in diet breath was observed for *Ossubtus*. Diet breadth of this species declined with body size (juveniles' $B = 6.58$; adults' $B = 1.78$). *Myleus* and *Tometes* had less variable diet breadth, with *Myleus* displaying lower values for juveniles ($B = 4.08$) than adults ($B = 4.84$) and *Tometes* displaying greater values for juveniles ($B = 6.02$) than adults ($B = 2.64$) (Supplementary Table S2).

Dietary overlap between groups was variable, ranging from 0.23 (between juvenile *Myleus* and adult *Ossubtus*) to 0.99 (between adults of *Ossubtus* and *Tometes*). *Myleus* and *Ossubtus* had low interspecific dietary overlap for both juveniles ($O_{ij} = 0.49$) and adults ($O_{ij} = 0.29$), whereas dietary overlap between *Ossubtus* and *Tometes* was high for juveniles ($O_{ij} = 0.83$) and adults ($O_{ij} = 0.99$) (Supplementary Table S2). Based on comparison with null model estimates, observed pairwise dietary niche overlap among all serrasalmid species and life stages was significantly higher than expected at random [observed mean value (0.60) > simulated value (0.51); $P < 0.05$].

Isotopic patterns

Stable isotope signatures of samples from different survey periods and sites were not significantly different for juvenile and adult *Myleus* (carbon: $H = 0.98$, $df = 2$, $P = 0.61$; nitrogen: $H = 1.80$, $df = 2$, $P = 0.41$), juvenile and adult *Tometes* (carbon: $H = 5.42$, $df = 4$, $P = 0.25$; nitrogen: $H = 4.78$, $df = 4$, $P = 0.31$), and adult *Ossubtus* (carbon: $H = 5.97$, $df = 4$, $P = 0.20$; nitrogen: $H = 7.59$, $df = 4$, $P = 0.11$). Juvenile *Ossubtus*, in contrast, showed significant differences for carbon ($H = 5.40$, $df = 1$, $P < 0.05$) between samples collected in September 2013 (two salted samples) and October 2015 (seven frozen samples). This difference for carbon was likely associated with inter-annual variation in isotopic ratios of food sources rather than preservation method; however, the two samples preserved in salt were excluded from subsequent analyses. Nitrogen isotopic ratios did not differ significantly among juvenile *Ossubtus* captured during different periods ($H = 1.67$, $df = 1$, $P = 0.20$). Carbon signatures had large overlap among species and ontogenetic stages (Table 5). In contrast, δ^{15}N was more differentiated among species and life stages (Fig. 5). δ^{15}N of juveniles and adults of *Myleus* and *Tometes* varied relatively little, whereas the range of values for *Ossubtus* differed considerably between juveniles and adults.

Juvenile and adult *Tometes* and *Myleus* had relatively large overlap in isotopic space, representing 42.9% of total ellipse area for *Tometes* and 45.7% of

Table 5 Range, mean, and standard deviations (SD) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), calculated trophic position (TP) according to Vanderklift & Ponsard (2003), and corrected standard ellipsis areas (SEAc) values for the three serrasalmid species from Xingu River basin

Species	Life stage	Range $\delta^{13}C$	Mean ± SD $\delta^{13}C$	Range $\delta^{15}N$	Mean ± SD $\delta^{15}N$	TP	SEAc
Myleus	J	− 33.0 to − 27.1	− 31.3 ± 2.3	9.8–10.6	10.1 ± 0.2	3.15	1.83
	A	− 33.7 to − 27.9	− 30.5 ± 1.8	9.5–11.2	10.4 ± 0.4	3.24	2.52
Ossubtus	J	− 26.3 to − 19.4	− 22.2 ± 2.7	9.4–9.9	9.7 ± 0.2	2.97	2.05
	A	− 32.9 to − 24.0	− 27.9 ± 2.2	9.6–12.7	11.1 ± 0.7	3.54	4.91
Tometes	J	− 36.2 to − 21.4	− 28.1 ± 3.7	8.8–10.4	9.5 ± 0.4	2.91	3.99
	A	− 32.6 to − 23.9	− 28.2 ± 2.5	8.9–9.9	9.4 ± 0.3	2.88	1.71

