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Morphological divergences as drivers of diet segregation between two sympatric species of *Serrapinnus* (Characidae: Cheirodontinae) in macrophyte stands in a neotropical floodplain lake

Correspondence:
Bruna Caroline Kotz Kliemann
bruna.kliemann@unesp.br

Bruna Caroline Kotz Kliemann[1], Eliana Maria Galdioli[2],
Andréa Bialetzki[2,3] and Rosilene Luciana Delariva[4]

Diet and morphology of *Serrapinnus notomelas* and *Serrapinnus* sp.1 were investigated across ontogeny, as a way to elucidate the key elements linked to the resource partitioning (a main driver for species coexistence). Fish sampling was conducted monthly between October 2010 and March 2012. Individuals were captured, identified, and classified into juvenile or adult. Our results show ontogenetic and interspecific differences in feeding abilities and morphological traits. Differences in body shape (relative area of the dorsal fin, length of head, height of the caudal peduncle, the aspect ratio of the pectoral and pelvic fin) favored divergent swimming performances (more maneuverability in *S. notomelas* and continuous swimming to *Serrapinnus* sp.1). We also observed divergences in trophic apparatus traits and correlations with different diets. In this context, it is highlighted that understanding the relationship between morphology and diet can assist in elucidating the processes that permeate the coexistence between sympatric species, and between ontogenetic periods. Besides, the relevant contribution of the measures of the trophic apparatus (gill raker length, the number of teeth cuspids, and intestinal coefficient) in trophic segregation seems to be a strong evidence in favor of the proposed discriminatory and predictive capacities of these traits.

Keywords: Diet, Ecomorphology, Interspecific variability, Ontogeny, Trophic apparatus.

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[1] Universidade Estadual Paulista (UNESP), Instituto de Biociências, Campus Botucatu, Programa de Pós-graduação em Ciências Biológicas/Zoologia. Laboratório de Ecologia de Peixes, Rua Monção, 226, Zona Norte, 15385-000 Ilha Solteira, SP, Brazil. (BCKK) bruna.kliemann@unesp.br (corresponding author).

[2] Laboratório de Ictioplâncton-Núcleo de Pesquisas em Limnologia Ictiologia e Aquicultura (Nupélia), Centro de Ciências Biológicas (CCB), Universidade Estadual de Maringá (UEM), Avenida Colombo, 5790, 87020-900 Maringá, PR, Brazil. (AB) bialetzki@nupelia.uem.br; (EMG) emgaldioli@uem.br.

[3] Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais (PEA), Departamento de Biologia, CCB, UEM. Avenida Colombo, 5790, 87020-900 Maringá, PR, Brazil.

[4] Universidade Estadual do Oeste do Paraná, Lieb – Laboratório de Ictiologia, Ecologia e Biomonitoramento. Programa de Pós-Graduação em Conservação e Manejo de Recursos Naturais, Rua Universitária, 2069, 85819-110 Cascavel, PR, Brazil. (RLD) rosilene.delariva@unioeste.br.

Dieta e morfologia de *Serrapinnus notomelas* e *Serrapinnus* sp.1 foram investigadas ao longo da ontogenia, como forma de elucidar os principais elementos ligados à partição de recursos (principal fator para a coexistência entre espécies). Foram realizadas coletas de peixes mensalmente entre outubro de 2010 e março de 2012. Os indivíduos foram capturados, identificados e classificados em juvenis ou adultos. Nossos resultados mostram diferenças ontogenéticas e interespecíficas na alimentação e características morfológicas. Diferenças na forma corporal (área da nadadeira dorsal, comprimento da cabeça, altura do pedúnculo caudal, proporção das nadadeiras peitorais e pélvicas) favoreceram desempenhos de natação divergentes (maior manobrabilidade para *S. notomelas* e natação contínua para *Serrapinnus* sp.1). Também observamos divergências nas características do aparato trófico e correlações com diferentes dietas. Nesse contexto, destaca-se que compreender a relação entre morfologia e dieta pode auxiliar na elucidação dos processos que permeiam a coexistência entre espécies simpátricas e entre períodos ontogenéticos. Além disso, a relevante contribuição das medidas do aparato trófico (número de cúspide nos dentes, rastros branquiais e coeficiente intestinal) na segregação trófica parece ser uma forte evidência a favor das propostas de capacidades discriminatórias e preditivas dessas características.

Palavras-chave: Aparato trófico, Dieta, Ecomorfologia, Ontogenia, Variabilidade interespecífica.

INTRODUCTION

Macrophyte stands in floodplain areas have been suggested as essential for the biological diversity maintenance, since they provide structurally complex habitats (Agostinho *et al*., 2007; Dibble, Thomaz, 2009; Yofukuji *et al*., 2021). These environments offer favorable abiotic conditions such as lower light and temperature, acting as food sources and refuge against predators (Colares *et al*., 2013; Cunha *et al*., 2019; Yofukuji *et al*., 2021). Besides, they offer substrates that are suitable for spawning and foraging, thus supporting a greater diversity of organisms (Delariva *et al*., 1994; Quirino *et al*., 2015; Yofukuji *et al*., 2021). In addition to species that temporarily use macrophyte stands, such as larvae and juveniles of large species, smaller-sized species with a short life cycle live in these environments (Prado *et al*., 2016). These smaller-sized species that inhabit the macrophyte stands are residents, dependent and influenced by the dynamics provided by the macrophyte stands, including possible interspecific interactions (*i.e*., competition for trophic resource) (Cunha *et al*., 2019; Yofukuji *et al*., 2021). In this regard, the complexity of the conditions and resources noted in macrophytes acts as robust environmental filters influencing the selection of traits adjusted to the demands of food and shelter over time (Prado *et al*., 2016; Cunha *et al*., 2019; Quirino *et al*., 2021).

