



## Spatial diet overlap and food resource in two congeneric mullet species revealed by stable isotopes and stomach content analyses

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**Abstract:** Food partitioning among coexisting species in different habitats remains an important research topic in trophic ecology. In this work, we combined carbon and nitrogen stable isotope ratios and stomach content analyses to investigate differences in diet and niche overlap of two congeneric juvenile mullet species (*Mugil curema* and *Mugil liza*) coexisting in a marine surf-zone and an estuarine zone in southern Brazil (29°S). These habitats have contrasting levels of food availability, especially in terms of prey diversity, with higher microalgae diversity in the estuary than in the marine surf-zone. In these contrasting conditions, we predicted that both mullet species will have (a) higher niche overlap and smaller niche breadth at the marine surf-zone due to the common exploration of highly abundant surf-zone diatoms and (b) lower niche overlap and higher niche breadth inside the estuary due to selective feeding on more diverse food resources. Isotope niche areas (measured as standard ellipse areas) were higher in the estuary (6.10 and 6.18) than in the marine surf-zone (3.68 and 3.37) for both *M. curema* and *M. liza*, respectively. We observed an overlap of 52% in isotopic niches of both species in the marine surf-zone and none in the estuary. We also found contrasting patterns in the diet composition between species according to the habitat. At the marine surf-zone, diatoms of the classes Bacillariophyceae and Coscinodiscophyceae dominated (> 99%) the food content of both mullet species. In contrast, green algae, cyanobacteria, dinoflagellates and flagellates comprised the diet of both species in the estuary. These results could be explained by spatial differences in food availability (especially regarding diversity of microalgae) between both habitats. At the marine site, both species explored the most abundant microalgae available (mostly the surf-zone diatom *Asterionellopsis* cf. *guyanusae* and fragments of *Coscinodiscus*), whereas in the estuary both species shifted their diets to explore the greater diversity of microalgae resources. Overall, our findings revealed that niche partitioning theory could not fully predict changes in breadth and overlap of food niches of estuarine dependent fish species with complex life cycles encompassing marine to estuarine systems with contrasting food availabilities.

**Abbreviations:** ICMBio – Brazilian National Environmental authority; SCA – Stomach Content Analysis; SEA<sub>B</sub> – Bayesian Standard Ellipse Areas; SEA<sub>C</sub> – Small Sample size-corrected standard Ellipse Areas; SIA – Stable Isotope Analysis; SIBER – Stable Isotope Bayesian Ellipses in R; UPGMA – Unweighted Pair Group Method with Arithmetic mean; TL – Total Length.

**Nomenclature:** FishBase catalog (2018).

### Introduction

Species coexistence and food partition among species across different habitats have been investigated since the pioneer work of Elton (1927), but still remain as a main research topic in trophic ecology (e.g., Winemiller et al. 2015, Dupke et al. 2016, Fitzgerald et al. 2017). Several theories are used to explain the main mechanisms and controlling driving forces of these interactions (Pianka 1976, Winemiller and Layman 2005). Niche partitioning theory, for instance, predicts that stable coexistence of competing species will occur through niche differentiation that reduces overlap among competitors. Hence, consumers are expected to adjust feeding to reduce

niche overlap (e.g., narrowing their diet breadth by selective feeding) with competitors in scenarios of relatively food scarcity (Pianka 1976). Accordingly, field studies have shown that fishes of tropical streams may change from small and distinct food niches during periods of lower resource availability (dry season) to broader overlapping food niches in more productive periods (wet season) (e.g., Correa and Winemiller 2014).

However, predictions of niche partitioning theory are difficult to be observed in nature because variability in food resources in space or time can influence the feeding behavior of consumers (Gerking 1994, Bastos et al. 2017). Hence, a species may explore a restrict number of food resources (specialist diet) in a habitat simply because these items are the

most abundant or because there are no alternative resources available. In contrast, this same species may have a broad food niche (generalist diet) in a habitat that has a greater variety of food resources available. This spatial (or temporal) effect of resource availability on species feeding strategy is not always taken into account when investigating the use of food resources by consumers (Fox and Morrow 1981).