Classified according to the ontogeny into juveniles (J) and adults (A)

total ellipses area for *Myleus*. Juvenile *Tometes* occupied a broader isotopic space (SEA_C = 3.99‰) than adults (SEA_C = 1.71‰) (Table 5). *Myleus* occupied a relatively small isotopic space for both juveniles (SEA_C = 1.83‰) and adults (SEA_C = 2.52‰). Juvenile and adult *Ossubtus* had no isotopic overlap, and juveniles occupied a smaller isotopic space than adults (SEA_C; 2.05‰ versus 4.91‰, respectively; Table 5). Both juvenile and adult *Ossubtus* did not overlap with any group (Fig. 5). Based on $\delta^{15}N$ values, and assuming all else being equal with regard to isotopic signatures of basal resource of food chains supporting these consumers, trophic positions ranged from 2.88 for adult *Tometes* to 3.54 for adult *Ossubtus* (Table 5). Juvenile and adult *Tometes* had significantly different trophic positions ($H = 4.34$, $df = 5$, $P = 0.03$), with TP = 2.91 and 2.88, respectively. Juvenile and adult *Ossubtus* also had significantly different trophic positions ($H = 7.09$, $df = 5$, $P < 0.001$), with juveniles having the lowest TP (2.97), and adults having the highest among all species and life stages (TP = 3.54). *Myleus* had an intermediate position (juvenile TP = 3.15, adult TP = 3.24) without a significant difference between life stages ($H = 0.94$, $df = 5$, $P = 0.35$) (Table 5).

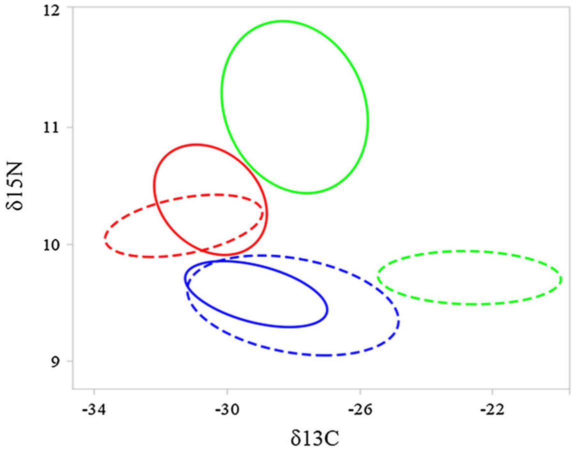

Fig. 5 Isotopic niches of juvenile (dashed lines) and adult (solid lines) *Myleus* (red ellipses), *Ossubtus* (green ellipses), and *Tometes* (blue ellipses) collected from rapids during the low-water period in lower Xingu. Standard ellipse areas based on axis 1 and 2 scores were estimated using Stable Isotope Bayesian Ellipses in R (Jackson et al., 2011). Ellipses represent 95% confidence intervals of juveniles (individual data points not shown for clarity)

Discussion

The herbivorous serrasalmids that coexist in rapids of the lower Xingu during the low-water period were well separated along gradients of morphological traits associated with feeding and swimming performance. Varying degrees of morphological differentiation were observed between juveniles and adults of each species. Morphological and trophic differentiation was greatest between *Ossubtus* and the other two species, and *Ossubtus* also revealed greatest ontogenetic differentiation, which may partially reflect differences in microhabitat use. *Ossubtus* seems to be more strictly confined to rapids habitats than *Myleus* or *Tometes* (Andrade et al., 2016c). Both juvenile and adult *Ossubtus* possess a subinferior mouth, as opposed to the terminal mouth position of *Myleus* and *Tometes*, as well as a shallower (more streamlined) body that should further reduce drag in fast-flowing water. Juvenile *Ossubtus* have higher values for the aspect ratio of the pectoral fin and lower values of body depth when compared with adults, suggesting that juveniles are particularly well adapted to inhabit fast-flowing water (Andrade et al., 2016c). The relatively narrow caudal peduncle of juvenile *Ossubtus* may further increase their swimming efficiency in fast water. *Myleus* and *Tometes* have relatively greater body depths and narrower caudal peduncles, which are less efficient for swimming in fast currents, but greatly enhance maneuverability. This body shape would be advantageous in rapids complexes that contain areas with slower water velocities where foraging can involve lateral movements without displacement from hydraulic resistance.

There were some discrepancies between dietary and isotopic patterns. Dietary overlap between life stages was high for both *Myleus* and *Tometes*. In contrast, *Ossubtus* had low dietary overlap between juveniles and adults, and the isotopic space occupied by juveniles also was different than the space occupied by adults. For *Myleus* and *Tometes*, the isotopic spaces of juveniles and adults overlapped extensively. If we assume that δ^{15}N accurately reflects vertical trophic position, *Tometes* in both life stages and juvenile *Ossubtus* occupied the lowest trophic positions. This inference contrasts with our dietary analysis that indicated *Tometes* and juvenile *Ossubtus* consumed the largest fractions of macroinvertebrates and therefore should occupy higher trophic positions. Based on δ^{15}N, *Myleus* occupied an intermediate trophic position and adult *Ossubtus* occupied the highest position among these three species, corroborating the expectation based on its shorter gut length. Conversely, based on dietary analysis, adult *Ossubtus* consumed mostly aquatic macrophytes, and therefore should occupy a low trophic position. This helps to understand the dietary difference among these species, but not variation in trophic position. Trophic position generally is negatively correlated with gut length in fishes (Wagner et al., 2009). Among frugivorous serrasalmids, those species with relatively shorter guts had higher trophic positions (Correa & Winemiller, 2014). Adult *Ossubtus* have a shorter relative gut length and higher estimated trophic position than *Myleus* and *Tometes*, as well as conspecific juveniles.

