

1 Title: Use of multielement stable isotope ratios to investigate ontogenetic movements of
2 *Micropogonias furnieri* in a tropical Brazilian estuary

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23

24 Abstract

25 The whitemouth croaker, *Micropogonias furnieri*, is a long-lived fish of high commercial
26 importance in the western Atlantic Ocean. Here, we used stable isotope ratios of carbon, sulfur,
27 and nitrogen and isotopic niche metrics (SIBER) to study feeding habits and track habitat use
28 by whitemouth croakers in Guanabara Bay, an estuary in Rio de Janeiro state, Brazil. Our
29 results highlighted size-related habitat segregation, with small juvenile fishes (<30 cm) residing
30 mostly inside estuaries and large adult fishes (>60 cm) feeding mainly in Continental Shelf
31 waters. Medium adult fishes (30-60 cm) appear to feed in multiple coastal and Continental
32 Shelf habitats. Moreover, their feeding ecology showed strong temporal differences, linked
33 with seasonal and, to a lesser extent, interannual variation in oceanographic features of the
34 ecosystem in which they live. Overall, these differences in ecological features suggest that (1)
35 adult and juvenile whitemouth croakers should be treated as different components of the food
36 web and (2) the conservation of these habitats should be prioritized to better manage and sustain
37 the coastal fisheries in Guanabara Bay.

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40 Keywords: whitemouth croaker, Guanabara Bay, Brazilian estuary, SIBER, isotopic niche.

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43 Introduction

44

45 The Sciaenidae, popularly known as croakers or drums due to the sound they produce using
46 muscles associated with the swim bladder, are a widely distributed fish family throughout the
47 world (Nelson 2006). Croakers constitute commercially important resources in the western
48 Atlantic Ocean and are the major fishery resource in Brazil, as they represent 22% of marine

49 and 9% of freshwater fish landings (Chao *et al.* 2015). The whitemouth croaker,
50 *Micropogonias furnieri* (Desmarest 1823) (Perciformes, Sciaenidae), is widely distributed in
51 the coastal waters of the western Atlantic Ocean, occurring from the Yucatán Peninsula
52 (Mexico, 28° N) to the Gulf of San Matías (Argentina, 41° S) (Isaac 1988; Vazzoler 1991).
53 However, despite its wide latitudinal distribution, this species becomes abundant from Rio de
54 Janeiro state (RJ) at 23° S latitude southwards to the shelf of Uruguay (Vazzoler 1991). In
55 Brazil, it constitutes one of the main demersal resources with an average annual catch of 42
56 000 metric tons between 2000 and 2015 (Chao *et al.* 2015; FAO 2017). In addition, the species
57 also represents one of the most important fishery resources in coastal demersal fisheries in
58 Uruguay and Argentina (Vasconcellos and Haimovici 2006; FAO 2017).

59 The whitemouth croaker occurs in coastal waters over large range of salinities (0.1-35) and
60 temperature (11-31.6°C) (Vazzoler 1991; Carneiro *et al.* 2005), inhabiting over sandy and
61 muddy bottoms, mainly at depths of up to 50 m and occasionally to 100 m (Carneiro *et al.*
62 2005). The population distribution of whitemouth croaker in the southeastern and southern
63 regions of Brazil has been investigated since the 1970s. Studies on morphometric and
64 biological features (Vazzoler 1991; Puchnick-Legat and Levy 2006) and population genetics
65 using protein allozymes (Vazzoler *et al.* 1985; Vazzoler and Phan 1989) have suggested the
66 presence of two separate populations: one located between 23°S and 29°S (population I -
67 southeast) and another between 29°S and 33°S (population II - south) (Vazzoler 1971, 1991;
68 Isaac 1988). Recent genetic work using polymorphic nuclear *loci* (microsatellite and intron size
69 polymorphisms) have confirmed clear differences between the two populations, reinforcing
70 that they should be considered as distinct stocks for management purposes (Vasconcellos *et al.*
71 2015).