Comparison of food habits and diet overlap of coexisting species among habitats with contrasting levels of food availability in terms of abundance and prey diversity are useful to provide empirical evidence to better understand the mechanisms controlling diet variation and food consumption across ecosystems (Winemiller and Layman 2005). One challenge of comparative feeding studies among habitats is to measure the width and overlap of food niches in a standardized and meaningful manner (Bearhop et al. 2004). A traditional approach to describe diet and determine food niche breadth has been stomach content analysis (SCA) of consumers (Nielsen et al. 2017). Although it may achieve high taxonomic resolution, this method can be biased due to the difficulty to determine the origin of partially digested food items and the impossibility to evaluate the true assimilation of nutrients derived from ingested food. In the past decades, stable isotope analysis (SIA) has been considered useful natural markers to reconstruct consumers' diet and food assimilation patterns (Layman et al. 2012). More recently, isotopic niche metrics have been proposed as useful proxies to evaluate the breadth and overlap of food niches (Bearhop et al. 2004, Newsome et al. 2007, Jackson et al. 2011). It is currently recognized that the combination of both techniques (SCA and SIA) provides more accurate representations of consumers' diet and food resource use (Layman et al. 2012, Condini et al. 2015).

Fishes have been considered an adequate model to investigate spatial changes in food resources use and diet overlap due to their diversity and high abundance across virtually all aquatic systems (Gerking 1994). Many fishes have complex life histories encompassing the use of contrasting aquatic systems with marked changes in food availability along their life cycle (Wootton 1999). Mullet species are good examples of fishes occurring from tropical to temperate zones that explore contrasting habitats along their life history. They usually spawn in the sea and their juveniles seek shallow protected waters in estuaries and coastal lagoons, where they feed upon highly abundant resources. Most mullet species have diets comprised by large amounts of detritus, benthic invertebrates, green filamentous macroalgae and microalgae (Cardona 2015). SCA has been traditionally used to describe their diet composition, a technique that can be highly time-consuming in mullet species feeding mostly on microalgae (Cardona 2001, Mai et al. 2018). A few studies have applied SIA to investigate trophic ecology of mullets (Lebreton et al. 2011, Le Loc'h et al. 2015), but only a handful of studies combined SCA and SIA techniques (e.g., Carassou et al. 2017). For instance, Carassou et al. (2017) employed SIA and SCA (and other natural markers) to investigate spatiotemporal changes in the diet of the freshwater mullet *Myxus capensis* in a river-estuary interface in South Africa. The authors highlighted the importance of a multiple technique approach to describe

the diet of mullets because different species within the family Mugilidae may rely on different food sources within the microphytobenthos or organic detrital matter pools (Le Loc'h et al. 2015, Carassou et al. 2017).

In the present work, we combined SIA and SCA techniques to investigate differences in diet and niche overlap of two congeneric juvenile mullet species (*Mugil curema* and *Mugil liza*) in a marine surf-zone and after their recruitment into an estuarine zone in southern Brazil (29°S). These habitats have contrasting levels of food availability, especially in terms of prey diversity. Estuaries along this region are characterized by high biomass of submerged aquatic vegetation (mainly seagrass and floating macroalgae), great diversity of benthic invertebrates and microalgae and large amounts of detritus (Odebrecht et al. 2010a, 2014, Hoeinghaus et al. 2011). In contrast, the marine surf-zone along this sandy coastline is characterized by high *in situ* phytoplankton production and microalgae blooms, dominated by frequent and dense accumulations of diatoms (Odebrecht et al. 2010b, 2014). In these contrasting scenarios of food availability, we predicted that both mullet species will have higher niche overlap and smaller niche breadth at the marine surf-zone due to the common exploration of the highly abundant surf-zone diatoms, whereas they will have lower niche overlap and higher niche breadth inside the estuary due to selective feeding on more diverse food resources.