Differences in trophic position inferred from isotopic and dietary analysis could be due to variation in trophic fractionation values between species or ontogenetic stages. Some herbivores have been shown to have trophic fractionation values for δ^{15}N higher than 2.54, the value used for our study (Caut et al., 2009; German & Miles, 2010). This would result in an overestimate of trophic position for strictly herbivorous species and may explain the high trophic position found for adult *Ossubtus* despite the large amount of macrophytes found in their stomachs. Discrepancies between dietary and isotopic data also could result from temporal variation in δ^{15}N of aquatic macrophytes. Stable isotope ratios of muscle tissue reflect assimilation of material consumed over a timescale of several weeks to months (Vander Zanden et al., 2015), whereas dietary analysis represents a snapshot of food resources consumed by fish minutes before capture. The isotopic turnover rate is faster for white muscle tissue than other tissues such as scales or bones; however, complete muscle turnover can take up to three months (Busst & Britton, 2017). That means that the isotopic signature in muscle tissues of the evaluated fish species in our study should reflect the diet several weeks before the fish's capture. Hydraulic conditions of local habitats also might affect isotopic fractionation associated with plant physiological processes (Correa & Winemiller, 2014), such as material exchanges at the cell-water boundary. Thus, we cannot rule out that isotopic ratios of rapids-dwelling herbivores might reflect food resources consumed from a different location or perhaps even from the same location but under different flow conditions. Faster growth rates of juveniles (Vander Zanden et al., 2015) should result in faster isotopic turnover of juvenile tissues and better reflection of contemporary local conditions. Consequently, the isotopic ratios of adult *Ossubtus* might reflect assimilation of food consumed a few months prior when aquatic macrophytes had different isotopic ratios or alternative food resources were exploited. However, Zuluaga-Gómez et al. (2016), studying Xingu fishes, inferred that phytomicrobenthos was the most important basal source supporting biomass of rapids-dwelling serrasalmids. Zuluaga-Gómez et al. (2016) only studied adult fishes, and because phytomicrobenthos samples probably contain some combination of benthic algae and microfauna (Zeug & Winemiller, 2008), this source could partially account for the relatively high δ^{15}N of adult *Ossubtus*.

Despite the lack of congruence between dietary and isotopic results, both datasets revealed clear separation between *Ossubtus* and the other two serrasalmids, especially among adults. All three species feed within rapids, but not all food resources are autochthonous in origin, and terrestrial plant material and terrestrial arthropods can drift through these habitats. *Myleus* and *Tometes* displayed large dietary overlap, but relatively low overlap in isotopic space. Compared to *Ossubtus*, juveniles and adults of these species are trophic generalists with similar morphologies and diets. *Ossubtus* diet breadth was greater for juveniles than adults, but the isotopic niche space occupied by juveniles was smaller than that occupied by adults. In contrast, dietary and isotopic patterns were congruent for *Myleus* and *Tometes*, with adults and juveniles overlapping extensively in both dietary and isotopic space. Adult *Tometes* feed heavily on Podostemaceae, but do not appear to target this resource to the same degree as *Ossubtus*. Juvenile *Ossubtus* consumed mostly aquatic macroinvertebrates and Podostomaceae, whereas adults *Ossubtus* apparently avoid consuming aquatic invertebrates that use these plants as habitat.

