

## Original Article

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# Use of stable isotopes in the evaluation of fish trophic guilds from a tropical hypersaline lagoon

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## Abstract

Environmental factors, size-related isotopic changes of the most abundant species and isotopic niche overlap were investigated using stable isotopes in order to evaluate spatial changes of fish trophic guilds in the Araruama Lagoon. Based on 440 muscle samples, 17 fish species were grouped into five trophic guilds. Mean salinity was above 40 at both sites sampled and a significant spatial difference was observed. The highest  $\delta^{13}\text{C}$  mean value was observed for an omnivorous species, whereas the lowest carbon signatures were found for the three fish species belonging to the planktivorous guild. Analysis of the carbon signature of fish species in lower trophic levels showed influence of salinity variation, whilst size appeared to play a role for others. A narrow  $\delta^{15}\text{N}$  difference was observed, but the piscivorous fish species showed the highest  $\delta^{15}\text{N}$  values. The Standard Ellipses Analysis (SEA) detected spatial differences and varying degrees of isotopic niche overlap among trophic guilds, but the percentages of most overlaps (<60%) suggest that, to some extent, the guilds had a unique isotopic niche space. These results are in agreement with data previously reported for the Araruama Lagoon, that found the same prey items with varying relative importance among the most abundant species. Further studies are necessary to understand how the interaction between salinity and other factors, such as migration patterns, changes in prey availability, changes in contribution of primary sources and changes in baseline isotopic signatures could affect the stable isotope signatures shown here.

## Introduction

Coastal lagoons are common ecosystems of global occurrence, occupying ~10% of the world's coastline (Moreno *et al.*, 2010). These ecosystems are transition zones between land and sea, known for high productivities (on average  $300\text{ g C m}^{-2}\text{ y}^{-1}$ ) supported by several factors, such as a high number of primary producers, high input of nutrients and efficient recycling of matter (Mouillot *et al.*, 2005; Vizzini & Mazzola, 2008). These ecosystems are exposed to wide variations in temperature and salinity (Bintz *et al.*, 2003), which in turn may have consequences for the local food web by changing its trophic structure (Bruno *et al.*, 2013; Rakhesh *et al.*, 2015).

Fish are present from lower to top levels of coastal lagoon food webs, and previous studies have shown that fish respond to spatial and/or temporal variations of abiotic factors and the biotic community in a number of different ways, from changes regarding habitat use to ontogeny and feeding habits (Daviás *et al.*, 2013; Mont'Alverne *et al.*, 2016; Sánchez-Hernández *et al.*, 2017; Muro-Torres *et al.*, 2019).

To help improve understanding of how changes in fish assemblages take place, the use of trophic guilds, defined by Root (1967) as 'a group of species that exploit the same resources in a similar way', has become popular. The benefits of this approach in trophic structure studies are two-fold. First, trophic guilds are considered basic building blocks of a community, meaning that by applying the concept it is possible to group species based on ecological roles that will ultimately represent the different compartments of a community. Second, it allows for easy comparison among studies, since trophic guilds of different communities are very similar even when species compositions differ (Simberloff & Dayan, 1991).

Stable isotope analysis (SIA) can be used in trophic studies to infer resource assimilation by consumers since, as isotopic ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) from producers pass from one trophic level to the next, their heavy isotope content tends to increase due to fractionation during metabolism (Peterson & Fry, 1987). While  $\delta^{13}\text{C}$  signatures of primary producers can be distinct enough for this element to serve as a tracer for different sources of carbon,  $\delta^{15}\text{N}$  values go through a constant enrichment from the lower to the upper levels in a chain or food web, therefore providing an indication of trophic level (Peterson & Fry, 1987; Post, 2002; Bearhop *et al.*, 2004).



The isotopic niche, useful as an indicator of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  dispersal and resource use (Newsome *et al.*, 2007; Jackson *et al.*, 2011), will change as a response to variations in environmental factors, trophic structure and resources exploitation over time and space. For example, Fry (2002) and Harrod *et al.* (2005) suggested a positive correlation between salinity and  $\delta^{13}\text{C}$  values of fish, while Davias *et al.* (2013) observed the influence of environmental factors over the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of three fish species in Chesapeake Bay, USA.

On the Brazilian coastline, lagoons are more frequent in the south-eastern and southern regions. Most of them are choked lagoons, i.e. connected to the sea by a single, narrow and shallow tidal channel, with restricted water exchange (Kjerfve, 1986). The Araruama Lagoon (AL) occupies an area of 220 km<sup>2</sup>, which makes it the largest permanent hypersaline lagoon in South America. It is surrounded by five towns with a permanent population of ~470,000 (IBGE, 2018), with the population increasing five-fold during the summer holidays (PROLAGOS, 2016). Domestic waste discharged into the lagoon, although treated by five sewage plants (two of them tertiary treatment), contains large amounts of nitrogen and phosphorus (Braga *et al.*, 2003), and blooms of macroalgae and cyanobacteria have been recorded (Clementino *et al.*, 2008). Despite these increased environmental troubles for the AL, few comprehensive studies are available. As a first attempt, using stable isotopes, to understand how the trophic fish guilds interact with each other and how salinity variation influences the fish population of the AL, the present study will provide valuable information for future attempts of ecosystem management.

Fish trophic guilds, with focus on ontogeny of the most abundant species and isotopic niche overlap were investigated using stable isotopes in order to evaluate the spatial changes of the fish trophic guilds from the AL. Specifically, this study aims to answer the following questions: (1) How do possible variations in fish assemblages, size of the focal species and environmental factors influence the isotopic niche of the trophic guilds? (2) Do trophic guilds show spatial changes regarding their isotopic niche? (3) Do these trophic guilds exhibit isotopic niche overlap and, if they do, what is the degree of the overlap?

## Materials and methods

### Study site and sampling

Sampling was conducted in the AL, located on the south-east coast of Brazil (22°49' and 22°57'S 42° and 42°23'W). The lagoon has an elliptic shape that could be divided into seven inlets limited by internal sand spits. The maximum width and length are 14 and 33 km, respectively. The lagoon has a microtidal regime and the mean depth is 2.5 m. The local climate is semi-arid with low rainfall volumes during the year (900 mm annual mean) and high evaporation rates (1400 mm) promoted by frequent and intense north-east winds (Barbieri, 1975). The only connection to the sea is by the 5.5 km long Itajuru Channel, located to the east of the lagoon, which associated with the climate, balance of rainfall/evaporation, low input of continental discharges (the local watershed has only two permanent low-volume discharge rivers), and long renewal time of its water (84 days) are responsible for the hypersaline (historical mean salinity of 52) regime waters (Kjerfve *et al.*, 1996; Moreira-Turcq, 2000; Souza *et al.*, 2003). The mean temperatures of the surface and bottom layer of the water column are 28.4°C and 24.4°C, respectively (André *et al.*, 1981).