## Material and methods

### Study area

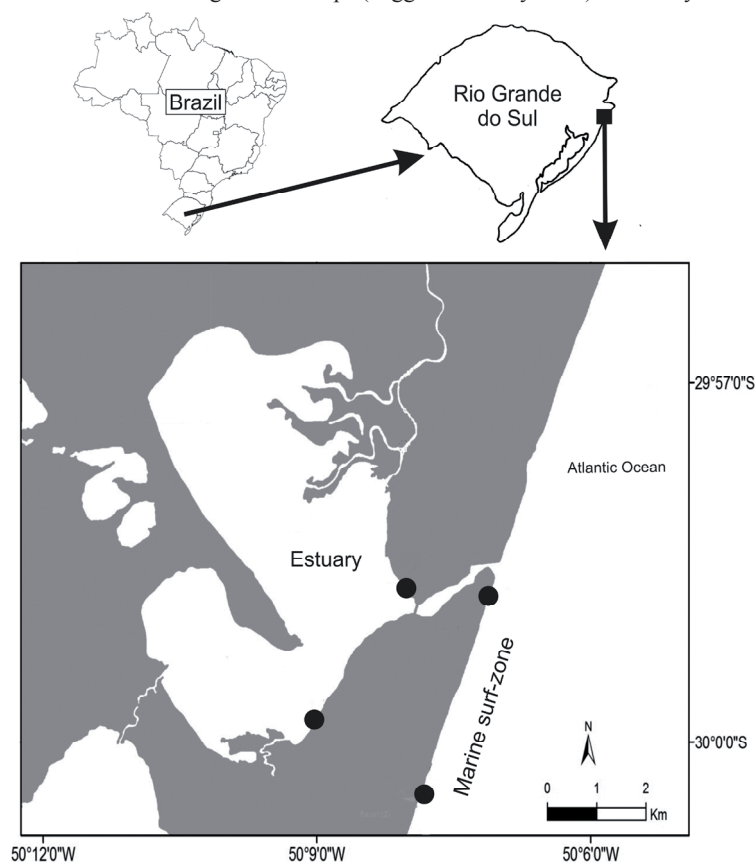
We carried out the study at the Tramandai-Armazém estuarine complex and its adjacent marine surf-zone located in southern Brazil (29°58'S; 50°08'W) (Fig. 1). The estuary has an area of approximately 30 km<sup>2</sup> and is comprised of two embayments and a main channel, from which the salinity intrusion enters the estuarine zone, especially when south winds predominate. The estuary has depths ranging from 1 to 5 m and is under the influence of a micro-tidal regime (Loitzenbauer and Mendes 2012). The most representative vegetation are marsh plants (e.g., *Juncus acutus*, *Rhynchospora gigantea*, *Scirpus olneyi*), the widgeon grass *Ruppia maritima*, floating macrophytes (e.g., *Eichhornia crassipes*) and terrestrial grasses along the estuarine margins (Hoeinghaus et al. 2011). The marine surf-zone has characteristics of a dissipative beach being directly exposed to waves with medium to high energy and may receive freshwater discharge from coastal streams draining freshwater wetlands. In contrast with the estuary, as described in a more southern (32°S) location (Odebrecht et al. 2010a), the surf zone is characterized by the dominance of phytoplankton, being absent other primary producers as aquatic macrophytes, macroalgae beds and seagrasses (AFSG, personal observation). Regarding anthropogenic impacts, the study area faces increasing environmental pressures from furniture industry, *Pinus* afforestation, rice irrigation, cattle rising, sand extraction and unplanned human occupation of the coastal zone (Malabarba et al. 2013).

### Field collections and sampling processing

We sampled mullet species on March 2015 using beach seine hauls, casting net and gillnets (see Garcia et al. 2006 for gears' dimensions and operation procedures) under field collection permit n° 47567-1 provided by the Brazilian National Environmental authority (ICMbio).

Individuals for isotopic analysis were kept frozen until later processing in laboratory, where they were dissected to obtain approximately 5 g of anterodorsal muscular tissues per sample. We rinsed each sample with distilled water, placed in a sterile Petri dish, and dried in an oven at 60°C to a constant weight (minimum of 48 h). Dried samples were ground to a fine powder with a mortar, and pestle and stored in Eppendorf tubes. We weighed subsamples (~1 mg) and pressed them into ultra-pure tin capsules (Costech Analytical Technologies) and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen isotope ratios. The carbon standard was Pee Dee Belemnite limestone, and the nitrogen standard was atmospheric nitrogen. We expressed results in delta notation (parts per thousand deviation from a standard material):  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}})-1]*1000$ , where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . We applied mathematical normalization on  $\delta^{13}\text{C}$  values to control for potential effects of lipid contents (DeNiro and Epstein 1977) using the equation proposed by Post et al. (2007):  $\Delta\delta^{13}\text{C} = -3.32 + 0.99 * \text{C:N}$ . We applied this correction for those samples with C:N higher than 3.5 (Post et al. 2007).