Our findings suggest that food resource partitioning is not a major mechanism for coexistence of herbivorous serrasalmid fishes inhabiting rapids during the annual low-water period in the lower Xingu River. Some resources, such as Podostomaceae and aquatic insect larvae, are abundant in rapids during the low-water period. Interspecific dietary differences were most associated with the greater importance of Podostomaceae for adult *Ossubtus* and *Tometes* when compared to both juvenile and adult *Myleus* that consumed more allochthonous plants and relatively little Podostomaceae (Supplementary Table S1). Similarly, juvenile *Ossubtus* and *Tometes*, which coexist in rapids within macrophyte beds or behind rocks that provide hydraulic refugia (Andrade et al., 2016a, c), had broad diets, whereas juvenile *Ossubtus* fed mostly on aquatic macroinvertebrates (Supplementary Table S1). In addition, juvenile *Tometes* fed more heavily on allochthonous plants than juveniles of the other two species. Future research should examine how seasonal variation in resource quantity and quality influences the trophic ecology and habitat use patterns of these fishes. High discharge during the wet season creates harsh hydraulic conditions in rapids even for rheophilic species, and likely changes availability of various food resources and microhabitats that provide refuge from swift currents. Among the three species studied here, *Ossubtus* appears to be most specialized for living and feeding in rapids. High dietary overlap between *Myleus* and *Tometes* does not preclude niche partitioning along alternative niche dimensions, such as the exploitation of resources in different microhabitats either temporally, or spatially. Indeed, previous work on fish assemblages within rapids of the Xingu found patterns of functional diversity suggesting many species were adapted to exploit similar resources in a variety of microhabitats (Fitzgerald et al., 2017).

Interspecific morphological differences also seem to reflect variation in foraging behavior. The smaller eyes and larger olfactory chamber of *Ossubtus* suggest that vision may be less important than olfaction when searching for food. In teleost fishes, a larger olfactory chamber often is associated with a greater number of olfactory folds that enhance the sense of smell (Døving et al., 1977). Considering that the Xingu is a clearwater river with very low turbidity and good visibility, this characteristic of *Ossubtus* may be associated with nocturnal foraging, whereas *Myleus* and *Tometes* probably forage diurnally. Indeed, most specimens of *Ossubtus* were collected at night, while *Myleus* and *Tometes* were most frequently captured during daylight hours. These diel differences in foraging behavior may enhance niche segregation among species.

Microhabitat use by fishes has been shown to vary in response to natural flood pulses (Kluender et al., 2017), and hydrological modification has been found to shift basal resources supporting aquatic food webs and altering stable isotope ratios (Delong & Thoms, 2016). River impoundment results in longer food chains within the reservoir, which corresponds to more trophic transfers between basal resources and top predators (Hoeinghaus et al., 2008). Operation of the Belo Monte Hydroelectric Complex is impacting the hydrology and habitats in the lower Xingu River, and threatens populations of fishes and other aquatic organisms, especially those adapted to live in rapids (Sabaj Pérez, 2015; Fitzgerald et al., 2018). *Ossubtus* and *Tometes* are particularly vulnerable because they are endemic to the basin (Andrade et al., 2016c; Winemiller et al., 2016). Hydroelectric dams have been shown to impact the ecology of rapids in other South American rivers (Horeau et al., 1998), and it is likely that the ecology and population dynamics of serrasalmids and other rapids-dwelling species of the Xingu will change over the next several years. Future studies should analyze samples collected over an entire annual cycle as well as the same season across multiple years. More research is needed to examine spatial and temporal variation in the isotopic ratios of potential food resources and aquatic consumer taxa. In addition, laboratory experiments are needed to improve understanding of isotopic turnover rates of tissues of consumers and resources in rapids as well as fluvial habitats generally. In conclusion, herbivorous serrasalmids from rapids of the Lower Xingu River revealed ontogenetic dietary shifts and high trophic niche overlap during the dry season, suggesting that food resources may not be limiting or else these species feed within different microhabitats. In addition to providing some of the first ecological information from this poorly studied system, this study reinforces the need for approaches that analyze multiple data sources and spatiotemporal scales.

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Compliance with ethical standards

The study complied with approved institutional protocol for animal use in research TAMU AUP IACUC 2014-0234.

Conflict of interest The authors declare that they have no conflict of interest.

References

Andrade, M. C., T. Giarrizzo & M. Jégu, 2013. *Tometes camunani* (Characiformes: Serrasalmidae), a new species of phytophagous fish from the Guiana Shield, rio Trombetas basin, Brazil. Neotropical Ichthyology 11: 297–306.

Andrade, M., A. Jesus & T. Giarrizzo, 2015. Length-weight relationships and condition factor of the eaglebeak pacu *Ossubtus xinguense* Jégu, 1992 (Characiformes, Serrasalmidae), an endangered species from Rio Xingu rapids, northern Brazil. Brazilian Journal of Biology 75: S102–S105.

Andrade, M. C., M. Jégu & T. Giarrizzo, 2016a. *Tometes kranponhah* and *Tometes ancylorhynchus* (Characiformes: Serrasalmidae), two new phytophagous serrasalmids, and the first *Tometes* species described from the Brazilian Shield. Journal of Fish Biology 89: 467–494.