Fish were collected at sampling stations positioned in two sectors of the lagoon (Figure 1), following the model proposed by Slack-Smith *et al.* (1977) that identified environmentally distinct

sectors in the lagoon based on depth, sedimentary features and salinity. Sampling stations were located in the outer (site 1) and inner zone (site 2). In order to obtain the best possible representation regarding length variability of the species, fish were sampled using a set of gill nets (15, 30 and 45 mm between opposing knots) in February, May, July and October 2011. The nets were placed at eight sampling stations and retrieved after 24 h. The fish sampled were frozen and taken to the laboratory for identification, measurement of total length (the distance between the tip of the snout to the tip of the longer lobe of the caudal fin) and subsequent muscle extraction.

At the same sites where fish sampling occurred, dissolved oxygen (mg l<sup>-1</sup>), temperature (°C), salinity and pH of the water were measured using a multi-parameter gauge (HANNA model HI9828). Due to a low catch during summer, a temporal analysis was not possible and only a spatial data analysis was performed. Fish species were grouped in trophic guilds following previous work developed in the same area (Saad, 2003, Almeida-Silva *et al.*, 2015, Cruz *et al.*, 2018). For logistical reasons, it was not possible to collect other components of the food web.

### Stable isotope analysis

Isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) was determined from a fragment of a dorsal muscle of each fish specimen. For each species, a subsample comprised of several specimens were chosen that covered the size variation observed to be representative for that species. Samples were freeze-dried, homogenized and ~0.5 mg was weighed and analysed with a continuous-flow mass spectrometer (Thermo Finnigan Delta V Plus) coupled to an elemental analyser and a Thermo ConFlo III interface. Results are expressed as delta ( $\delta$ ), in parts per thousand (‰), relative to Pee Dee Belemnite for  $\delta^{13}\text{C}$  and atmospheric N<sub>2</sub> for  $\delta^{15}\text{N}$ , according to equation (1):

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3 \quad (1)$$

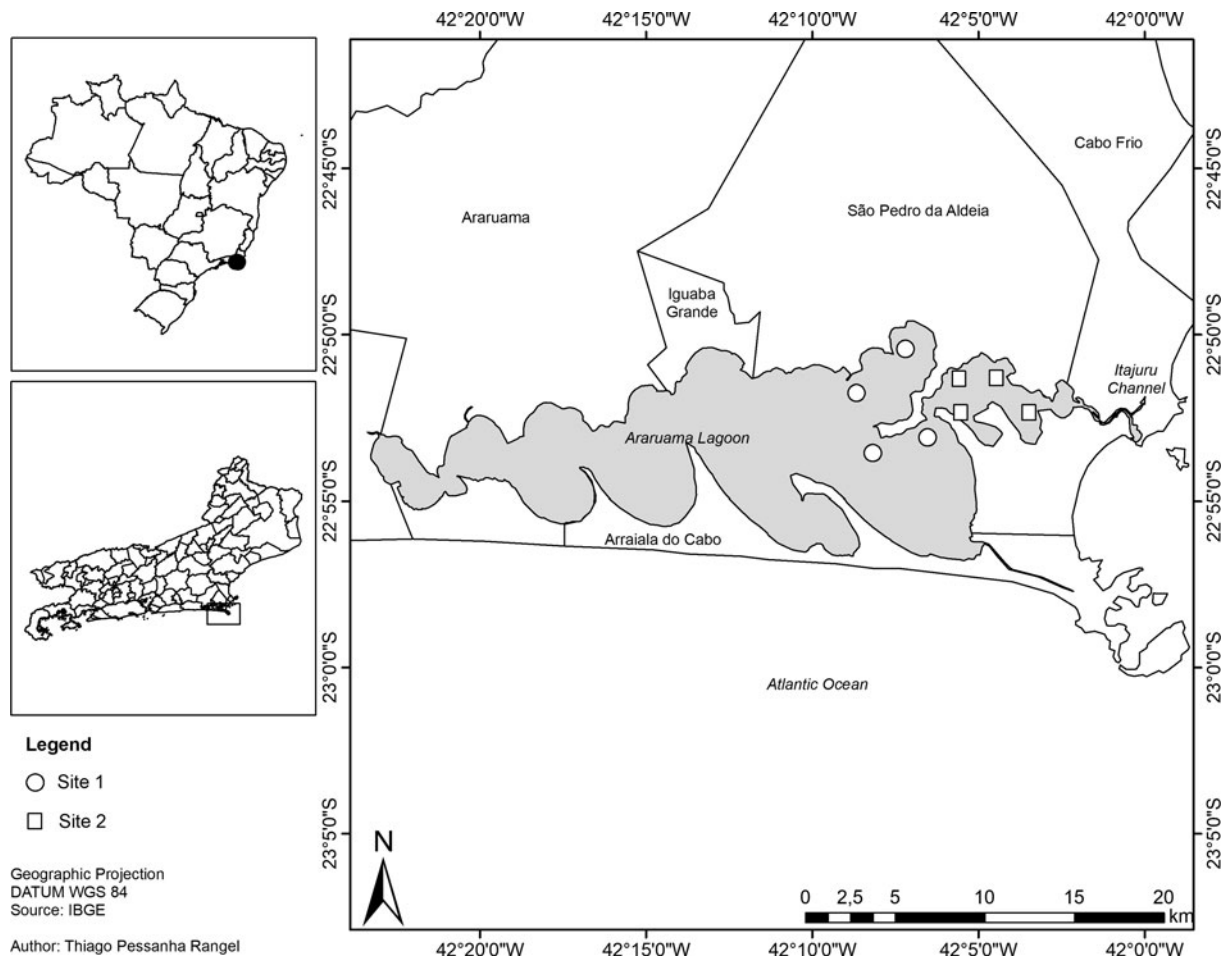
where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the corresponding ratios of rare to common isotopes (<sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N) in the samples and international standards, respectively (Peterson & Fry, 1987). The analytical precision was ± 0.3‰ for  $\delta^{15}\text{N}$  and 0.2‰ for  $\delta^{13}\text{C}$ .

Lipids are depleted in <sup>13</sup>C and high levels of this compound in fish samples, evidenced by a C:N ratio ≥ 3.5, may compromise  $\delta^{13}\text{C}$  results and interpretation (Kilujen *et al.*, 2006). However, elemental composition of the samples analysed indicated a low C:N ratio (≤ 3.5) and therefore a lipid extraction was not necessary.