According to the classical niche theory, the effects of competition can be reduced and the coexistence can be favored when the differentiation occurs in, at least, one dimension of the niche (Hutchinson, 1961; Leray *et al*., 2019). Spatial, temporal, and trophic dimensions are considered predominant factors in the structure of the communities, with food being the most important for fish, since it modulates basal activity levels (Schoener, 1974; Ross, 1986; Gerking, 1994; Dehnhard *et al*., 2020). Thereby, partitioning food resources is seen as a mediator process that decreases the probability of local extinction of the inferior competitor while enables long-term species' coexistence (Moncayo-Estrada *et al*., 2011; Walker *et al*., 2013).

Resource partitioning is shaped by distinct evolutionary processes. The morphology and ecological opportunities are interpolated and underlying pathways (Alexander, 1967; Chase, Leibold, 2003; Silva *et al*., 2016; Neves *et al*., 2021). More conservative intrinsic aspects, such as morphological traits, shape and constrain an organism's ability to perform tasks related to resource use (Carroll, 2001). The body shape reveals a lot about the locomotion performance, which, in a way, is the basis of how fishes can access food resources (Gatz, 1979; Gerking, 1994). Some studies have indicated that the body morphology reflects differences in swimming performances, such as continuous swimming, acceleration, and maneuverability (Winemiller, 1991; Casatti, Castro, 2006). It also reflects the net energy obtained from an incorporated food source as well as the best performance in the capture, cost and efficiency in the selection, digestion, and absorption (Gerking, 1994). In this sense, aspects of the trophic apparatus - such as the number of teeth cuspids, number and length of gill rakers, and intestine length - can be excellent indicators in elucidating dietary preferences (Bonato *et al*., 2017; Ohara *et al*., 2017; Delariva, Neves, 2020; Nascimento *et al*., 2020). Despite such a strong theoretical background (Winemiller, 1991; Carroll, 2001; Casatti, Castro, 2006), studies showing a direct link between the diet divergence and specific morphological adaptations are still scarce. Then, the combined use of morphological traits can be a robust proxy for determining potential divergent uses of habitat and obtaining of food in structured habitats, such as macrophyte stands.

Differentiation in the foraging niche can also occur at the expense of changes in morphology during the ontogenetic development, especially when it is derived from allometry (Gerking, 1994). In fact, morphological traits can shape the diet through their influence on the feeding capability of a fish (Lukoschek, McCormick, 2001; Sánchez-Hernández, Cobo, 2016). Such relationships have been largely documented for most fish clades (Winemiller, 1991; Willis *et al*., 2005). For example, larger head and mouth and longer fins allow the consumption of larger food items and foraging over long distances, respectively (Winemiller, 1991; Willis *et al*., 2005). Smaller food items, such as phytoplankton, zooplankton, and insects, are consumed in the early stages of life cycles or by smaller size classes (Neves *et al*., 2015; Kliemann *et al*., 2019; Sánchez-Hernández *et al*., 2019). Besides, it has been empirically well established that different impositions of energy requirements between juveniles and adults can determine changes in the diet throughout the development (Gerking, 1994; Keppeler *et al*., 2015). Therefore, changes in the foraging capacity and consequent food selectivity occur in a way that corresponds to functional expectations throughout the life cycle (Keppeler *et al*., 2015).

In order to provide support for the niche divergence hypothesis, studying the ontogenetic differentiation of sympatric and congeneric species is especially useful. *Serrapinnus notomelas* (Eigenmann, 1915) and *Serrapinnus* sp.1 are the most abundant species in isolated lakes of the upper Paraná River (Delariva *et al*., 1994; Piana *et al*., 2006). Furthermore, these small fishes are residents of the macrophyte stands (Pelicice *et al*., 2005; Quirino *et al*., 2018). Despite several studies on diet and types of preferential macrophytes used by *Serrapinnus* species in the upper Paraná River floodplain (Pelicice *et al*., 2005; Piana *et al*., 2006; Alves *et al*., 2011; Carniatto *et al*., 2012; Quirino *et al*., 2015, 2018), information on the morphological traits and their implications for the swimming performance (*sensu* Casatti, Castro, 2006) and the consequent shaping of the genus feeding is lacking.

Here, we focus on the diet and morphology of *S. notomelas* and *Serrapinnus* sp.1 variation across ontogeny, to elucidate the key elements linked to the resource partitioning and, therefore, understand the main driver of the coexistence of these species in macrophyte stands. Specifically, we tested whether: i) There are differences in the diet and in the trophic niche breadth between species and ontogeny; ii) Differences in ecomorphological indices related to swimming occur between species and ontogeny; and iii) Trophic apparatus (gill raker length, the number of teeth cuspids, and intestinal coefficient) differs between species. Since according to the classic niche theory, the variation in a niche dimension can avoid competition and favor coexistence (Hutchinson, 1961), we predicted that these species should differ in both morphology and diet during ontogeny. We expect to find differences in ecomorphological indices related to fin areas, body height and peduncle. When divergent, these morphological traits reflect in a greater efficiency in continuous swimming or maneuverability. They are also key indicators for the access of the fish to prey in macrophyte stands. We also hope to find differences in the trophic apparatus, especially those traits related to the acquisition (number of teeth cuspids and gill rakers) and the absorption of food (intestine length). Correlations between combined morphological traits and the diet can primarily reveal the microhabitat occupation by individuals (juveniles and adults) and species.

MATERIAL AND METHODS

Study area. This study was conducted in one lake permanently connected to Ivinheima River, a tributary of the upper Paraná River floodplain (Fig. 1). The Finado Raimundo Lake (22°47'40"S 53°32'14"W) is shallow (3 m on average) and has approximately 3,700 m length and 400 m width. The lake presents extensive macrophyte stands colonizing its littoral region. *Eichhornia azurea*, a floating aquatic macrophyte, is the predominant species, and *Eichhornia crassipes*, less abundant, is a free-floating species like the first concerning morphology. *Polygonum* spp., an emergent macrophyte also presents a great abundance.