Andrade, M. C., M. Jégu, & T. Giarrizzo, 2016b. A new large species of *Myloplus* (Characiformes, Serrasalmidae) from the Rio Madeira basin, Brazil. Zookeys 153–167.

Andrade, M. C., L. M. Sousa, R. P. Ota, M. Jégu & T. Giarrizzo, 2016c. Redescription and geographical distribution of the endangered fish *Ossubtus xinguense* Jégu 1992 (Characiformes, Serrasalmidae) with comments on conservation of the rheophilic fauna of the Xingu River. PLoS ONE 11: e0161398.

Arrington, D. A. & K. O. Winemiller, 2002. Preservation effects on stable isotope analysis of fish muscle. Transactions of the American Fisheries Society 131: 337–342.

Birindelli, J. L. O. & H. A. Britski, 2013. Two new species of *Leporinus* (Characiformes: Anostomidae) from the Brazilian Amazon, and redescription of *Leporinus striatus* Kner 1858. Journal of Fish Biology 83: 1128–1160.

Burress, E. D., 2014. Cichlid fishes as models of ecological diversification: patterns, mechanisms, and consequences. Hydrobiologia 748: 7–27.

Busst, G. M. A., & J. R. Britton, 2017. Tissue-specific turnover rates of the nitrogen stable isotope as functions of time and growth in a cyprinid fish. Hydrobiologia, https://doi.org/10.1007/s10750-017-3276-2.

Carvalho, L. N., J. Zuanon, & I. Sazima, 2007. Natural history of amazon fishes. International Commission on Tropical Biology and Natural Resources 1–32.

Caut, S., E. Angulo & F. Courchamp, 2009. Variation in discrimination factors (Δ15 N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology 46: 443–453.

Chakrabarty, P., & W. L. Fink, 2011. Piranha 3D. By Alexandre Aja (director). Copeia 2011: 181.

Clarke, K. R., & R. N. Gorley, 2015. PRIMER v7: User Manual/Tutorial. Plymouth, 296.

Conway, K. W., N. K. Lujan, J. G. Lundberg, R. L. Mayden & D. S. Siegel, 2012. Microanatomy of the paired-fin pads of ostariophysan fishes (Teleostei: Ostariophysi). Journal of Morphology 273: 1127–1149.

Correa, S. B. & K. O. Winemiller, 2014. Niche partitioning among frugivorous fishes in response to fluctuating resources in the Amazonian floodplain forest. Ecology 95: 210–224.

Correa, S. B., K. O. Winemiller, H. López-Fernández & M. Galetti, 2007. Evolutionary perspectives on seed consumption and dispersal by fishes. BioScience 57: 748–756.

Correa, S. B., R. Betancur-R, B. de Mérona & J. W. Armbruster, 2014. Diet shift of red belly pacu *Piaractus brachypomus* (Cuvier, 1818) (Characiformes: Serrasalmidae), a Neotropical fish, in the Sepik-Ramu River Basin, Papua New Guinea. Neotropical Ichthyology 12: 827–833.

Correa, S. B., K. Winemiller & D. Cárdenas, 2016. Isotopic variation among Amazonian floodplain woody plants and implications for food-web research. Biota Neotropica 16: e20150078.

Delong, M. D. & M. C. Thoms, 2016. Changes in the trophic status of fish feeding guilds in response to flow modification. Journal of Geophysical Research: Biogeosciences 121: 949–964.

Dias, T. S. & C. B. Fialho, 2011. Comparative dietary analysis of *Eurycheilichthys pantherinus* and *Pareiorhaphis hystrix*: two Loricariidae species (Ostariophysi, Siluriformes) from Campos Sulinos biome, southern Brazil. Iheringia. Série Zoologia 101: 49–55.

Døving, K. B., M. Dubois-Dauphin, A. Holley & F. Jourdan, 1977. Functional anatomy of the olfactory organ of fish and the ciliary mechanism of water transport. Acta Zoologica 58: 245–255.

Fitzgerald, D. B., K. O. Winemiller, M. H. Sabaj Pérez & L. M. Sousa, 2017. Seasonal changes in the assembly mechanisms structuring tropical fish communities. Ecology 98: 21–31.

Fitzgerald, D. B., M. H. Sabaj-Pérez, L. M. Sousa, A. P. Gonçalves, L. R. Py-Daniel, N. K. Lujan, J. Zuanon, K. O. Winemiller & J. G. Lundberg, 2018. Diversity and community structure of rapids-dwelling fishes of the Xingu River: Implications for conservation amid large-scale hydroelectric development. Biological Conservation 222: 104–112.