We preserved individuals for stomach content analysis in 10% formaldehyde and later in 70% alcohol. In laboratory, we dissected each individual and removed its stomach. The food content of each stomach was analyzed following the guidelines suggested by Cardona (2015). It was not practical to employ gravimetric or volumetric prey measurements due to the presence of detritus and sand in most of the stomachs. Therefore, we opted to evaluate resource exploitation patterns using the frequency of occurrence of prey species (Cardona 2015). The food content of one to three specimens of each species at each region was combined into a unique sample to enable its quantitative analysis, summing up four samples of each species in each region, with the exception of *M. liza* who had five samples analyzed in the marine surf-zone. Table appendix 1 shows the total number of specimens (n=30) used in each sample and location and their individual total length (TL) in millimeters. Each food content sample was mixed with ethanol and stirred. One aliquot was immediately collected with a micropipette and microalgae were counted at  $\times 400$  magnification under a light microscope using a Fuchs Rosenthal chamber (Cardona 2015). The counts were carried out until reaching at least 100 individuals (cells, colonies or filaments) in each sample. Cell fragments of *Coscinodiscus* were counted separately. Microalgae were identified to the lowest practical taxonomic level based on specialized literature. Additional subsamples were acid cleaned and mounted on glass slides using Naphrax™ (Brunel Microscopes Ltd., Chippenham, UK) in toluene and examined at  $\times 1000$  under a light microscope (Biggs and Kilroy 2000) to identify diatoms.



**Figure 1.** Map of Brazil and its southernmost state (Rio Grande do Sul) where is located the Tramandaí-Armazém estuarine complex. Full circles (lower panel) denote the sampling stations at the marine surf-zone and within the estuary where mullet individuals were sampled.

Our goal was to provide an overall comparison of food intake for each species in each region to contrast with isotope evidences and not to provide a full and detailed dietary study of each species. Therefore, we believe our analysis of only four samples (summing up 30 individuals) of each species in each region was sufficient to provide such comparison (Appendix, Table A1). The great majority (77%) of analyzed specimens were juveniles (total length, TL, less than 50 mm). Regardless of a few larger individuals, we polled all specimens together for analysis because diet is very similar for specimens > 50 mm (Drake et al. 1984).

#### Data analyses

As a measure of the isotope niche (Newsome et al. 2007), we used small sample size-corrected standard ellipse areas ( $SEA_C$ ) containing ca. 40% of all data to measure and compare isotopic niche between species in each region (Jackson et al. 2011). We also computed Bayesian standard ellipse areas ( $SEA_B$ ) ( $n = 200$  iterations) to produce a range of probable values (95%, 75% and 50%) for the calculated standard ellipses (Jackson et al. 2011). Overlap in  $SEA_C$  between mullet species in each region was used as a measure of isotopic niche partitioning. Isotopic niche overlap was expressed as a proportion of the area of overlap between two  $SEA_C$  and its own  $SEA_C$  (Catry et al. 2016). All analyzes were carried out using the SIBER package v 2.1.3 (Jackson et al. 2011) in R.

We employed cluster analysis to evaluate the similarity in diet composition of *M. curema* and *M. liza* separately for each region based on Euclidean distances and the agglomerative UPGMA method on a matrix comprised of dietary information ( $\log_{10}(\text{abundance}+1)$ ) of each sample. We calculated the correlation between the original distance matrix