The connectivity of the lake with the main river channel promotes environments with fluctuations in the water level, which favors the establishment of macrophytes with high and intermediate complexity (Thomaz *et al*., 2009). These, in turn, are more stable habitats and present higher density and richness of fish species, including species of the genus *Serrapinnus* (Dias *et al*., 2017a).

Sampling. Fish sampling was conducted monthly between October 2010 and March 2012, however only in July, August, and September 2011 (dry season, Quirino *et al*., 2018) was possible to observe the co-occurrence of the two species of *Serrapinnus* selected for this study in the macrophyte stands, with a similar number of individuals for each group (juveniles and adults) (Fig. 2).

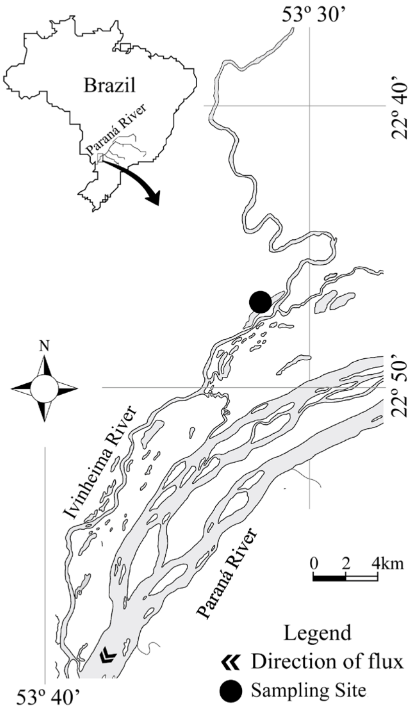


FIGURE 1 | Study area: location of sampling site in the upper Paraná River floodplain, Mato Grosso do Sul State, Brazil.

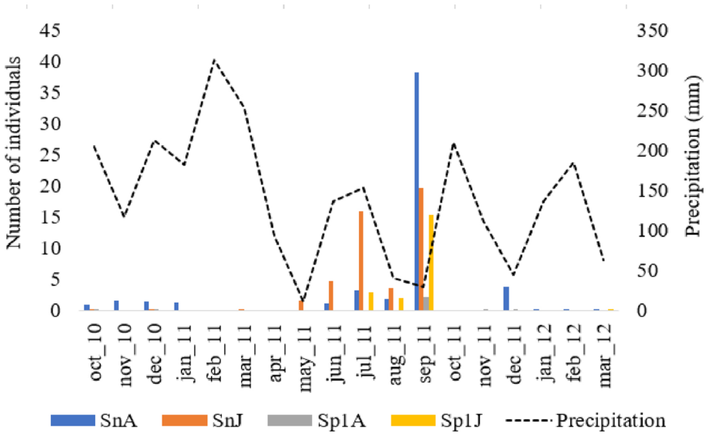


FIGURE 2 | Number of individuals from *Serrapinnus notomelas* and *Serrapinnus* sp.1 sampled concerning precipitation (mm) between October/2010 and March/2012 in a lake in the upper Paraná River floodplain, Brazil. SnA = *S. notomelas* Adult; SnJ = *S. notomelas* juvenile; Sp1A = *Serrapinnus* sp.1 adult; Sp1J = *Serrapinnus* sp.1 juvenile.

In the Finado Raimundo Lake, three macrophyte stands about two meters long were selected randomly after visual inspection of the coastal region. For each stand, a slow and cautious approach was performed so as not to scare off fish (active sampling) and using a sieve (dimensions = 1.5 mx 1.0 m; mesh size = 0.5 mm; Nakatani *et al*., 2001). It was pushed under the macrophyte stands and swiftly lifted to the surface to trap fish, resulting in three samples/month (one each stand). The juveniles and adults caught (total number/month) were euthanized with clove oil and fixed in 4% formaldehyde buffered with calcium carbonate. The project was approved by the Committee for Animal Ethics and Experimental at Universidade Estadual de Maringá (UEM) under protocol 123/2010. License for collection nº 09/2005 (IMAP – Instituto de Meio Ambiente Pantanal, N° 23/304974/2002).

Laboratory procedures. The individuals were identified according to Ota *et al*. (2018) and the confirmation of the identity of the specimens collected was made by a specialist in the group (Dr. Carla Simone Pavanelli, Nupélia/UEM), including the distinction of *Serrapinnus* sp.1 from the other species. The individuals were classified in juvenile or adult (Fuiman, Werner, 2002) according to the size of the first maturation, and this value was obtained in Vazzoler (1996). Voucher specimens were deposited in the fish collection Nupélia/UEM (*S. notomelas*, NUP 11249 and NUP 4173; *Serrapinnus* sp.1, NUP 7571).

Diet. The stomach contents of 121 individuals were analyzed, under a stereomicroscope and optical microscope, to identify food items consumed by individuals. The food items were quantified using the volumetric method (displacement of each food item from the stomach contents that is measured, usually on some type of graduated measuring device) (Hyslop, 1980). In a gridded Petri dish, the food items were compressed with glass slides until 1 mm height. The number of quadrants occupied by each food item on the dish was multiplied by 0.001 to obtain volumes in milliliters as proposed by Hellawell, Abel (1971). Food items were identified to the lowest possible taxonomic level according to the literature (Bicudo, Bicudo, 1970; Mugnai *et al*., 2010).

Morphology. Thirty individuals of each species and development period (juvenile and adult) were used for morphology, except when the total number of individuals was less (*i.e*., when the total number of individuals was less than 30, we used the number of individuals available). To evaluate the body morphology, we performed 27 linear morphometric measurements and areas related to the trunk, fin, head, eye, and mouth. Morphological variables were measured, whenever possible, on the left side of specimens. Linear morphometric measurements were obtained with the aid of a digital caliper (precision of 0.01 mm) while the areas were calculated in the AutoCAD 2015 software. The fins and eyes were drawn in plastic material, digitized, and inserted into the program (https://www.autodesk.com/education/free-software/featured, accessed February 27, 2019).