72 Several aspects of the whitemouth croaker's life history have been well studied in population
73 II, including its food and feeding habits, age and growth, reproductive biology, and length

74 distribution (Muelbert and Weiss 1991; Macchi *et al.* 2003; Albuquerque *et al.* 2009, 2010,
75 2012; Olsson *et al.* 2013; Costa *et al.* 2014). Studies on the movement patterns of population
76 II have shown seasonal migrations by adults to open water following the displacement of the
77 convergence between the Brazilian and Falkland currents, with higher densities between 31°S
78 and 35°S in the summer and between 27°S and 31°S in the winter (Isaac 1988). In addition to
79 these seasonal migrations, in temperate regions of Brazil (population II), whitemouth croaker
80 displays ontogenetic habitat shifts being estuarine dependent during early life stages, while
81 adults can spawn in the estuaries or in shallow coastal areas (Mendoza-Carranza and Vieira
82 2008; Albuquerque *et al.* 2012; Costa *et al.* 2014). Most fish species undertake movement
83 patterns during their life cycle and defining these ontogenetic movements plays a key role in
84 understanding their ecology, which is important for both conservation and exploitation
85 management purposes. Although knowledge has been generated on whitemouth croaker
86 biology in Brazilian waters (population II), information is still scarce for population I, whose
87 distribution includes RJ (Isaac 1988; Vazzoler 1991).

88 To investigate ontogenetic movements in fishes, a diversity of approaches can be used varying
89 from abundance and size frequency distributions to methods using artificial tags and natural
90 tags, *i.e.*, trace elements and stable isotopes (Gillanders *et al.* 2003; Gillanders 2009). Although
91 information obtained from natural tags can be more equivocal to interpret than that from
92 artificial tags, the use of natural tags has increased in recent years, since this approach is
93 suitable for use with early larval stages and small specimens as well as larger fish (Thorrold *et*
94 *al.* 2002; Reis-Santos *et al.* 2015).

95 Stable isotope ratios of light biogenic elements have proved to be valuable tools for the study
96 of trophic relationships in marine ecosystems as well as providing insights into animal
97 migration (Hobson 1999; Das *et al.* 2000; Kolasinski *et al.* 2009; Botto *et al.* 2011; MacKenzie
98 *et al.* 2011; Bisi *et al.* 2012, 2013). Stable isotope ratios serve as natural tags, as their natural

99 distribution reflects and integrates the history of physical and metabolic processes of the
100 environment (Peterson and Fry 1987; Herzka 2005). The isotopic ratios of carbon (typically
101 expressed as $\delta^{13}\text{C}$) are used to establish the sources of organic matter that support food webs
102 (McCutchan *et al.* 2003). The fractionation of carbon isotopes during photosynthesis by the
103 producers of the food web helps to identify food sources originating from different systems,
104 *i.e.*, terrestrial versus marine, coastal versus oceanic, or benthic versus pelagic (Peterson and
105 Fry 1987; Boutton 1991). Also, sulfur isotope ratios ($\delta^{34}\text{S}$ values) can be used as a second tracer
106 of organic matter sources that is independent of the carbon isotopic distribution (Connolly *et*
107 *al.* 2004). Nitrogen stable isotope ratios ($\delta^{15}\text{N}$ values) can also be used to trace organic matter
108 sources but are more commonly applied to provide information on the position occupied by a
109 species in a trophic web, as nitrogen isotopes show predictable stepwise increases in values
110 from prey to consumer (DeNiro and Epstein 1981).

111 Through the measurement of stable isotopes of carbon, nitrogen, and sulfur, this study aimed
112 to investigate differences in spatial distribution patterns among three size classes of
113 whitemouth croaker in Guanabara Bay estuary, an important fishery area in RJ. Defining the
114 preferred habitats of the different whitemouth croaker size classes within the estuary may help
115 to understand ontogenetic movement patterns during their life cycle, providing information for
116 more efficient management of the fishery.

117

118 Materials and Methods

119

120 Study area

121 The Rio de Janeiro state (RJ) coast, in southeastern Brazil, is under high anthropogenic pressure
122 because it is an important urban and industrial center for Brazil (Molisani *et al.* 2007; Baptista-
123 Neto *et al.* 2013). Despite the anthropogenic disturbance, RJ ranks in third place amongst

124 Brazilian marine fisheries, with the whitemouth croaker constituting one of the most important
125 target species in the state (FIPERJ 2015).

126 The Guanabara Bay (GB) (22°03'S-22°41'S, 43°01'W-43°16'W) (Fig. 1–IBGE 2016), located
127 within the metropolitan area of RJ, has remarkable ecological, social, and economical
128 importance for the Brazilian southeastern region. Despite the impact of diverse anthropogenic
129 activities, including input of sewage and industrial waste in its drainage basin, GB is one of the
130 most species-rich tropical estuarine ecosystems on the Brazilian coast and continues to sustain
131 important fisheries and a large number of fishers (Jablonski *et al.* 2006; Dorneles *et al.* 2013;
132 Silva Jr. *et al.* 2016). GB has a total surface area of 384 km² extending a maximum distance of
133 28 km on its east-west axis and 30 km on its north-south axis, with depths ranging from less
134 than 1 m in marginal regions to more than 50 m in the central channel. The bay holds about 2
135 x 10⁹ m³ of water, with water volume maintained by the inflow from 91 rivers and channels, in
136 addition to the exchange with the Atlantic Ocean through the narrow bay entrance in
137 semidiurnal tide cycles with an amplitude of up to 1.4 m (Kjerfve *et al.* 1997; Catanzaro *et al.*
138 2004).