### Statistical analyses

Principal component analysis (PCA) was applied on the environmental data matrix in order to identify spatial patterns of the water variables measured.

Differences in the isotopic values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) among trophic guilds were tested separately with non-parametric analysis of variance (Kruskal–Wallis), followed by a *posteriori* Dunn's test. For each abundant species (N > 30) with at least 10 specimens sampled on each site, a linear regression between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values was performed to check for a possible relationship between the variables. In addition, a linear regression between  $\delta^{15}\text{N}$  and total length was also performed to check for the possibility of changes in trophic level during the life cycle of the species, suggesting ontogeny. Differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the same trophic guild between sites were tested and a non-



**Fig. 1.** Map showing the location of Araruama Lagoon in Brazil (above on the left), its location in Rio de Janeiro State (below on the left) and the study area showing sampling stations at site 1 (outer zone) at site 2 (inner zone).

parametric test was performed when the homoscedasticity of variances was not observed.

To investigate the trophic structure of the fish assemblage in both sites, six community-wide metrics were applied and are described by Layman *et al.* (2007) as follows: (1)  $\delta^{15}\text{N}$  Range (NR): Distance (i.e. maximum  $\delta^{15}\text{N}$  – minimum  $\delta^{15}\text{N}$ ) between the two species with the most enriched and the most depleted  $\delta^{15}\text{N}$  values. NR represents the vertical structure (trophic position) within a food web. (2)  $\delta^{13}\text{C}$  Range (CR): Distance (i.e. maximum  $\delta^{13}\text{C}$  – minimum  $\delta^{13}\text{C}$ ) between the two species with the most enriched and the most depleted  $\delta^{13}\text{C}$  values. CR values would increase when there are multiple basal resources with varying  $\delta^{13}\text{C}$  values. (3) Total Area (TA): Convex hull area encompassed by all species in a  $\delta^{13}\text{C}$  –  $\delta^{15}\text{N}$  bi-plot space representing a measure of the total niche space occupied and an indicator for the total extent of trophic diversity in a food web. (4) Mean Distance to Centroid (CD): Average Euclidean distance of each species to the  $\delta^{13}\text{C}$  –  $\delta^{15}\text{N}$  centroid, described as the mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for all species in the food web. It provides a measure of the average degree of trophic diversity within a food web and it may be more reliable than TA when there are outlier species differentially affecting the latter. (5) Mean Nearest Neighbour Distance (MNND): Mean of the Euclidean distances to each species' nearest neighbour in a  $\delta^{13}\text{C}$  –  $\delta^{15}\text{N}$  bi-plot space. Food webs with proportionally more species characterized by similar trophic modes will exhibit an increased trophic redundancy and a smaller MNND. (6) Standard Deviation of Nearest Neighbour Distance (SDNND): A measure of the evenness of species packing in a  $\delta^{13}\text{C}$  –  $\delta^{15}\text{N}$  bi-plot space. Low SDNND values indicate a more

even distribution of trophic niches. Differences in the CD and MNND values between communities at sites 1 and 2 were tested separately with a *T*-test.

The corrected standard ellipse area (SEAc) was calculated to represent the isotopic niche width of the community of each site and of the different trophic guilds. The SEAc is insensitive to bias associated to small sample size and the ellipses were created with a 40% probability of containing data samples obtained posteriorly (Jackson *et al.*, 2011). An ellipses' overlap analysis via Bayesian model based on maximum likelihood estimation was also performed among trophic guilds for each site. All community metrics, SEAc and overlap percentages were calculated with the SIBER package (Stable Isotope Bayesian Ellipses tool in R, Jackson *et al.*, 2011) available in the software R (R Core Team, 2017).

Results were considered significant at  $P < 0.05$ . All statistical analyses were conducted using Statistica 8.0, Graphpad 3.1 and the supplement ActionStat for Microsoft Excel.

## Results

### Abiotic factors

Mean values of dissolved oxygen and temperature did not vary greatly between sites (Table 1), and a significant difference was not observed (*t*-test:  $t = 0.58$ ,  $df = 30$ ,  $P > 0.05$  and  $t = -1.20$ ,  $df = 30$ ,  $P > 0.05$  for DO and temperature, respectively). Mean salinity was above 40 at both sites and significantly higher at site 2 (Mann–Whitney *U* test,  $U = 186.5$ ,  $Z = -4.37$ ,  $P < 0.001$ ). Mean

**Table 1.** Mean values and standard deviation of abiotic factors for both areas sampled

Factors	Site 1	Site 2
Salinity	41.1 ± 3.3 <sup>a</sup>	45.0 ± 1.7 <sup>b</sup>
Dissolved oxygen (mg l <sup>-1</sup> )	7.1 ± 2.2	6.7 ± 2.3
pH	8.8 ± 0.3 <sup>a</sup>	8.6 ± 0.5 <sup>b</sup>
Temperature (°C)	24.0 ± 2.2	25.0 ± 2.4

Superscript letters show significant differences among sites ( $P < 0.05$ ). Data were pooled from all sample periods.

water pH was very similar between the two sampling areas. Still, a low within-site variation contributed to a significantly higher value at site 1 (Mann–Whitney  $U$  test,  $U = 354.0$ ,  $Z = 2.12$ ,  $P < 0.05$ ).

Eigenvalues greater than 1.0 were observed for two principal components (PC-1 and PC-2) that together explained 68.9% of the data variability (Table 2). Salinity was positively correlated to the first axis, whereas water pH was negatively correlated. PC1 showed a significant difference between the two areas ( $t = -2.26$ ,  $df = 30$ ,  $P < 0.05$ ). Dissolved oxygen and temperature were positively correlated to PC2, but its scores did not differ significantly between areas ( $t = 0.39$ ,  $df = 30$ ,  $P > 0.05$ ).

#### Fish species composition, $\delta^{13}C \times \delta^{15}N$ and TL $\times \delta^{15}N$ of the focal species

Overall, 440 muscle samples were analysed, representing 17 fish species that were grouped into five trophic guilds, as follows: three planktivorous, three invertivorous, one omnivorous, five piscivorous and five detritivorous (Table 3). The highest  $\delta^{13}C$  mean value was observed for an omnivorous species, *Diapterus rhombeus* (Cuvier, 1829), whereas the lowest carbon signatures were found for the three fish species belonging to the planktivorous guild, as follows: *Brevoortia pictinata* (Jenyns, 1842), *Opisthonema oglinum* (Lesueur, 1818) and *Sardinella janeiro* (Eigenmann, 1894). The species *O. oglinum* together with the invertivorous *Achirus lineatus* (Linnaeus, 1758) also showed the lowest  $\delta^{15}N$  value, while the highest  $\delta^{15}N$  signature was observed for the piscivorous fish *Menticirrhus americanus* (Linnaeus, 1758) (Table 3).