From the linear morphological measurements and the measured areas, 16 ecomorphological indices were calculated. These indices indicate the habitat use, locomotion, and foraging and are relevant because they minimize the effect of body size, focusing mainly on body shape and structures (Winemiller, 1991). Additionally, we measured traits related to the trophic apparatus, whose characteristics can be considered a proxy for the performance of food capture and processing: number of teeth cuspids, gill rakers, and intestine length. The number of teeth cuspids was counted for each specimen with the assistance of a stereoscopic microscope. The first left gill arch was carefully extracted from everyone and the length of the gill rakers was measured with the support of an optical microscope. The digestive tract was dissected, and the intestinal length was measured using a digital caliper (accuracy: 0.01 mm).

Morphological traits (ecomorphological indices and trophic apparatus) information is provided in **S1**.

Data analysis. The volume (ml) matrix of the food items consumed was used for the analysis of diet data. To verify differences in diet between species and ontogeny (juvenile and adult) was used the Permutational Multivariate Analysis of Variance (PERMANOVA). This analysis using the Bray-Curtis dissimilarity index, with 9,999 random permutations (Anderson, 2001). Additionally, the Similarity Percentage Method (SIMPER) was used to verify which food items contributed to the difference observed between species (*S. notomelas* and *Serrapinnus* sp.1) and ontogeny (juvenile and adult). All possible pairs of samples were compared using the Bray-Curtis measure (Clarke, 1993).

To test for differences in the trophic niche between ontogeny (juvenile and adult) and between the species (*S. notomelas* and *Serrapinnus* sp.1), the trophic niche breadth was calculated using the Permutational Analysis of the Multivariate Dispersion (PERMDISP; Anderson, 2006). Moreover, through this analysis, the variation in the diet among individuals of a population is observed since the increase in niche breadth in a population can occur if all individuals increase the niche breadth or if the variation in diet among individuals of the population increases (Abbey-Lee *et al*., 2013). In this way, the PERMDISP shows differences in the diet breadth by measuring the spatial dispersion of the diets of the population, using a matrix of similarity of diet (Correa, Winemiller, 2014). By PERMDISP, the distance to the centroid of a group defined a priori is calculated, in this case the species–ontogeny, through a Principal Coordinate Analysis (PCoA). The calculation of the centroid of the group was performed using the dissimilarity measure of Bray-Curtis, allowing the comparison of the average dissimilarity in *n* individual observations within the group. Here, distance to the centroid (D) corresponds to trophic niche breadth. The assumption was that differences in the distance between species indicate that some species have more restricted or broader diets than others (Correa, Winemiller, 2014; Silva *et al*., 2017). To test the null hypothesis that the trophic niche breadth did not differ among the groups (species/ontogeny: juvenile and adult), an F statist was calculated to compare the average distance of each sample to the median of the group. Subsequently, the *p*-value was obtained through 9,999 permutations of the residuals of the least-squares (Anderson, 2006). Post hoc pairwise comparisons were made by Tukey's honest significant difference method.

To verify possible morphological divergences in body shape and trophic traits, we used the Canonical Variate Analysis (CVA). For this analysis the morphological data for 94 individuals was used, with a similar proportion of individuals of each period of development (26 juveniles and 20 adults of *S. notomelas*, 28 juveniles and 20 adults of *Serrapinnus* sp.1). Nineteen morphological traits were included in the CVA. To equal weight each trait, the data were standardized using a z-transformation; thus, the mean of each measure was equal to 0, and its standard deviation was equal to 1 (Villéger *et al*., 2010). Canonical Variate Analysis (CVA) is used to describe differences among the group means (Zelditch *et al*., 2004), and allows us to find the number of significant dimensions of the group space. In this way, the first axes account for all relevant information, and the other axes represent only noise (Mardia *et al*., 1979). To verify if the morphological differences were correlated with diet, Spearman's Correlation Coefficients were estimated for all morphological traits and main food items displayed in the SIMPER.

The level of statistical significance for all analyses was $p < 0.05$. The PERMANOVA, SIMPER, and CVA were performed in the PAST program (ver. 2.08, Paleontological Statistics Software, https://palaeo-electronica.org/2001 _1/past/issue1_01.htm accessed February 19, 2019). PERMDISP and Spearman's correlation coefficients were run using the R Programming Environment (R Foundation for Statistical Computing, Vienna, Austria) with the help of the *Vegan* (Oksanen, 2015), *Corrplot* (Wei, Simko, 2017), and *Hmisc* package (Harrell Jr, 2020).

RESULTS

The stomach contents of 121 individuals belonging to two species, *S. notomelas* and *Serrapinnus* sp.1, were analyzed (Tab. 1). From the stomach content analysis, different feeding patterns was observed, with *S. notomelas* individuals consuming algae from several taxa, and *Serrapinnus* sp.1 (mostly juveniles) exhibited higher proportions of animal origin items, such as Copepoda and other zooplankton (Tab. 1).

TABLE 1 | Food items consumed by juveniles and adults of *Serrapinnus notomelas* and *Serrapinnus* sp.1 in a lake in the upper Paraná River floodplain, Brazil. In parenthesis there are the number of analyzed stomachs. Values are based on the percentage data of the volume of food items. *Represents values less than 0.01. The most consumed food items are in bold.