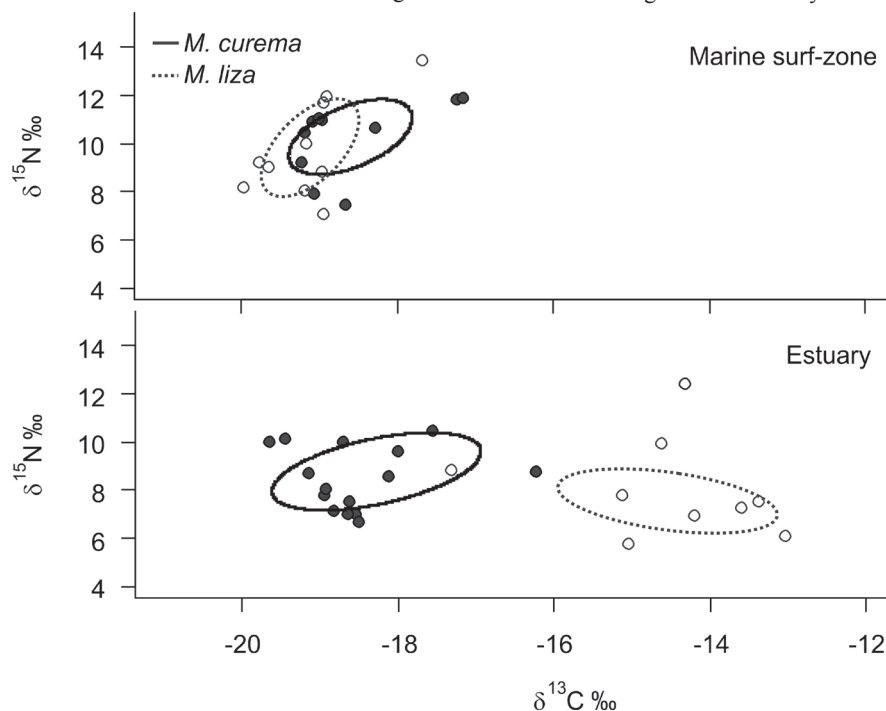
and the cophenetic matrix to evaluate how well the dendrogram preserved the pairwise distances between the original unmodeled data points (Rohlf and Fisher 1968). We run these analyses using the vegan package in R (Oksanen et al. 2017). We also analyzed the niche overlap based on diet composition between the two mullet species at each region using the Morisita-Horn index following the formula:  $C_H = 2 * (\sum_i p_{ij} * p_{ik}) / (\sum_i p_{ij}^2 + \sum_i p_{ik}^2)^{-1}$ , where  $C_H$  is the diet niche overlap between species  $j$  and  $k$ ,  $p_{ij}$  is the proportion of resource  $i$  from the total resources used by species  $j$ ,  $p_{ik}$  is the proportion of resource  $i$  from the total resources used by species  $k$ ,  $n$  is the total number of resources (Krebs 1999, Cardona 2001).

#### Results

##### Differences in isotopic composition and isotope niches

All (44) individuals sampled in the marine surf-zone (*M. curema* = 10, *M. liza* = 10) and estuary (*M. curema* = 16, *M. liza* = 8) had their carbon ( $\delta^{13}C$ ) and nitrogen ( $\delta^{15}N$ ) stable isotope ratios analyzed. All specimens were juveniles with total length (TL, mm) lower than 100, with exception of *M. liza* that ranged from 57 to 341 within the estuary. The comparison of average  $\delta^{13}C$  and  $\delta^{15}N$  values between species revealed statistically significant differences only for  $\delta^{13}C$  at the estuary (KW-H (1;20) = 7;  $p < 0.008$ ), with *M. liza* showing higher average values than *M. curema* (-15.43 vs. -18.27, respectively).

Standard ellipse areas ( $SEA_C$ ) of both *M. curema* and *M. liza* had higher values in the estuary (6.10 and 6.18, respectively) than in the marine surf-zone (3.68 and 3.37) (Fig. 2). Bayesian estimation of these ellipses areas ( $SEA_B$ ) corroborated this pattern of greater isotope ellipses in the estuary at 75% and 50% levels of credibility, but also revealed greater variability at 95% credibility intervals mainly for



**Figure 2.** Carbon ( $\delta^{13}C$ ) and nitrogen ( $\delta^{15}N$ ) stable isotope ratios and standard ellipse area (SEA) for *Mugil curema* (full line) and *Mugil liza* (dashed line) individuals sampled in the marine surf-zone and in the estuary of Tramandaí-Armazém estuarine complex.

*M. liza* (Fig. 3). Overlap in isotopic niches between species based on  $SEA_C$  changed markedly between regions, showing an overlap of 52% in the marine surf-zone and no overlap in the estuary (Fig. 2).