A  $\delta^{13}C \times \delta^{15}N$  bi-plot and a possible correlation between total length and  $\delta^{15}N$  was assessed for each abundant species ( $N > 30$ ) with at least 10 specimens sampled on each site (Figure 2). With the exception of *B. pictinata*, highest  $\delta^{13}C$  values observed for the species were found at site 1, as well as the lowest  $\delta^{15}N$  for all species. Specimens of *B. pictinata* and *Micropogonias furnieri* (Desmarest, 1823) showed a significantly higher TL and  $\delta^{15}N$  at site 2 (TL =  $218.5 \pm 11.0$  mm,  $\delta^{15}N = 12.7 \pm 0.4$  and TL =  $198.7 \pm 68.1$  mm,  $\delta^{15}N = 13.5 \pm 0.6$ , respectively), whereas mean  $^{13}C$  signatures observed for *E. argenteus*, *M. furnieri* and *E. gula* were significantly more enriched at site 1 ( $\delta^{13}C = -11.5 \pm 1.5$ ,  $\delta^{13}C = -12.7 \pm 0.8$ ,  $\delta^{13}C = -12.0 \pm 1.3$ , respectively) (Table 4). A positive significant correlation between total length and  $\delta^{15}N$  was observed for *B. pictinata*, *Eucinostomus argenteus* Baird & Girard, 1855 and *M. furnieri* (Figure 2).

#### Trophic guilds and niche overlap

Detritivorous fish were the most abundant at both sampling sites (Table 5), and represented about 60% ( $N = 236$ ) of the total individuals caught. Samples of omnivorous fish (both sites), and piscivorous fish (site 1), comprised less than 10 individuals.

**Table 2.** Results of the principal component axis (PCA) with correlations for each abiotic factor, eigenvalues ( $\lambda$ ) and percentage of explanation for each axis (correlation values above 0.4 are in bold according to Hair et al., 1984).

Factors	PC1	PC2
Salinity	<b>0.61</b>	-0.38
Dissolved oxygen (mg l <sup>-1</sup> )	0.31	<b>0.66</b>
pH	<b>-0.65</b>	-0.32
Temperature (°C)	-0.32	<b>0.56</b>
Eigenvalues ( $\lambda$ )	1.53	1.23
% of explanation	38.3	30.6

For both sampling sites, piscivorous fishes showed the highest  $\delta^{15}N$  ( $\delta^{15}N = 13.5 \pm 1.1$ ,  $\delta^{15}N = 14.4 \pm 1.4$  for site 1 and 2, respectively), which differed significantly from the remaining trophic guilds at site 2 (Kruskal–Wallis,  $MS = 0.93$ ,  $df = 128$ ,  $P < 0.001$ ). Apart from the omnivorous group, the lowest mean  $\delta^{15}N$  values of both sites were observed for planktivores, followed by the invertivorous guild (Table 5). Mean carbon values of the planktivorous guild were significantly lower than the remaining groups at site 2 ( $\delta^{13}C = -16.1 \pm 1.9$ , Kruskal–Wallis,  $MS = 3.23$ ,  $df = 128$ ,  $P < 0.001$ ). At site 1, the carbon signature of the planktivorous was only similar to the piscivorous fishes, probably due to a high standard deviation observed for both trophic groups (Table 5). Significant intra-guild differences in  $\delta^{13}C$  and  $\delta^{15}N$  values between sites were observed for the invertivorous guild ( $t$ -test,  $t = 6.78$ ,  $df = 101$ ,  $P < 0.0001$  and  $t$ -test,  $t = -2.70$ ,  $df = 101$ ,  $P < 0.01$ , respectively) and between  $\delta^{13}C$  values for the detritivorous guild ( $t$ -test,  $t = 7.21$ ,  $df = 234$ ,  $P < 0.0001$ ).

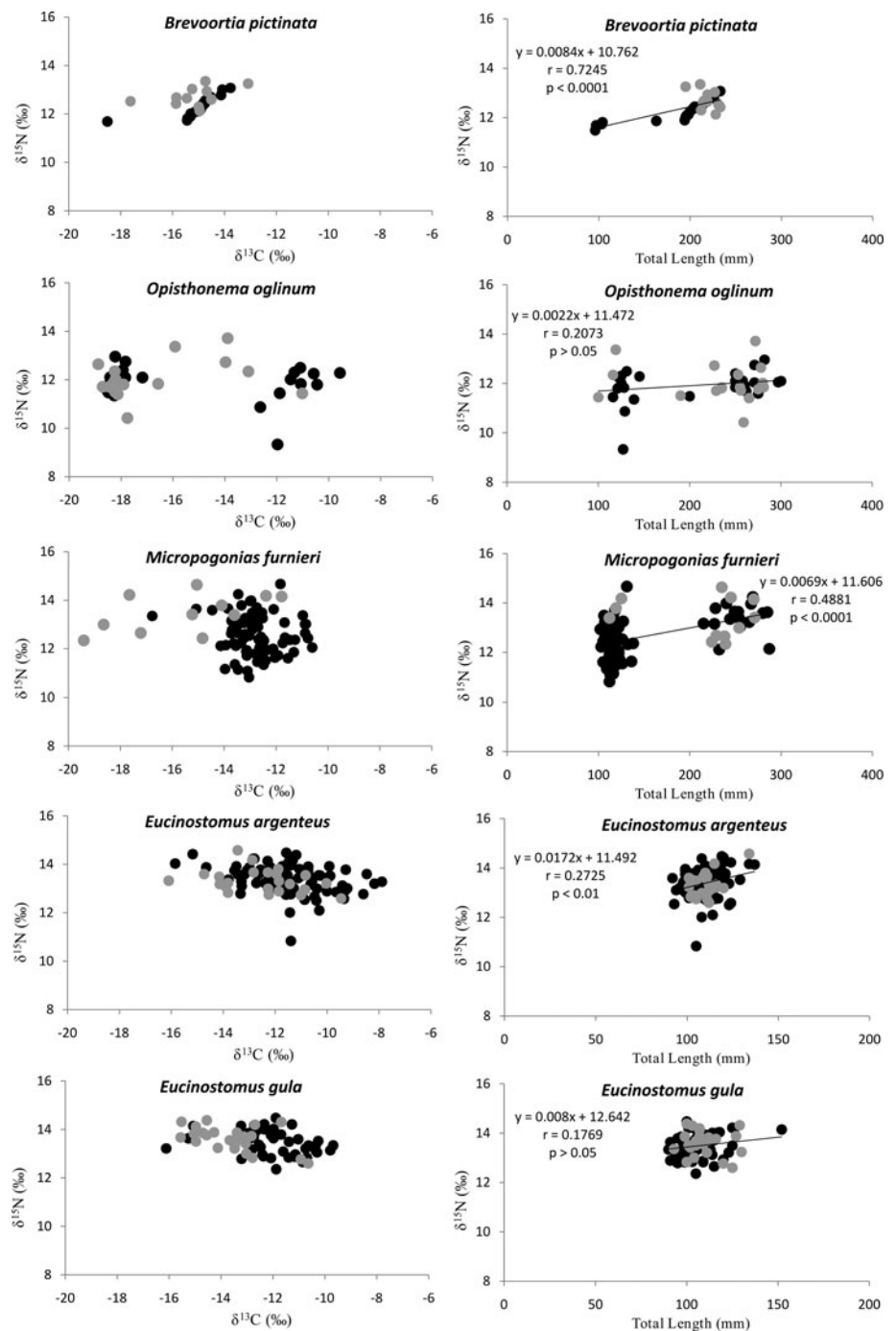
The spatial difference analysis of the trophic niche (Figure 3) showed that the detritivores showed the highest SEAc value ( $9.7\% \text{‰}^2$ ) at site 1, followed by the piscivorous ( $6.7\% \text{‰}^2$ ) and the planktivorous guild ( $6.0\% \text{‰}^2$ ). At site 2, the invertivores showed the largest trophic niche ( $7.7\% \text{‰}^2$ ), followed by the piscivorous ( $6.8\% \text{‰}^2$ ) and detritivorous guilds ( $3.7\% \text{‰}^2$ ). While the smallest SEAc at site 1 was observed for the omnivorous guild, it was not possible to calculate its trophic niche at site 2 due to a low number of specimens ( $N < 3$ ).