139

140 Sampling

141 Ethical approval was not required for this study, as all sampled whitemouth croaker samples
142 were obtained from commercial fishery landings in GB. Total length was used to divide the
143 samples into different size classes. Whitemouth croaker from population I are reported to reach
144 first sexual maturity (*i.e.*, L₅₀, length at which 50% of the fish are mature) at 27.5 cm (Vazzoler
145 1991); however, studies from different estuaries along the southeastern Brazilian coast have
146 reported a range of L₅₀ from 26.9 to 36.9 cm (Carneiro *et al.* 2005; Santos *et al.* 2015). Thus,
147 a total length of 30 cm was used in the present study as the approximate length for separating
148 fish into juveniles (< 30 cm) or adults (≥ 30 cm). In addition, a third size class was used for the

149 larger adult fish (> 60 cm) caught in the outer part of GB, since they exceed the theoretical
150 maximum size from population I (Vazzoler 1971).

151 In addition to sourcing fish from commercial fishers, small specimens (< 30 cm) were collected
152 by a bottom otter trawler dedicated to shrimp catching from December to March, *i.e.*, during
153 the austral summer (expressed from now on as summer) in 2013 and 2014 as well as from July
154 to August, *i.e.*, during the austral winter (expressed from now on as winter) in 2014. These
155 correspond to the two main seasons in RJ, *i.e.*, the warm rainy season and the cold dry season,
156 respectively (Paranhos and Mayr 1993). Each individual was weighed, measured, and
157 dissected. The aliquots sampled were kept frozen (-20°C) until analysis.

158 Fishes were separated into groups according to sampling season (summer and winter),
159 sampling year (2013 or 2014), and size class (small: < 30 cm, medium: 30-60 cm, and large: >
160 60 cm) and the numbers of specimens (*n*) in each group are presented in Table 1.

161

162 Stable isotope measurements

163 Dorsal white muscle samples were oven-dried over 3 days to constant mass at 60°C before
164 being ground into a homogeneous powder using a mortar and pestle. Approximately 4 mg of
165 dry powdered material was used for stable isotope analysis. Measurements of stable isotope
166 ratios were performed via continuous flow - elemental analysis - isotope ratio mass
167 spectrometry (CF-EA-IRMS) at the Laboratory for Oceanology, University of Liege
168 (Belgium), using a vario MICRO cube C-N-S elemental analyzer (Elementar Analysensysteme
169 GMBH, Hanau, Germany) coupled to an IsoPrime100 isotope ratio mass spectrometer
170 (Isoprime, Cheadle, United Kingdom). Isotopic ratios were expressed using the widespread
171 delta (δ) notation (Coplen 2011). Sucrose (IAEA-C-6: $\delta^{13}\text{C} = -10.8\text{‰} \pm 0.5\text{‰}$; mean \pm SD),
172 ammonium sulfate (IAEA-N-2: $\delta^{15}\text{N} = 0.4\text{‰} \pm 0.2\text{‰}$; mean \pm SD), and silver sulfide (IAEA-
173 S-1: $\delta^{34}\text{S} = -0.3\text{‰}$) were used as certified reference materials for measurement of stable isotope

174 ratios of carbon, nitrogen, and sulfur, respectively. All of these reference materials
175 (International Atomic Energy Agency, Vienna, Austria) are calibrated against the international
176 references Vienna Pee Dee Belemnite (for carbon), Atmospheric Air (for nitrogen), and Vienna
177 Canyon Diablo Troilite (for sulfur). Standard deviations on multibatch replicate measurements
178 of internal laboratory standards (sulfanilic acid and animal muscle tissue) analyzed interspersed
179 with samples (one replicate of each standard every 15 analyses) were 0.2‰ for both $\delta^{13}\text{C}$ and
180 $\delta^{15}\text{N}$ and 0.4‰ for $\delta^{34}\text{S}$, respectively.

181

182 Statistical analyses and data processing

183 Fishes were grouped according to sampling location, season, year, and size class (see Table 1).
184 Intergroup differences in carbon, nitrogen, and sulfur stable isotope ratios were investigated
185 through multiple comparisons. Since Shapiro-Wilk normality tests revealed that several data
186 sets did not follow a Gaussian distribution, nonparametric procedures were applied, *i.e.*, the
187 Mann-Whitney U test when two groups were compared and Kruskal-Wallis one-way ANOVA
188 followed by Dunn's post hoc test when three groups were compared. All tests were performed
189 using the statistical software GraphPad Prism 5.0.