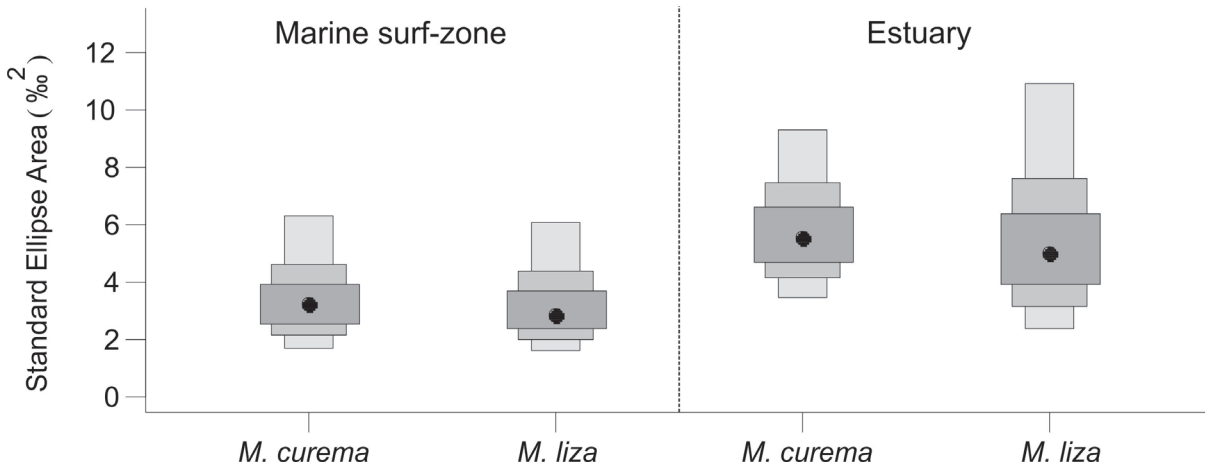
*Interspecific and between-habitat differences in food stomach contents*

The analysis of food contents of both species revealed contrasting patterns in diet composition and niche overlap ( $C_H$ ) between species according to the region. At the marine surf-zone, diatoms of the classes Bacillariophyceae and Coscinodiscophyceae dominated (> 99%) the food content of *M. curema* (60.1% and 39.2%, respectively) and *M. liza* (77.5% and 22.1%). In contrast, a greater variety of taxonomic groups, such as Chlorophyceae, Cyanobacteria and Dinophyceae, in addition to non-identified flagellates, contributed in different proportions to the diet of *M. curema* and *M. liza* in the estuary (Appendix, Fig. A1). Accordingly, the niche overlap ( $C_H$ ) was more than three-folds higher at the marine surf-zone (0.95) than in the estuary (0.27).

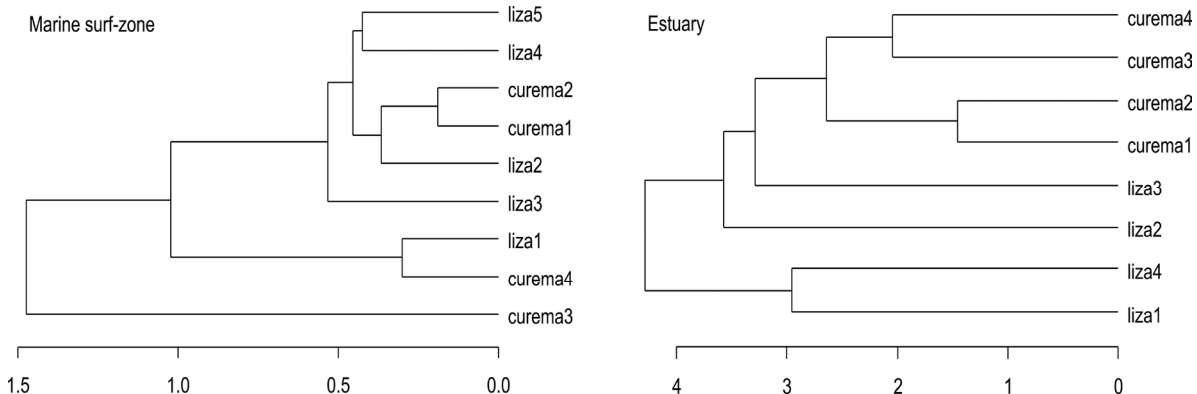
Cluster analysis revealed that *M. curema* and *M. liza* showed more dissimilar diets in the estuary than in the marine surf-zone (Fig. 4). At the marine surf-zone, *M. curema* and *M. liza* exhibited a diet comprised of a few distinct food items (five and six items, respectively) with a marked predominance of the surf-zone diatom *Asterionellopsis cf. gyunusae* (50.9% and 57.3%, respectively) in their stomach contents. In contrast, the diets of *M. curema* and *M. liza* were much richer and with no predominance of particular food items in the estuary (Fig. 5; Appendix, Table A2). Moreover, the number of food items in the diet of *M. liza* was more than two-folds higher than of *M. curema* (71 vs. 30 items) and there were also differences in the most consumed items by each species (Fig. 5; Appendix, Table A2).