Gatz, A. J., 1979. Ecological morphology of freshwater stream fishes. Tulane Studies in Zoology and Botany 21: 91–124.

Gatz, A. J., 1981. Morphologically inferred niche differentiation in stream fishes. The American Midland Naturalist 106: 10–21.

German, D. P. & R. D. Miles, 2010. Stable carbon and nitrogen incorporation in blood and fin tissue of the catfish *Pterygoplichthys disjunctivus*. Environemental Biology of Fishes 89: 117–133.

Gotelli, N. J., & A. M. Ellison, 2013. EcoSimR: null models for ecology, version 1.00, http://www.uvm.edu/~ngotelli/EcoSim/EcoSim.html.

Goulding, M., 1980. The fishes and the forest: Explorations in Amazonian Natural History. University of California Press, Berkeley.

Gracan, R., D. Zavodnik, P. Krstinic, B. Dragicevic & B. Lazar, 2016. Feeding ecology and trophic segregation of two sympatric mesopredatory sharks in the heavily exploited coastal ecosystem of the Adriatic Sea. Journal of Fish Biology 90: 1–18.

Hoeinghaus, D. J., K. O. Winemiller & A. A. Agostinho, 2008. Hydrogeomorphology and river impoundment affect food-chain length of diverse Neotropical food webs. Oikos 117: 984–995.

Horeau, V., P. Cerdan, A. Champeau & S. Richard, 1998. Importance of aquatic invertebrates in the diet of rapids-dwelling fish in the Sinnamary River, French Guiana. Journal of Tropical Ecology 14: 851–864.

IUCN, 2018. The IUCN Red List of Threatened Species. Version 2018-1. Downloaded on 7 July 2018.

Jackson, M. C. & J. R. Britton, 2014. Divergence in the trophic niche of sympatric freshwater invaders. Biological Invasions 16: 1095–1103.

Jackson, A. L., R. Inger, A. C. Parnell & S. Bearhop, 2011. Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80: 595–602.

Jégu, M. & P. Keith, 1999. Le bas Oyapock limite septentrionale ou simple etape dans la progression de la faune des poissons d'Amazonie occidentale. Comptes Rendus de l'Academie des Sciences 322: 1133–1143.

Jégu, M. & G. M. dos Santos, 2002. Révision du statut de *Myleus setiger* Müller & Troschel, 1844 et de *Myleus knerii* (Teleostei: Characidae: Serrasalminae) avec une description complémentaire des deux espèces. Cybium 26: 33–57.

Jégu, M. & J. Zuanon, 2005. Threatened fishes of the world: *Ossubtus xinguense* (Jégu 1992) (Characidae: Serrasalminae). Environmental Biology of Fishes 73: 414.

Jégu, M., G. M. dos Santos & E. Ferreira, 1989. Une nouvelle espèce du genre *Mylesinus* (Pisces, Serrasalmidae), *M. paraschomburgkii*, décrite des bassins du Trombetas et du Uatumã (Brésil, Amazonie). Revue d'Hydrobiologie Tropicale 22: 49–62.

Jégu, M., P. Keith, & E. Belmont-Jégu, 2002. Une nouvelle espèce de *Tometes* (Teleostei: Characidae: Serrasalminae) du bouclier guyanais, *Tometes lebaili* n. sp. Bulletin Francais De La Peche Et De La Pisciculture 23–48.

Jégu, M., P. Keith & P. Y. Le Bail, 2003. *Myloplus planquettei* sp. n. (Teleostei, Characidae), une nouvelle espèce de grand Serrasalminae phytophage du bouclier guyanais. Revue Suisse De Zoologie 110: 833–853.

Jepsen, D. B. & K. O. Winemiller, 2007. Basin geochemistry and isotopic ratios of fishes and basal production sources in four neotropical rivers. Ecology of Freshwater Fish 16: 267–281.

Junk, W. J. & M. G. M. Soares, 2001. Freshwater fish habitats in Amazonia: state of knowledge, management, and protection. Aquatic Ecosystem Health & Management 4: 437–451.

Kassambara, A., & F. Mundt, 2016. Package "factoextra". R package version 1.0.3, http://www.sthda.com/english/rpkgs/factoextra.

Kawakami, E. & G. Vazzoler, 1980. Método gráfico e estimativa de índice alimentar aplicado no estudo de alimentação de peixes. Brazilian Journal of Oceanography 29: 205–207.

Kluender, E. R., R. Adams & L. Lewis, 2017. Seasonal habitat use of alligator gar in a river-floodplain ecosystem at multiple spatial scales. Ecology of Freshwater Fish 26: 233–246.

Krebs, C. J., 1999. Ecological methodology. Benjamin Cummings, New York.