190 For fish groups with $n \geq 6$, ecological niches were explored using the SIBER (Stable Isotope
191 Bayesian Ellipses in R) method (Jackson *et al.* 2011). This approach involves the use of
192 standard ellipses (bivariate equivalent of standard deviation) (Jackson *et al.* 2011) to define
193 isotopic niches, *i.e.*, the space occupied by an animal population in a bivariate isotopic space.
194 Since variation in the isotopic composition of animals (*i.e.*, position of points in the isotopic
195 space) is driven by both consumed prey items (Jackson *et al.* 2011; Layman and Allgeier 2012)
196 and habitat use (Flaherty and Ben-David 2010), this isotopic niche can be used as a proxy of
197 the realized ecological niche. Size and position of ellipses carry complementary information
198 about animal ecology. A larger ellipse suggests that an animal population commonly uses more

199 trophic and habitat resources. Overlap between ellipses associated with different populations
200 suggests that these populations partly exploit the same food and (or) habitat resources. The
201 bigger the overlap, the more resources are shared by the two populations. Here, SIBER 2.0.3
202 was run in the R 3.2.2 statistical environment (R Core Team 2015). Two separate sets of
203 ellipses were constructed: one using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data and another using $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ data.
204 The areas of all ellipses were estimated using the SEA_c correction for small sample sizes, as
205 outlined in Jackson *et al.* (2011). The areas of these ellipses were also estimated using Bayesian
206 modelling (SEA_B , 10^6 iterations) and direct intergroup pairwise comparisons of SEA_B were
207 performed. Model solutions were presented using credibility intervals of probability density
208 function distributions. Pairwise comparisons were considered meaningful when the probability
209 of occurrence exceeded 95%.

210

211 Results

212

213 Stable isotopes ratios of carbon, nitrogen, and sulfur in whitemouth croaker

214

215 There were no significant differences in $\delta^{13}\text{C}$ values between winter and summer 2014 for the
216 small size class (Mann-Whitney test, $p > 0.05$) (Fig. 2A) or for the medium size class between
217 all three sampling periods (Kruskal-Wallis, $p > 0.05$) (Fig. 2A). As individuals from the large
218 class were only sampled in winter 2014, interseasonal comparisons between sampling periods
219 were not possible for this group. Significant differences in $\delta^{13}\text{C}$ values were found between
220 size classes in GB with less ^{13}C -depleted values recorded for small whitemouth croaker in
221 winter 2014 ($-15.2 \pm 0.9\%$; Dunn's test, $p < 0.05$) and summer 2014 ($-15.3 \pm 1\%$; Mann-
222 Whitney test, $p < 0.05$) (Fig.2A).

223 The $\delta^{15}\text{N}$ data for whitemouth croaker in GB indicated that small and medium fish were ^{15}N -
224 enriched in summer compared to winter 2014. $\delta^{15}\text{N}$ values for small fish in summer 2014 were
225 significantly higher than in winter 2014 (Mann-Whitney test, $p < 0.05$) (Fig. 2B). The same
226 pattern of ^{15}N enrichment in summer was also observed for the medium size class when
227 comparing the three sampling periods in GB (Kruskal-Wallis, $p < 0.05$); however, the $\delta^{15}\text{N}$
228 values were significantly higher in summer 2013 ($16.6 \pm 0.6\text{‰}$) than in summer 2014 ($14 \pm$
229 0.8‰) (Dunn's test; $p < 0.05$) (Fig. 2B). Comparisons between the different size classes within
230 the same sampling period demonstrated that the smallest whitemouth croaker were
231 significantly ^{15}N -depleted in comparison to medium and large specimens (winter: Dunn's test,
232 $p < 0.05$; summer: Mann-Whitney test, $p < 0.05$) (Fig.2B).

233 Examination of the sulfur isotope data demonstrated that fish from summer 2014 presented
234 significantly higher $\delta^{34}\text{S}$ values than those from winter 2014. This pattern was observed for
235 both small (summer 2014: $14.5 \pm 1.2\text{‰}$, winter 2014: $13.5 \pm 1.1\text{‰}$; Mann-Whitney test, $p <$
236 0.05 (Fig. 2C) and medium size classes (summer 2013: $14.5 \pm 0.6 \text{‰}$, winter 2014: $13.8 \pm 2\text{‰}$,
237 summer 2014: $15 \pm 0.8 \text{‰}$; Dunn's test, $p < 0.05$) (Fig. 2C). Although the large fish were only
238 sampled in winter 2014, they showed significantly higher $\delta^{34}\text{S}$ values compared to small and
239 medium size classes from the same sampling season (Dunn's test, $p < 0.05$) (Fig. 2C).