Food items	*Serrapinnus notomelas*		*Serrapinnus* sp.1	
	Adults (30)	Juveniles (26)	Adults (20)	Juveniles (45)
Bacillariophyta		6.70	0.69	0.31
Oedogoniophyta		**13.21**		
Chlorophyta	0.80	**7.78**	3.82	1.56
Cyanophyta	**88.94**	**59.44**	**75.90**	**18.90**
Rhodophyta	**4.05**		*	*
Aquatic plants		2.72	0.99	0.28
Cladocera	0.60		*	*
Copepoda	0.19	0.29	3.07	**30.77**
Rotifera	0.36		0.20	1.07
Ostracoda		1.31	1.49	
Testate amoebae		4.71		*
Other zooplankton		0.42	**5.51**	**43.87**
Diptera larvae (Chironomidae)	0.51		0.60	0.11
Nematoda			*	0.34
Porifera			0.05	
Bryozoa			0.30	
Fish scale				*
Aquatic insect				*
Terrestrial plants	*			*
Terrestrial insect			0.20	0.89
Detritus	**4.54**	3.41	**7.07**	1.88

Significant differences were observed in the diet between *S. notomelas* and *Serrapinnus* sp.1 (PERMANOVA two way, F = 7.67, p = 0.0001) and between juveniles and adults of both species (PERMANOVA two way, F = 4.88, p = 0.0001). Among the food items that composed the diet of species, Cyanophyta (algae) and zooplankton were the most relevant important items that contributed to the observed differences between species and ontogeny (juvenile and adult). Juveniles and adults of *S. notomelas* and adults of *Serrapinnus* sp.1 mainly consumed Cyanophyta (algae), whereas juveniles of *Serrapinnus* sp.1 mainly consumed other zooplankton (Tab. 2).

Significant differences were observed in the average niche breadth (distance to centroid) between species and ontogeny (juvenile and adult) (PERMDISP, F = 22.918, p < 0.01). Among the species, a greater average niche breadth was observed for *Serrapinnus* sp.1. However, considering the ontogeny for each species, average niche breadth was higher for juveniles of *S. notomelas* and adults of *Serrapinnus* sp.1 (Fig. 3).

TABLE 2 | Results of the dissimilarity analysis (SIMPER) for the proportion of food items of *Serrapinnus notomelas* and *Serrapinnus* sp.1 between juveniles and adults in a lake in the upper Paraná River floodplain, Brazil.

		S. notomelas	***Serrapinnus* sp.1**	
Food item		Cyanophyta (algae)	Cyanophyta (algae)	Others Zooplankton
Overall average dissimilarity		97.8	77.65	
Contribution %		77.33	35.74	31.55
Cumulative contribution %		77.33	35.74	67.28
Mean abundance:	juveniles	0.000874	0.000188	0.000438
	adults	0.00000995	0.000383	0.00002785

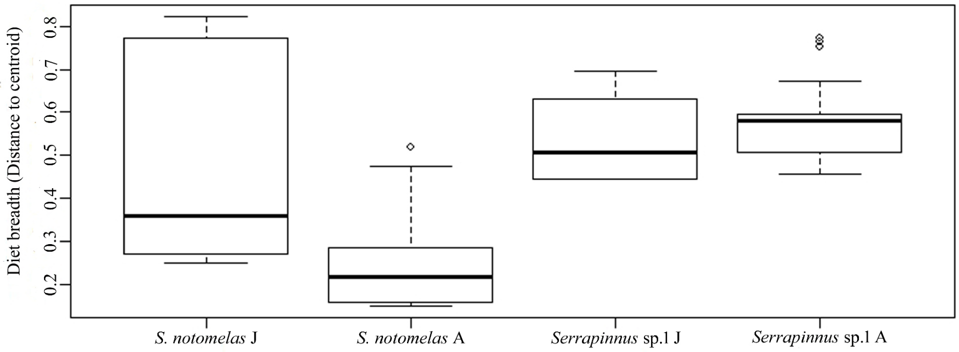


FIGURE 3 | Variation in the diet breadth of *Serrapinnus notomelas* and *Serrapinnus* sp.1 using PERMDISP, for the juveniles and adults in a lake in the upper Paraná River floodplain, Brazil. Boxes represent the 25th and 75th quartiles and demonstrate the individual variation of the trophic niche. The horizontal bars in each box represent the average niche breadth. Whiskers indicate the range and individual symbols indicate outliers. **J**=juveniles; **A**= adults.

Canonical Variate Analysis of morphological traits demonstrated inter-intraspecific differences. The negative CV1 (66.3%) scores segregated *S. notomelas* due to the intestinal coefficient, the relative area of the dorsal fin, relative length of head, and gill raker length. In contrast, the positive scores segregated *Serrapinnus* sp.1 due to the relative height of the caudal peduncle, the aspect ratio of the pectoral and pelvic fin. The CV2 (17.6%) scores segregated adults and juveniles, mainly for *S. notomelas*. Negative scores related to the *S. notomelas* juveniles with relative area of the dorsal fin, and relative position of eye; and *Serrapinnus* sp.1 adult with a relative height of mouth and relative height of head. Positive scores related to the intestinal coefficient with *S. notomelas* adult; and the aspect ratio of the pelvic fin, the relative width of the head and relative height of the peduncle with to *Serrapinnus* sp.1 juvenile (Tab. 3; Fig. 4).

TABLE 3 | Results of the Canonical Variate Analysis (CVA) of the morphological traits for *Serrapinnus notomelas* and *Serrapinnus* sp.1 in a lake in the upper Paraná River floodplain, Brazil. The eigenvalues for each axis and the percentage of the variance explained by each of CV1 and CV2 are shown. The five highest positive and negative values of the morphological traits were highlighted in bold and selected for interpretation.