The isotopic niche overlap measures for site 1 (Table 6) showed a complete segregation between the omnivorous guild and all others, except the detritivorous guild. When compared with site 1, the detritivorous guild presented a smaller SEAc at site 2, but also a higher percentage overlap with the piscivorous ( $58.3\%$ ) ellipses. The highest overlap at site 2 was observed between the invertivorous and planktivorous guilds ( $59.0\%$ ), but only  $24.5\%$  of the ellipse of the latter overlapped with that of the former. As observed at site 1, the ellipses of the planktivorous and detritivorous guilds presented a small overlap at site 2 ( $3.4\%$ ). A complete segregation at site 2 was only observed between the piscivorous and the planktivorous guilds.

A SIBER analysis considering all the trophic guilds of each site as one community was performed, and the results showed higher values of SEAc, total area (TA),  $\delta^{13}C$  range (CR), and MNND at site 1 (Figure 4 and Table 7). In theory, these results indicated that the community at site 1 had a larger feeding plasticity (TA), associated with a higher variability of food sources ( $\delta^{13}C$  range). On the other hand, a wider  $\delta^{15}N$  range (NR) observed at site 2 suggested a higher range of trophic level. Significantly smaller MNND values were found at site 2 ( $t = -6.92$ ,  $df = 439$ ,  $P < 0.0001$ ) compared with site 1, indicating a higher trophic redundancy. A significant difference in CD values between sites was not observed ( $t = 0.19$ ,  $df = 439$ ,  $P > 0.05$ ).

**Table 3.** Guild and species from a hypersaline lagoon in south-east Brazil, including number of specimens caught, mean, standard deviation and range of total length (mm),  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰)

Guild	species	n	Occurrence (N)		Total length – mm (mean $\pm$ SD) (Min–Max)	$\delta^{13}\text{C}$ (mean $\pm$ SD) (Min–Max)	$\delta^{15}\text{N}$ (mean $\pm$ SD) (Min–Max)
			Site 1	Site 2			
Planktivore	<i>Brevoortia pictinata</i> (Jenyns, 1842)	32	21	11	198.1 $\pm$ 39.0 (96.0 to 233.0)	–14.9 $\pm$ 0.7 (–17.6 to –13.1)	12.4 $\pm$ 0.5 (11.4 to 13.3)
	<i>Opisthonema oglinum</i> (Lesueur, 1818)	24	12	12	214.3 $\pm$ 66.5 (116.0 to 297.0)	–16.0 $\pm$ 3.2 (–18.9 to –9.6)	11.9 $\pm$ 0.7 (9.3 to 13.7)
	<i>Sardinella janeiro</i> (Eigenmann, 1894)	1	1		181.0	–17.8	11.2
Invertivore	<i>Achirus lineatus</i> (Linnaeus, 1758)	2		2	93.0 $\pm$ 9.9 (86.0 to 100.0)	–13.9 $\pm$ 2.2 (–15.4 to –12.4)	11.9 $\pm$ 0.7 (11.5 to 12.5)
	<i>Micropogonias furnieri</i> (Desmarest, 1823)	98	8	12	149.8 $\pm$ 60.2 (102.0 to 287.0)	–13.1 $\pm$ 1.7 (–18.6 to –10.6)	12.6 $\pm$ 0.8 (10.8 to 14.7)
	<i>Pogonias chromis</i> (Linnaeus, 1766)	3	3		255.7 $\pm$ 16.0 (240.0 to 272.0)	–14.3 $\pm$ 3.0 (–17.4 to –11.6)	12.7 $\pm$ 1.7 (11.0 to 14.5)
Omnivore	<i>Diapterus rhombeus</i> (Cuvier, 1829)	9	7	2	86.0 $\pm$ 7.5 (75.0 to 94.0)	–11.5 $\pm$ 1.0 (–13.1 to –10.0)	11.4 $\pm$ 0.2 (11.2 to 11.7)
Piscivore	<i>Caranx latus</i> Agassiz, 1831	1	1		201.0	–12.5	13.9
	<i>Centropomus undecimalis</i> (Bloch, 1792)	1		1	241.0	–10.2	15.2
	<i>Elops saurus</i> (Linnaeus, 1766)	15	1	14	314.0 $\pm$ 86.5 (213.0 to 524.0)	–13.5 $\pm$ 0.8 (–14.7 to –11.4)	15.2 $\pm$ 0.7 (13.5 to 15.8)
	<i>Menticirrhus americanus</i> (Linnaeus, 1758)	17	2	15	182.0 $\pm$ 45.1 (123.0 to 281.0)	–13.6 $\pm$ 2.0 (–16.4 to –9.9)	13.4 $\pm$ 1.4 (9.7 to 15.3)
	<i>Trichiurus lepturus</i> (Linnaeus, 1758)	1		1	804.0	–14.1	16.0
Detritivore	<i>Eucinostomus argenteus</i> Baird & Girard, 1855	108	85	23	109.1 $\pm$ 8.8 (92.0 to 137.0)	–11.7 $\pm$ 1.6 (–16.1 to –7.9)	13.4 $\pm$ 0.6 (10.8 to 14.6)
	<i>Eucinostomus gula</i> (Quoy & Gaimard, 1824)	70	45	25	107.9 $\pm$ 11.3 (90.0 to 152.0)	–12.6 $\pm$ 1.5 (–16.1 to –9.7)	13.5 $\pm$ 0.5 (12.4 to 14.5)
	<i>Eugerres brasiliensis</i> (Cuvier, 1830)	2	1	1	193.0 $\pm$ 3.5 (190.0 to 195.0)	–12.5 $\pm$ 0.01 (–12.6 to –12.5)	14.2 $\pm$ 0.5 (13.8 to 14.6)
	<i>Mugil curema</i> (Valenciennes, 1836)	55	51	4	157.7 $\pm$ 43.8 (123.0 to 295.0)	–11.0 $\pm$ 2.2 (–18.4 to –7.5)	9.4 $\pm$ 1.4 (7.3 to 14.4)
	<i>Mugil liza</i> (Valenciennes, 1836)	1	1		270.0	–8.9	8.6