Layman, C. A., D. A. Arrington, C. G. Montaña & D. M. Post, 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88: 42–48.

Legendre, P. & L. Legendre, 2012. Numerical ecology. Elsevier Science BV, Amsterdam.

Leitão, R. P., J. I. Sánchez-Botero, D. Kasper, V. Trivério-Cardoso, C. M. Araújo, J. Zuanon & É. P. Caramaschi, 2015. Microhabitat segregation and fine ecomorphological dissimilarity between two closely phylogenetically related grazer fishes in an Atlantic Forest stream, Brazil. Environmental Biology of Fishes 98: 2009–2019.

Leite, R. G. & M. Jégu, 1990. Régime alimentaire de deux espèces d'*Acnodon* (Characiformes, Serrasalmidae) et habitudes lépidophages de *A. normani*. Cybium 14: 353–360.

Lincoln, R. J., G. A. Boxshall & P. F. Clark, 1985. A dictionary of ecology, evolution, and systematics. Cambridge University Press, New York.

Loubens, G. & J. Panfili, 1997. Biologie de *Colossoma macropomum* (Teleostei: Serrasalmidae) dans le bassin du Mamoré (Amazonie bolivienne). Ichthyological Exploration of Freshwaters 8: 1–22.

Loubens, G. & J. Panfili, 2001. Biologie de *Piaractus brachypomus* (Teleostei: Serrasalmidae) dans le bassin du Mamoré (Amazonie bolivienne). Ichthyological Exploration of Freshwaters 12: 51–64.

Lowe-McConnell, R. H., 1987. Ecological studies in tropical fish communities. Cambridge University Press, Cambridge.

Lujan, N. K. & K. W. Conway, 2015. Life in the fast lane: a review of rheophily in freshwater fishes. In Riesch, R., M. Tobler & M. Plath (eds), Extremophile Fishes: Ecology, Evolution, and Physiology of Teleosts in Extreme Environments. Springer, Cham: 107–136.

Lujan, N. K., K. O. Winemiller & J. W. Armbruster, 2012. Trophic diversity in the evolution and community assembly of loricariid catfishes. BMC Evolutionary Biology 12: e124.

Meunier, F., Y. Fermon, M. Jégu & P. Keith, 2004. Les piranhas et les kumaru: diversité et biologie. In Meunier, F. (ed.), Piranhas Enivrés. Des poissons et des Hommes en Guyane, SFI/RMN: 35–41.

Montaña, C. G. & K. O. Winemiller, 2013. Evolutionary convergence in Neotropical cichlids and Nearctic centrarchids: Evidence from morphology, diet, and stable isotope analysis. Biological Journal of the Linnean Society 109: 146–164.

Montaña, C. G., K. O. Winemiller & A. Sutton, 2014. Intercontinental comparison of fish ecomorphology: null model tests of community assembly at the patch scale in rivers. Ecological Monographs 84: 91–107.

Moreira, S. S. & J. Zuanon, 2002. Dieta de *Retroculus lapidifer* (Perciformes: Cichlidae), um peixe reofílico do Rio Araguaia, estado do Tocantins, Brasil. Acta Amazonica 32: 691–705.

Mouchet, M. A., M. D. M. Burns, A. M. Garcia, J. P. Vieira & D. Mouillot, 2013. Invariant scaling relationship between functional dissimilarity and co-occurrence in fish assemblages of the Patos Lagoon estuary (Brazil): Environmental filtering consistently overshadows competitive exclusion. Oikos 122: 247–257.

National Red List, 2016. Brazilian Red List, http://www.nationalredlist.org. Accessed 25 July 2018.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, & H. Wagner, 2017. Package "vegan". Community Ecology Package Version 2.4-5. http://cran.r-project.org/web/packages/vegan.

Pagezy, H., & M. Jégu, 2002. Valeur patrimoniale de Serrasalminae herbivores du haut Maroni (Guyane Française): approches biologique et socioculturelle en pays Wayana. Bulletin Francais de la Peche et de la Pisciculture 49–69.

Pianka, E. R., 1973. The structure of lizard communities. Annual Review of Ecology and Systematics 4: 53–74.

R Development Core Team, 2017. R: A Language and Environment for Statistical Computing, http://www.r-project.org/.

Ross, S. T., 1986. Resource partitioning in fish assemblages: a review of field studies. Copeia 1986: 352–388.

Sabaj Pérez, M. H., 2015. Where the Xingu bends and will soon break. American Scientist 103: 395–397.