	CV1	CV2
Eigenvalue	7.48	1.98
% Variance	66.3	17.6
Morphology traits		
Compression index	-0.32	0.30
Depression index	-0.06	0.04
Relative length of caudal peduncule	0.19	-0.15
Relative height of caudal peduncule	**0.60**	0.50
Relative width of caudal peduncule	0.00	-0.12
Relative area of dorsal fin	**-0.45**	**-0.54**
Aspect ratio of caudal fin	0.18	0. 09
Aspect ratio of anal fin	0.07	-0.21
Aspect ratio of pectoral fin	**0.21**	0.28
Aspect ratio of pelvic fin	**0.39**	**0.52**
Relative length of head	**-0.43**	0.24
Relative height of head	**0.35**	**-0.42**
Relative width of head	0.07	**0.54**
Relative height of mouth	0.11	**-0.42**
Relative width of mouth	0.09	0.05
Relative position of eye	-0.04	**-0.29**
Multicuspid teeth	$-2.5E^{-26}$	$1.82E^{-26}$
Pentacuspid teeth	$1.2E^{-26}$	$9.1E^{-27}$
Intestinal coefficient	**-4.39**	**3.66**
Gill raker length	**-0.82**	-0.23

Spearman correlation coefficients between food items and morphological traits showed significant negative and positive correlations (Fig. 5). Algae presented a negative correlation with aspect ratio of the pelvic fin (r = -0.22; p = 0.03); and positive correlation with the relative area of dorsal fin (r = 0.28; p = 0.006). In turn, zooplankton was correlated negatively with compression index (r = -0.25; p = 0.01), the relative area of dorsal fin (r = -0.25; p = 0.01), multicuspid teeth (r = -0.34; p = 0.0007), and gill raker length (r = -0.26; p = 0.01); and positively correlated with relative height of caudal peduncle (r = 0.27; p = 0.006), aspect ratio of the anal fin (r = 0.24; p = 0.02), aspect ratio of the pectoral fin (r = 0.38; p = 0.0001), relative width of the head (r = 0.25; p = 0.01) and pentacuspid teeth (r = 0.34; p = 0.0007) (Fig. 5).

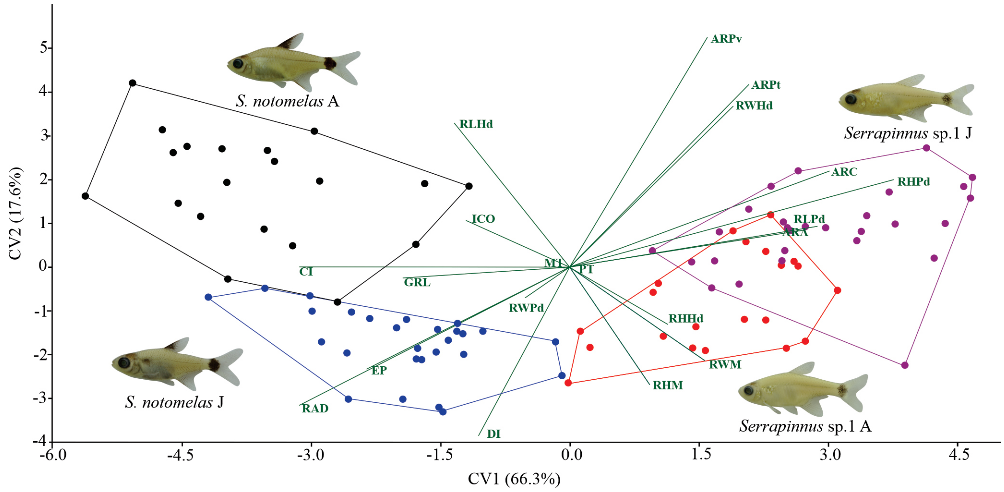
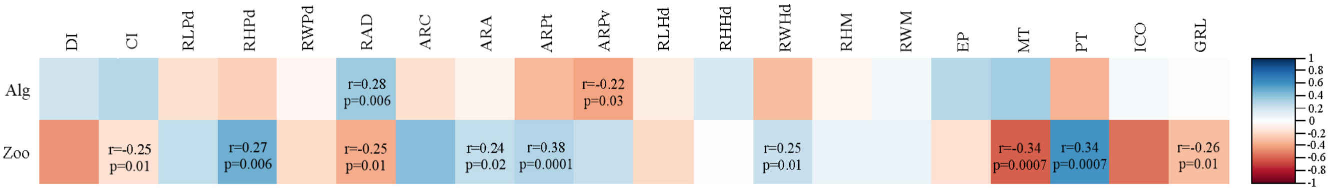


FIGURE 4 | Canonical variate analysis illustrating differences in morphological traits for the *Serrapinnus notomelas* and *Serrapinnus* sp.1 in a lake in the upper Paraná River floodplain, Brazil. **CI** – Compression index; **DI** – Depression index; **RLPd** – Relative lenght of caudal peduncule; **RHPd** – Relative height of caudal peduncule; **RWPd** –Relative width of caudal peduncule; **RAD** – Relative area of dorsal fin; **ARC** – Aspect ratio of caudal fin; **ARA** – Aspect ratio of anal fin; **ARPt** – Aspect ratio of pectoral fin; **ARPv** – Aspect ratio of pelvic fin; RLHd – Relative length of head; **RHHd** – Relative height of head; **RWHd** – Relative width of head; RHM – Relative height of mouth; **RWM** – Relative width of mouth; **EP** – Relative position of eye; **MT** – multicuspid teeth; **PT** – pentacuspid teeth; **ICO** – Intestinal coefficient; **GRL** – Gill raker length.