**Fig. 2.** Stable isotope bi-plot ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and correlation between total length (mm) and  $\delta^{15}\text{N}$  for *B. pictinata*, *O. oglinum*, *M. furnieri*, *E. argenteus* and *E. gula*.  $P < 0.05$  represents significant correlation (black circles – site 1, grey circles – site 2).

## Discussion

Stomach contents and biomass studies have been made at AL in order to understand how salinity would affect fish composition and trophic structure. While Almeida-Silva *et al.* (2015) concluded that hypersalinity was not a predominant factor influencing the trophic ecology of fish in this lagoon, Saad (2003) and Cruz *et al.* (2018) suggested that species composition, biomass and, consequently, food web structure across the ecosystem were under influence of a gradient that separated species according to their tolerances to salinity variation. In hypersaline lagoons, salinity is considered to be the main abiotic factor known to influence fish composition (Vegas-Sandejas & Santillana, 2004; Deegan *et al.*, 2010), and changes in species and guild composition will influence the food web structure, leading to feeding (stomach contents) and isotopic variation (O'Farrell *et al.*, 2014; Houssain *et al.*, 2016; Rosa *et al.*, 2016). The present case study

was the first attempt to identify possible fish trophic structure changes in AL using stable isotopes, and conclusions should be drawn with care.

A lower salinity year-round had been observed at site 1 (closer to the sea), with more constant, higher values at site 2 (Kjerfve *et al.*, 1996, Cruz *et al.*, 2018). These spatial trends regarding salinity were also observed herein, leading to a significant difference between sites ( $41.1 \pm 3.3$ ,  $45.0 \pm 1.7$ , Mann–Whitney  $U$  test,  $U = 186.5$ ,  $Z = -4.37$ ,  $P < 0.001$ , Table 1). Salinity variability can be relevant for the isotope values of the fish assemblage, since it may influence the carbon signature of the  $\text{CO}_2$ . The  $\text{CO}_2$ , responsible for the majority of the dissolved inorganic carbon (DIC) in the oceans, usually reflects the value of calcium carbonate (0–2‰). However, this is observed when the salinity is around 35 (Martinelli *et al.*, 2009). According to Fry (2002) and Gilikin *et al.* (2006), in regions with lower salinity values, and consequently less carbonate, DIC  $\delta^{13}\text{C}$  signatures tend to be more

**Table 4.** Main species from a hypersaline lagoon in south-east Brazil, including number of specimens caught, mean and standard deviation of total length (mm),  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰) for each site together with ANOVA results. Lower-case letters show significant difference of a species between two sites

Species	N	Mean $\pm$ SD					
		Total length (mm)		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
<i>Brevoortia pictinata</i>	32	188.3 $\pm$ 43.8 <sup>a</sup>	218.5 $\pm$ 11.0 <sup>b</sup>	-15.8 $\pm$ 3.8	-15.2 $\pm$ 1.1	12.3 $\pm$ 0.4 <sup>a</sup>	12.7 $\pm$ 0.4 <sup>b</sup>
<i>Opisthonema oglinum</i>	24	194.3 $\pm$ 68.9	234.1 $\pm$ 57.5	-15.4 $\pm$ 3.5	-16.9 $\pm$ 2.3	11.9 $\pm$ 0.7	12.0 $\pm$ 0.8
<i>Micropogonias furnieri</i>	117	136.9 $\pm$ 51.5 <sup>a</sup>	198.7 $\pm$ 68.1 <sup>b</sup>	-12.7 $\pm$ 0.8 <sup>a</sup>	-15.2 $\pm$ 2.2 <sup>b</sup>	12.5 $\pm$ 0.8 <sup>a</sup>	13.5 $\pm$ 0.6 <sup>b</sup>
<i>Eucinostomus argenteus</i>	108	108.7 $\pm$ 9.1	110.4 $\pm$ 7.3	-11.5 $\pm$ 1.5 <sup>a</sup>	-12.5 $\pm$ 1.6 <sup>b</sup>	13.4 $\pm$ 0.6	13.3 $\pm$ 0.5
<i>Eucinostomus gula</i>	70	107.1 $\pm$ 11.9	109.5 $\pm$ 9.9	-12.0 $\pm$ 1.3 <sup>a</sup>	-13.6 $\pm$ 1.3 <sup>b</sup>	13.4 $\pm$ 0.5	13.6 $\pm$ 0.5
ANOVA							
Total length (mm)		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$			
N	F	P	F	P	F	P	
<i>Brevoortia pictinata</i>	32	4.22	<0.05	0.24	0.62	7.91	<0.01
<i>Opisthonema oglinum</i>	24	1.80	0.18	2.55	0.12	0.48	0.49
<i>Micropogonias furnieri</i>	117	17.38	<0.0001	67.42	<0.0001	20.67	<0.0001
<i>Eucinostomus argenteus</i>	108	0.69	0.41	8.55	<0.01	0.25	0.61
<i>Eucinostomus gula</i>	70	0.77	0.38	23.52	<0.0001	1.84	0.19

**Table 5.** Trophic guilds from a hypersaline lagoon in south-east Brazil, including site of occurrence, number of specimens, and mean and standard deviation of  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰)