Santos, G. M., S. S. Pinto & M. Jégu, 1997. Alimentação do pacu-cana, *Mylesinus paraschomburgkii* (Teleostei, Serrasalmidae) em rios da Amazônia brasileira. Revista Brasileira de Biologia 57: 311–315.

Sazima, I., 1983. Scale-eating in characoids and other fishes. Environmental Biology of Fishes 9: 87–101.

Sazima, I. & F. A. Machado, 1990. Underwater observations of piranhas in western Brazil. Environmental Biology of Fishes 28: 17–31.

Skinner, M. M., A. A. Martin & B. C. Moore, 2016. Is lipid correction necessary in the stable isotope analysis of fish tissues? Rapid Communications in Mass Spectrometry 30: 881–889.

Stewart, D. J. & T. R. Roberts, 1976. An ecological and systematic survey of fishes in the rapids of the lower Zaïre or Congo River. Bulletin of the Museum of Comparative Zoology 147: 239–317.

Tófoli, R. M., R. M. Dias, G. H. Zaia Alves, D. J. Hoeinghaus, L. C. Gomes, M. T. Baumgartner & A. A. Agostinho, 2017. Gold at what cost? Another megaproject threatens biodiversity in the Amazon. Perspectives in Ecology and Conservation 15: 129–131.

Trindade, M. E. D. & R. Juca-Chagas, 2008. Diet of two serrasalmin species, *Pygocentrus piraya* and *Serrasalmus brandtii* (Teleostei: Characidae), along a stretch of the rio de Contas, Bahia, Brazil. Neotropical Ichthyology 6: 645–650.

Vander Zanden, M. J., M. K. Clayton, E. K. Moody, C. T. Solomon & B. C. Weidel, 2015. Stable isotope turnover and half-life in animal tissues: a literature synthesis. PLoS ONE 10: e0116182.

Vanderklift, M. A. & S. Ponsard, 2003. Sources of variation in consumer-diet δ15 N enrichment: A meta-analysis. Oecologia 136: 169–182.

Verwaijen, D., R. Van Damme & A. Herrel, 2002. Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. Functional Ecology 16: 842–850.

Vitorino Júnior, O. B., C. S. Agostinho & F. M. Pelicice, 2016. Ecology of *Mylesinus paucisquamatus* Jégu & Santos, 1988, an endangered fish species from the rio Tocantins basin. Neotropical Ichthyology 14: e150124.

Wagner, C. E., P. B. McIntyre, K. S. Buels, D. M. Gilbert & E. Michel, 2009. Diet predicts intestine length in Lake Tanganyika's cichlid fishes. Functional Ecology 23: 1122–1131.

Winemiller, K. O., 1991. Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions. Ecological Monographs 61: 343–365.

Winemiller, K. O., P. B. McIntyre, L. Castello, E. Fluet-Chouinard, T. Giarrizzo, S. Nam, I. G. Baird, W. Darwall, N. K. Lujan, I. Harrison, M. L. J. Stiassny, R. A. M.

Silvano, D. B. Fitzgerald, F. M. Pelicice, A. A. Agostinho, L. C. Gomes, J. S. Albert, E. Baran, J. Petrere M., C. Zarfl, M. Mulligan, J. P. Sullivan, C. C. Arantes, L. M. Sousa, A. A. Koning, D. J. Hoeinghaus, M. Sabaj, J. G. Lundberg, J. Armbruster, M. L. Thieme, P. Petry, J. Zuanon, G. T. Vilara, J. Snoeks, C. Ou, W. Rainboth, C. S. Pavanelli, A. Akama, A. van Soesbergen, & L. Saenz, 2016. Balancing hydropower and biodiversity in the Amazon, Congo, and Mekong. Science 351:128–129.

Zaia Alves, G. H., D. J. Hoeinghaus, G. I. Manetta & E. Benedito, 2017. Dry season limnological conditions and basin geology exhibit complex relationships with $\delta^{13}C$ and $\delta^{15}N$ of carbon sources in four Neotropical floodplains. PLoS ONE 12: e0174499.

Zeug, S. C. & K. O. Winemiller, 2008. Evidence supporting the importance of terrestrial carbon in a large-river food web. Ecology 89: 1733–1743.

Zuanon, J. & I. Sazima, 2002. *Teleocichla centisquama*, a new species of rapids-dwelling cichlid from Xingu River, Amazonia (Perciformes: Cichlidae). Ichthyological Exploration of Freshwaters 13: 373–378.

Zuluaga-Gómez, M. A., D. B. Fitzgerald, T. Giarrizzo & K. O. Winemiller, 2016. Morphologic and trophic diversity of fish assemblages in rapids of the Xingu River, a major Amazon tributary and region of endemism. Environmental Biology of Fishes 99: 647–658.