	DI	CI	RLPd	RHPd	RWPd	RAD	ARC	ARA	ARPt	ARPv	RLHd	RHHd	RWHd	RHM	RWM	EP	MT	PT	ICO	GRL
Alg						r=0.28 p=0.006				r=-0.22 p=0.03										
Zoo		r=-0.25 p=0.01		r=0.27 p=0.006		r=-0.25 p=0.01		r=0.24 p=0.02	r=0.38 p=0.0001				r=0.25 p=0.01				r=-0.34 p=0.0007	r=0.34 p=0.0007		r=-0.26 p=0.01

FIGURE 5 | Graph of Spearman's correlation coefficient calculated between the morphological traits indicated by CVA and main food items consumed by *Serrapinnus notomelas* and *Serrapinnus* sp.1 in a lake in the upper Paraná River floodplain, Brazil. Values of r and p indicate the correlation and statistical significance, respectively. Positive correlations are represented by blue color and negative correlations by red color. ALG – algae; ZOO – zooplankton; DI – Depression index; CI – Compression index; RLPd – Relative lenght of caudal peduncule; RHPd – Relative height of caudal peduncule; RWPd – Relative width of caudal peduncule; RAD – Relative area of dorsal fin; ARC – Aspect ratio of caudal fin; ARA – Aspect ratio of anal fin; ARPt – Aspect ratio of pectoral fin; ARPv – Aspect ratio of pelvic fin; RLHd – Relative length of head; RHHd – Relative height of head; RWHd – Relative width of head; RHM – Relative height of mouth; RWM – Relative width of mouth; EP – Relative position of eye; MT – multicuspid teeth; PT – pentacuspid teeth; ICO – Intestinal coefficient; GRL – Gill raker length.

DISCUSSION

Inter and intraspecific (juveniles and adults) differences in diet, trophic niche breadth, and morphological traits confirm the differentiation of trophic niche between the sympatric and congeneric species that we evaluated here. In this way, despite the morphological similarity (phylogenetic conservatism; characids with compressed bodies, lateral eyes, and lateral pectoral fins), inter and intraspecific differences in body shape favor different swimming abilities (more efficient continuous swimming or maneuverability) with implications for the segregation of microhabitat and food use within the macrophyte stands. Moreover, we observed relevant relationships between the diet and the trophic apparatus morphology (*i.e.*, gill raker length, the number of teeth cuspids, and intestinal coefficient). Overall, our results reveal that the species studied exhibited morphological differences that resulted in food resource partitioning, corroborating the predictions of the niche theory (Hutchinson, 1961; Leray *et al*., 2019). Since the natural selection acts upon species that can maintain themselves in an environment while they exploit it (Chase, Leibold, 2003), the observed segregation and high seasonality in the availability of resources in these macrophyte stands (Quirino *et al*., 2015) should permeate the dynamics of competitors (intra and interspecific) and favor the coexistence of species.

Serrapinnus notomelas and *Serrapinnus* sp.1 consumed mostly algae and zooplankton, respectively. Adults and juveniles of *S. notomelas* and adults of *Serrapinnus* sp.1 mainly consumed algae, while juveniles of *Serrapinnus* sp.1 mainly consumed zooplankton. Algae and zooplankton are abundant in macrophyte stands (Colares *et al*., 2013; Quirino *et al*., 2015). Algae proliferate in the periphyton that is formed in the roots and stems of aquatic macrophytes (Biolo, Rodrigues, 2013; Quirino *et al*., 2015), whereas microcrustaceans use roots and stems as shelter against predation (Colares *et al*., 2013; Quirino *et al*., 2015). Therefore, *S. notomelas* and *Serrapinnus* sp.1 individuals explored resources that are typically available in macrophytes in a different way, thus differing in foraging habits. Variations in the main food consumption have been reported for this genus in other studies conducted in the same region. For example, for *S. notomelas*, seasonal variations in the diet were observed with the predominant consumption of invertebrates (Quirino *et al*., 2015) and zooplankton (Quirino *et al*., 2018).

The average trophic niche breadth differed between species and ontogeny. The higher average niche breadth of *Serrapinnus* sp.1 revealed that individuals, mostly adults, consumed a greater variety of food. These findings, in addition to the higher consumption of animal items, indicate more trophic flexibility and omnivorous habit for *Serrapinnus* sp.1. In contrast, *S. notomelas* seems to be more specialized on plants, since the diet has low variability; besides, intra-population variation was low too, in accordance to what is expected for this trophic guild (Gerking, 1994). The broad trophic niche and high trophic flexibility may favor the coexistence between species (Chase, Leibold, 2003; Neves *et al*., 2021). The flexibility of omnivorous fishes in changing their diet when faced with environmental variation, allows the trophic niche differentiation (Chase, Leibold, 2003). The expansion or contraction of the trophic niche favors resource partitioning, thus allowing the coexistence and avoiding/decreasing the effects of competition, especially when it comes to congeners species (Van Valen, 1965; Bolnick *et al*., 2010; Neves *et al*., 2021). In this perspective, we suggest that the greatest trophic niche breadth observed for *Serrapinnus* sp.1 can be a partitioning resource mechanism with *S. notomelas*. Thereby, considering the morphological limitations, the trophic niche was expanded to supply energy demands and avoid trophic competition with *S. notomelas*.

A relationship between the diet and the morphological attributes, since the morphology influences foraging strategies and tactics, has been demonstrated by several studies in fishes (Bonato *et al*., 2017; Portella *et al*., 2017; Ornelas-García *et al*., 2018; Kliemann *et al*., 2019; Delariva, Neves, 2020). According to Alexander (1967), the morphological mechanisms used by a fish to obtain food represent adaptations that allow them to take advantage of the specific prey. Interspecific differences in morphological traits for the species under study, as well as the significant correlation between the diet and the morphological traits, demonstrate that dietary divergences were favored by different performances in the locomotion and in the feeding abilities (prey capture, selection, and absorption).