**Discussion**

Our findings corroborated our predictions of spatial changes in niche breadth and overlap of congeneric mullet species occurring in a marine surf-zone and an estuarine region. We observed lower isotope niche area for both species



**Figure 3.** Bayesian standard ellipse areas for *Mugil curema* and *M. liza* individuals sampled in the marine surf-zone and in the estuary of Tramandaí-Armazém estuarine complex. Black dots represent the mode and boxes present the 50%, 75% and 95% credible intervals.



**Figure 4.** Results of the cluster analysis showing the classification of *Mugil curema* and *M. liza* based on food items found in each sample (numbered from 1 to 4; one to three individuals from each species in each region were combined in composite samples, see Appendix, Table A1) at the marine surf-zone and estuary of Tramandaí-Armazém estuarine complex. See Figure 5, for the diet composition of each species in each location.



diatom dominated the food content of both mullet species at the marine surf-zone, followed by fragments of the diatom *Coscinodiscus* spp. This diatom-based diet resulted in lower isotope niches for both consumers, suggesting that a restricted number of primary carbon sources sustain these juvenile mullets at the marine surf-zone.

However, in contradiction with the niche partitioning theory (Pianka 1976), we did not observe niche segregation between *M. curema* and *M. liza* in the habitat with comparatively lower food diversity (i.e., the marine surf-zone). This apparent discrepancy could be explained by different factors. Firstly, although the availability of food resources at the marine surf-zone may be comparatively lower than in the estuary, there is still high abundance of food items such that probably there was no food limitation for both consumers. Although dominated by a few species, the *in situ* phytoplankton production is high (1.647 mg m<sup>-3</sup>) and characterized by dense blooms (10<sup>9</sup> cells l<sup>-1</sup>) of surf-zone diatoms in this marine coastline (Odebrecht and Garcia 1997, Odebrecht et al. 2010b). Hence, it seems plausible to assume that there were enough food resources to be exploited by both mullet species. In such condition, as predicted, we did not observe the effects of niche segregation (Pianka 1976). A high degree of overlap in food niches has also been observed in other marine areas with high *in situ* productivity (e.g., upwelling ecosystems), where food resources may not be a limiting factor and inter-specific competition apparently is not an important factor in structuring fish assemblages (Abdellaoui et al. 2017). Secondly, even in a situation of limited food resources, other factors could allow non-competitive coexistence despite of the observed high food niche overlap between mullets in the marine surf-zone. For instance, Cardona (2001) observed that food niche overlap among coexisting mullet species remained similar along the year despite seasonal changes in food availability, including seasons with food limitation. According to this author, an alternative explanation may be related with the reproductive biology of mullets, as they spawn off-shore and their young-of-the-year are dispersed over a wide geographic area. In these Estuarine Marine Migrant species with a complex life cycle encompassing marine to estuarine systems, recruitment at a particular habitat (e.g., surf-zone, estuary) may be decoupled from growth performance of the species in the area and, hence, competitive interaction and niche segregation do not operate even when food supply is limited (Wootton 1999, Cardona 2001).

As predicted, niche breadths increase substantially inside the estuary for both mullet species. Their diet shifted from almost exclusively diatoms (Bacillariophyceae and Coscinodiscophyceae) to a higher variety of food items such as green algae, cyanobacteria and flagellates. Hence, the diet of both species seemed to shift from a more specialist in the marine surf-zone to a more generalist diet inside the estuary. Such change in feeding behavior may be explained by the abundance and great diversity of microalgae commonly found in estuaries (Day et al. 2012). For instance, prior studies on microalgae diversity in Patos Lagoon estuary (situated in the same coastal plain and ~375 km apart of our study area) revealed the presence of several groups of freshwater

and marine species of diatoms, cyanobacteria, chlorophytes, dinoflagellates, and other flagellates in the water column and high biomass of epiphytic microalgae (mainly pennate diatoms *Cocconeis*, *Synedra*, *Amphora*) on the leaves and shoots of seagrasses (Seeliger 1997, Odebrecht et al. 2010b). Our evidences obtained from stable isotope and stomach content analyses highlighted the trophic plasticity of the two studied mullet species, which apparently shift their diet in response to the more diverse microalgae availability inside the estuary.