Site	N	Trophic guild	Mean $\pm$ SD $\delta^{13}\text{C}$ (‰)	Mean $\pm$ SD $\delta^{15}\text{N}$ (‰)
1	34	Planktivore	-15.5 $\pm$ 3.8 <sup>a</sup>	12.1 $\pm$ 0.8 <sup>ab</sup>
	89	Invertivore	-12.7 $\pm$ 1.1 <sup>bcs</sup>	12.5 $\pm$ 0.8 <sup>ab</sup>
	7	Omnivore	-11.7 $\pm$ 1.0 <sup>bcd</sup>	11.5 $\pm$ 0.2 <sup>b</sup>
	4	Piscivore	-13.8 $\pm$ 2.1 <sup>abc</sup>	13.5 $\pm$ 1.1 <sup>a</sup>
	183	Detritivore	-11.3 $\pm$ 1.7 <sup>ds</sup>	12.2 $\pm$ 2.1 <sup>ab</sup>
2	23	Planktivore	-16.1 $\pm$ 1.9 <sup>a</sup>	12.4 $\pm$ 0.7 <sup>ab</sup>
	14	Invertivore	-13.9 $\pm$ 1.8 <sup>bs</sup>	13.0 $\pm$ 1.0 <sup>bs</sup>
	2	Omnivore	-11.0 $\pm$ 1.4 <sup>c</sup>	11.3 $\pm$ 0.1 <sup>a</sup>
	31	Piscivore	-13.4 $\pm$ 1.6 <sup>c</sup>	14.4 $\pm$ 1.4 <sup>c</sup>
	53	Detritivore	-13.2 $\pm$ 1.6 <sup>cs</sup>	13.3 $\pm$ 0.7 <sup>b</sup>

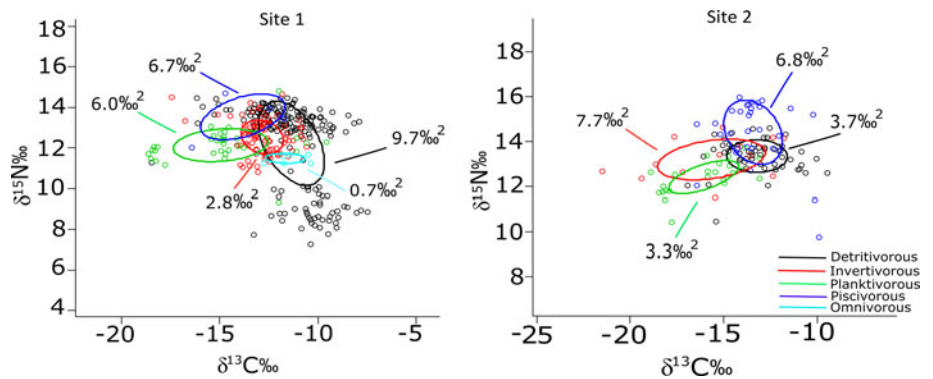
Superscript letters show a significant difference among guilds in each site. § indicates significant difference in  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰) for the same guild between sites.

negative, while a higher salinity leads to higher carbon isotope values. Consequently, salinity fluctuations will promote changes in primary carbon sources, and will eventually influence organisms on higher trophic levels. Still, the opposite was observed, since all three most abundant species (*E. gula*, *E. argenteus* and *M. furnieri*), with significant  $\delta^{13}\text{C}$  spatial difference (Table 4) showed lower values ( $\delta^{13}\text{C} = -13.6 \pm 1.3$ ,  $\delta^{13}\text{C} = -12.5 \pm 1.6$  and  $\delta^{13}\text{C} = -15.2 \pm 2.2$  for *E. gula*, *E. argenteus* and *M. furnieri*, respectively) in the more saline site 2 compared with site 1; this may be a consequence of a greater contribution of a  $\delta^{13}\text{C}$  depleted pelagic source (such as phytoplankton), a lower participation of enriched carbon sources (such as C4 plants) to the diet of the

fish species or even a combination of the two (Bouillon *et al.*, 2011). Also, a spatial shift in diets supported by  $\delta^{13}\text{C}$  significant different baselines was proposed by Litvin & Weinstein (2004) and Harrod *et al.* (2005) as a possibility for distinct  $\delta^{13}\text{C}$  signatures found for fish species along a salinity gradient. The latter, associated with different contributions of carbon sources between sites, would explain a significant spatial difference in  $\delta^{13}\text{C}$  values of *E. argenteus* and *E. gula*.

A greater salinity standard deviation at site 1 (Table 1) was expected, since it is considered a transitional zone between the sea and the inner lagoon (Bidegain & Bizerril, 2002). A salinity variation would mainly influence carbon primary sources and organisms in lower trophic levels (Doi *et al.*, 2013, Davias *et al.*, 2013), and this seems to be an explanation for the two highest  $\delta^{13}\text{C}$  standard deviations observed for the planktivores *B. pictinata* and *O. oglinum* collected at site 1 (Table 4). Considering this is the site closest to the sea, the greater  $\delta^{13}\text{C}$  standard deviation of these two planktivorous species could also be a consequence of signatures of specimens migrating from the sea, which would have associated with a lower constant salinity (~35) compared with site 2.

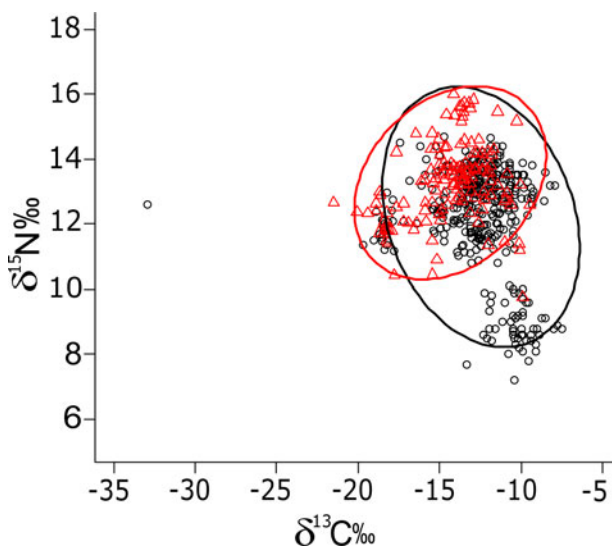
The effect of a salinity variation appeared to be less clear in species of other guilds, and maybe different factors should be considered. For example, size (total length) seemed to be relevant to variations found in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the invertivore *M. furnieri*. For this species, significantly smaller specimens (TL = 136.9  $\pm$  51.5) were observed at site 1, followed by a significant spatial difference in  $\delta^{15}\text{N}$  (Table 4). These results suggest that the larger, adult individuals, mainly observed at site 2, may occupy a higher trophic level, and a positive correlation between total length and  $\delta^{15}\text{N}$  (Figure 2) corroborates this assumption. Also, a study conducted at AL by Almeida-Silva *et al.* (2015) found a higher trophic plasticity in adults that could lead to the larger variation in  $\delta^{13}\text{C}$  values observed in site 2, if *M. furnieri* would begin feeding on prey linked to different carbon sources. In fact, the spatial



**Fig. 3.** Stable isotope bi-plots representing the isotopic niche areas of the trophic guilds at sites 1 and 2. Values in ‰<sup>2</sup> indicate the corrected standard ellipse areas (SEAc).