Serrapinnus notomelas exhibited a larger dorsal fin, which involves a better stabilization capacity in deflections, thus providing lower resistance to perform maneuvers such as dorsoventral or lateral movements (Gosline, 1971). Besides, it allows the exploration of structurally complex habitats, as is the case of macrophyte stands in lentic waters (Esguícero, Arcifa, 2010; Prado *et al*., 2016). Thus, it can be inferred that the ability to stabilize deflections and greater maneuverability facilitates the consumption of algae that are associated with the stems and roots of macrophytes. This inference is corroborated by the high consumption of algae exhibited by this species and the positive correlation of this item with the relative area of the dorsal fin. By contrast, *Serrapinnus* sp.1 had higher peduncles and larger pectoral and pelvic areas than *S. notomelas*. The high peduncle implies less is maneuverability, and broad pectoral and pelvic fins favor continuous swimming at higher speeds and provide a greater ability to balance (Gatz, 1979; Wainwright *et al*., 2002). So, since microcrustaceans extensively move between the roots and stems of macrophytes (Choi *et al*., 2015), broad pectoral fins increase the swimming speed and enhance foraging efficiency in this habitat. Hence, the swimming performance of *Serrapinnus* sp.1 favors the capture of these organisms. The correlation between zooplankton and the anal fin aspect ratio, as well as the relative height of the caudal peduncle, reinforces these findings.

Associated with the swimming performance, differences in morphological traits related to the food capability (that is, obtaining, selecting, and processing food in the digestive tract; *sensu* Gerking, 1994) may additionally explain the dietary segregation here reported. The peculiarities of the trophic apparatus (gill raker length and intestinal coefficient) were the main traits that differed between the species. Longer gill rakers, as observed for *S. notomelas,* were correlated with the higher consumption of algae. More specialized structures, such as elongated intestine have been listed as adaptive responses to extract nutrients from plants (poorer in readily assimilable energy) (Gerking, 1994). Likewise, the long and more juxtaposed gill rakers give advantages in filtering algae (Gatz, 1979; Kramer, Bryant, 1995; Villéger *et al*., 2010), an abundant resource in macrophyte stands.

Another important morphological characteristic that was correlated with the diet was the number of teeth cuspids. Algivorous species tend to have multicuspid teeth for cutting or tearing (Gibson, 2015; Ohara *et al*., 2017), which are in accordance to observed for *S. notomelas*. In contrast, species with few cusps, often between three or five, tend to be omnivorous (Winemiller, 1991), as in the case of *Serrapinnus* sp.1.

We also observed differences in diet and morphology throughout ontogeny, corroborating with several authors who reported these variations and related them to the energy requirement during development (see Neves *et al*., 2015; Dias *et al*., 2017b; Schilling *et al*., 2017; Kliemann *et al*., 2019). Body size and the demand for energy increase with the growth of the fish. Thus, morphological changes may occur that allow the consumption of a greater range of resources, whether in size or quantity of food, which meet energy demand (Werner, Gilliam, 1984; Scharf *et al*., 2000; Marsh *et al*., 2017). Diet and morphology adjustments between adults and juveniles were verified mainly for *Serrapinnus* sp.1. We observed a wider head and mouth and the consumption of Diptera larvae, Bryozoa, and Porifera (more energy-efficient resources) in adults. Additionally, we found a greater average trophic niche breadth for adults, reinforcing that there is the exploration of a wide variety of habitats and the consumption of items of larger sizes as the fish grows (Chase, Leibold, 2003; Ward *et al*., 2006; Tupinambás *et al*., 2015; Schilling *et al*., 2017).

When compared to adults, juveniles of *S. notomelas* had larger dorsal fins and juveniles of *Serrapinnus* sp.1 had larger pelvic fins. Moreover, juveniles of *S. notomelas* had dorsally positioned eyes when compared to adults. These morphological traits are related to the ability to balance, stabilize in deflections, and capture prey in the water column (Gosline, 1971; Gatz, 1979; Pouilly *et al*., 2003). This set of divergences suggests ontogenetic segregation in the habitat use, which can be a strategy to avoid intra-interspecific competition (Hutchinson, 1961; Leray *et al*., 2019). It is inferred those adults and juveniles of both species explored different habitats, with the water column mainly inhabited by juveniles. These findings are important from the perspective of conservation. Considering that macrophyte stands are strongly dependent on the water level and seasonal dynamics (Thomaz *et al*., 2009), atypical changes in these dynamics can affect juveniles and adults of one or another species in a divergent way. Furthermore, this segregation in the habitat use implies differentiation of the trophic niche, reinforcing the mechanisms for inter-intraspecific coexistence predicted by the niche theory.

In summary, our results show differences in morphological traits and correlations with different feeding habits. We infer that the correlation between the locomotor and feeding abilities may result from interactions of the individuals, with their preys and habitats. These associations increase opportunities for independent diversification, and it may explain the species coexistence in macrophyte stands, corroborating with the niche theory. Moreover, the ontogenetic differences in diet and morphology observed for the two species, indicate a segregation in the habitat use. The information from the trophic apparatus (intestinal coefficient, gill raker length, and the number of teeth cuspids) is valuable to demonstrate the trophic segregation. In our analysis, the combined use of morphological traits is an excellent way to objectively identify cases of adaptive and evolutionary divergence between phylogenetically closely related species.

Finally, we encourage future studies to evaluate other niche dimensions (space, time) that can help favoring the coexistence (Leray *et al*., 2019) between pairs of congeners species. In addition, there is a lack of studies considering factors unevaluated here, such as seasonality, spatiality, resources availability, and influence of predators, which can interfere in the behavior of individuals (Hart *et al*., 2016). Also, the isotopic niche approach is also valuable because it can reveal the assimilation of food resources and the metabolic state (Post, 2002; Carvalho *et al*., 2017).

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AUTHOR'S CONTRIBUTION

Bruna Caroline Kotz Kliemann: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing-original draft, Writing-review and editing.

Eliana Maria Galdioli: Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing-original draft, Writing-review and editing.

Andréa Bialetzki: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

Rosilene Luciana Delariva: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

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COMPETING INTERESTS

The authors no declare competing interests.

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