In contrast with our initial expectation, the increase in niche breadth inside the estuary did not occur in similar magnitude for both mullet species. In fact, we observed that *M. liza* had a diet two-folds richer than *M. curema* in the estuary. A possible hypothesis to explain such between-species differences in diet richness could be related with the exploitation of distinct microhabitats within the estuary by each species. These microhabitats could be associated with differences in depths and/or particle size preferences (Cardona 2015). Prior studies in Colombia, for example, revealed distinct preference for mean particle size in the substrate associated with foraging activity, with lower particle sizes for *M. curema* (163 µm) and higher for *M. liza* (401 µm) (Osorio-Dualiby 1988). Such particle size preferences could reduce inter-specific competition between congeneric mullet species in estuaries (Marais 1980, Cardona 2015). Further, differences in the composition of microalgae in the stomach contents may indicate that the mullet species are foraging in distinct depths or at least in different regions of the estuary that have sediments with distinct properties and, thus, distinct compositions of microalgae. For instance, the microalgal community of intertidal sandy areas is composed of few species than the community that develops on fine sediments (i.e., epipelon) of deeper areas (Round 1984) and is dominated by mats of cyanobacteria (Stal 2001), as we found for *M. curema*. Moreover, some typical epipellic diatoms, such as *Nitzschia*, *Navicula*, *Tryblionella* and *Terpsinoe*, predominant in the sediments of another estuary in the same coastal plain (Bergesch et al. 1995, Silva et al. 2010), were not found in the stomach content of *M. curema*. This species also consumed a low diversity of flagellates, commonly found in the epipelon (Round 1984), in comparison to *M. liza*. Other explanation may be related with differences in zoogeographical distribution and residence time inside the estuary. In contrast to *M. curema* that has a more tropical distribution and peak in abundance in the studied subtropical estuary (29°C) only during warmer periods, *M. liza* occurs year-round in subtropical estuaries of the Southwestern Atlantic (Mai et al. 2018). It is possible, therefore, that for subtropical estuaries *M. liza* is better adapted than *M. curema* to explore different microhabitats and a great variety of food resources. Further field studies comparing microhabitat uses and foraging activity rates in subtropical vs. tropical estuaries are needed to evaluate these hypotheses.

Overall, dietary analyses based on stomach content analysis have often revealed a high similarity in the diet of sympatric mullet species in both their planktophagous fry stage and the juvenile/adult sedimentivorous stage (Cardona 2015). However, recently work based on stable isotope evidences has suggested food niche segregation among congeneric mul-

let species in estuaries (Le Loc'h et al. 2015), which agrees with our stable isotope results. Although we did not find differences in average nitrogen isotope ratios between species, we observed differences in carbon isotope ratios suggesting they assimilated distinct carbon primary sources inside the estuary, which could be associated with feeding on distinct groups of microalgae. In fact, our stomach content results showed that *M. liza* explores a wider range of microalgae in the estuary.

In summary, our evidences based on stable isotope and stomach content analyses revealed marked changes in the breadth and overlap of food niches of congeneric mullets between a marine surf-zone and an estuary. This pattern could be explained by spatial differences in food availability (especially regarding diversity of microalgae) between both habitats. At the marine surf-zone, both species explored the most abundant microalgae available, mostly the surf-zone diatom *Asterionellopsis* cf. *guyunusae*. However, after recruiting into the estuary, their diets became richer and their isotope niches segregated, probably due to the exploration of different estuarine microhabitats.

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## Appendix

**Table A1.** Number of specimens and total length of *Mugil curema* and *Mugil liza* used in each sample of stomach content analyzed at the marine surf-zone and estuary of Tramandaí-Armazém estuarine complex.

**Table A2.** Average numerical abundance of microalgae found in the stomach contents of *Mugil curema* and *Mugil liza* at the marine surf-zone and estuary of Tramandaí-Armazém estuarine complex.

**Figure A1.** Diet composition and relative frequency of occurrence of classes of microalgae (in addition to non-identified flagellates) observed in the stomach content of individuals of *Mugil curema* and *M. liza* sampled in the marine surf-zone and in the estuary of Tramandaí-Armazém estuarine complex. The file may be downloaded from [www.akademai.com](http://www.akademai.com).

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