**Table 6.** Overlapping SEAc (%) between trophic guilds in both sites (the values indicate the percentage the ellipses from the guilds in the columns overlap with the ones from the guild in the row)

Site1	Detritivorous	Invertivorous	Planktivorous	Piscivorous	Omnivorous
Detritivorous	–	15.6	2.0	13.4	8.6
Invertivorous	54.2	–	38.3	33.5	0.0
Planktivorous	3.2	17.5	–	24.0	0.0
Piscivorous	32.8	14.8	25.1	–	0.0
Omnivorous	92.3	0.0	0.0	0.0	–
Site2	Detritivorous	Invertivorous	Planktivorous	Piscivorous	
Detritivorous	–	57.3	3.4	31.4	
Invertivorous	25.7	–	24.5	19.1	
Planktivorous	5.0	59.0	–	0.0	
Piscivorous	58.3	22.8	0.0	–	



**Fig. 4.** Stable isotope bi-plots representing the isotopic niche areas of the fish communities of both sampling sites in Araruama Lagoon. Values in ‰<sup>2</sup> indicate the corrected standard ellipse areas (SEAc).

differences observed for *M. furnieri* can be related to size, different prey being assimilated at both sites or a combination of these two, and this is yet to be investigated.

The SEAc analysis detected spatial differences and varying degrees of isotopic niche overlap among trophic guilds (Figure 3, Table 6), and this seems mainly promoted by a narrower standard deviation and spatial changes in δ<sup>13</sup>C values of the trophic guilds (Table 5). This was observed for the carbon

**Table 7.** Layman’s metrics obtained for the fish assemblages from both sampling areas in Araruama Lagoon, including number of specimens, δ<sup>13</sup>C‰ (CR range), δ<sup>15</sup>N‰ (NR range), total area (TA), mean distance to centroid (CD), mean nearest neighbour distance (MNND) and standard deviation of the mean nearest neighbour distance (SDNND)

	Site 1	Site 2
N	279	117
CR range	5.2	4.3
NR range	2.1	3.1
TA	7.1	4.5
CD	1.3	1.5
MNND	1.7	1.4
SDNND	0.8	0.6

signatures of the planktivorous species, where a wider standard deviation at site 1 led to a larger SEAc for the guild and a larger δ<sup>13</sup>C range (CR) at a community-level when compared with site 2 (Figure 4, Table 7). The invertivorous guild (composed mostly by *M. furnieri*) at site 2 showed a SEAc more than two times the area observed for site 1, probably due to the trophic plasticity of adult *M. furnieri* (Almeida-Silva *et al.*, 2015) reflected in the larger δ<sup>13</sup>C standard deviation of the guild in site 2. As a consequence, the degree of overlap between the invertivorous and the other guilds was higher (Figure 3, Table 6), as well as the trophic redundancy (MNND values, Table 7) of the community in that site. Compared with site 1, closer δ<sup>13</sup>C values and smaller SEAc for the detritivorous and the planktivorous guild in site 2 also



seems to be the reason for a larger degree of isotopic niche overlap between them.

Changes in the isotopic niches based on carbon values were expected due to different ranges observed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Considering the  $\delta^{13}\text{C}$  values published by Bouillon *et al.* (2011), a range between  $-7.5$  and  $-18.9\text{‰}$  shows that fish species from different trophic guilds prey spatially on carbon sources with distinct  $\delta^{13}\text{C}$  signatures. While for  $\delta^{15}\text{N}$ , a range between  $9.4$  and  $15.8\text{‰}$  suggests that fish species from AL have a limited variability regarding trophic position (Post, 2002). As an example, there were no significant differences in the  $\delta^{15}\text{N}$  mean values of the planktivorous, invertivorous, detritivorous and piscivorous guilds at site 1, contributing to a higher isotopic niche overlap among them (Shaw *et al.*, 2016).

Despite the narrow  $\delta^{15}\text{N}$  range observed and with the exception of a high degree of overlap ( $\sim 92\%$ ) between the omnivorous and the detritivorous guild at site 1 (Figure 3, Table 6), the overlap percentages ( $<60\%$ ) revealed by the SEA analysis suggest that, to some extent, the guilds have unique isotopic niche spaces. These results are in agreement with analyses of stomach content data from previous studies from AL that showed the same prey items with varying relative importance among the most abundant species. For example, while crustaceans and molluscs were the main prey categories observed for *M. furnieri* (Cruz *et al.*, 2018), they were less important but still present in the stomachs of the detritivorous *Eucinostomus* spp. and the planktivorous *O. glinum*. The same was observed regarding polychaetes, suggested as the main food item for *E. gula*, and rare but still present for *M. furnieri* and *O. glinum* (Almeida-Silva *et al.*, 2015; Cruz *et al.*, 2018). Further analyses regarding prey assimilation by the fish species are necessary in order to properly correlate stomach contents and isotopic signatures.

The present case study was the first attempt to evaluate the fish trophic structure from the AL using stable isotopes, and there was evidence of spatial differences. Salinity variation seemed to affect  $\delta^{13}\text{C}$  values of the two most abundant planktivorous species, and this influence can be seen at a guild and at a community-level. Size seemed to play an important role for *M. furnieri*, leading to a spatial difference in the isotopic niche of the invertivore guild. Spatial differences regarding isotopic niche overlap between guilds are most affected by  $\delta^{13}\text{C}$  values, while a narrow  $\delta^{15}\text{N}$  standard deviation detected suggests the presence of fish species from different trophic guilds in the same trophic level.

The results presented herein are the first evidence of how salinity influences fish composition and trophic structure at an isotopic level. Coupled with stomach content data from the literature, this study provides new data that may be used in the future to help decision making regarding ecosystem management. This is especially true for ecosystems under high anthropogenic pressure, such is the case with the AL. Nevertheless, further studies are necessary in order to understand how other factors may affect fish composition, feeding behaviour and stable isotope signature. Variation in isotopic signatures at different levels (species, trophic guild and community) may also be related to migration patterns, changes in prey availability, changes in contribution of primary sources, changes in baseline isotopic signatures or even any combination of the above. In the present study, data were not available for these assessments that need to be addressed in future work in order to elucidate the role of other factors in fish trophic structure of hypersaline lagoons.

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