240

241 Stable isotope ellipses - relative positions

242 SIBER results suggested that the core isotopic niche of medium whitemouth croakers sampled
243 in summer 2013 was markedly separated from that of any other group when looking at carbon
244 and nitrogen ellipses (Fig. 3A, black ellipse) but was strongly overlapping with most groups
245 when considering the carbon and sulfur ellipses (Fig. 3B). When compared to carbon and sulfur
246 ellipses associated with the same size class (*i.e.*, medium fish), the summer 2013 ellipse was
247 completely included within the winter 2014 ellipse (Fig. 3B, green ellipse) and overlapping for

248 0.72 ‰² (*i.e.*, 79% of its area) with summer 2014 (Fig. 3B, blue ellipse). The overlap between
249 medium fish sampled in winter and summer 2014 was considerable for both carbon and
250 nitrogen (1.42 ‰², *i.e.*, 53% of the smallest ellipse area) (Fig. 3A, green and blue ellipses) and
251 carbon and sulfur (2.45 ‰², *i.e.*, 63% of the smallest ellipse area) (Fig. 3B, green and blue
252 ellipses) core isotopic niches. The carbon and nitrogen ellipses for the small size class in winter
253 (Fig. 3A, red ellipse) and summer 2014 (Fig. 3A, grey ellipse) were strongly overlapping (2.69
254 ‰², *i.e.*, 78% of the smallest ellipse area). Overlap between isotopic niches for small fishes
255 between seasons was also present, to a lesser extent, for carbon and sulfur ellipses (overlap:
256 1.40 ‰², *i.e.*, 43% of the smallest ellipse area) (Fig. 3B, red and grey ellipses). In winter 2014,
257 both carbon and nitrogen (Fig. 3A) and carbon and sulfur (Fig. 3B) ellipses of small (red
258 ellipses) and medium (green ellipses) fish showed considerable overlap (1.87 ‰² or 40% of
259 the smallest ellipse area for carbon and nitrogen and 1.92 ‰² or 59% of the smallest ellipse
260 area for carbon and sulfur, respectively) (Fig. 3). This trend seemed less strong in summer
261 2014, where overlap between small (grey ellipses) and medium (blue ellipses) fish was more
262 moderate (0.58 ‰² or 22% of the smallest ellipse area for carbon and nitrogen and 1.76 ‰² or
263 46% of the smallest ellipse area for carbon and sulfur, respectively) (Fig. 3). In winter 2014,
264 overlap between isotopic niches of small (red ellipses) and large (orange ellipses) fish was very
265 low for carbon and nitrogen ellipses (0.08 ‰² or 4% of the smallest ellipse area) (Fig. 3A) and
266 nil for carbon and sulfur ellipses (Fig. 3B), respectively. Finally, in the same season, ellipses
267 associated with medium (green) and large (orange) fish were strongly overlapping when
268 looking at carbon and nitrogen data (1.72 ‰² or 82% of the smallest ellipse area) (Fig. 3A) but
269 much more separated when looking at carbon and sulfur data (overlap: 0.59 ‰² or 21% of the
270 smallest ellipse area) (Fig. 3B).

271

272 Standard ellipse areas

273 Areas of the standard ellipses associated with each fish group varied widely, with SEAc values
274 ranging from 0.57 ‰² (carbon and nitrogen ellipse of medium fishes from winter 2014) (Figs.
275 3A and 4A) to 11.15 ‰² (carbon and sulfur ellipse of medium fishes from winter 2014) (Figs.
276 3B and 4B). Medium fishes from winter 2014 had the widest isotopic niche by far, with a larger
277 niche than any other group in nearly all model solutions (99.80% of model solutions for carbon
278 and nitrogen data, > 99.99% of model solutions for carbon and sulfur data) (Fig. 4).
279 Interestingly, the model suggested that the isotopic niche of medium fishes sampled in summer
280 was larger in 2014 than in 2013 for both carbon and nitrogen and carbon and sulfur data (>
281 99.99% of model solutions in each case) (Fig. 4). The carbon and nitrogen isotopic niche of
282 small fishes was larger in winter 2014 than in summer 2014 in 99.81% of model solutions (Fig.
283 4A), but this trend was not seen in carbon and sulfur niches, as the winter ellipse was only
284 larger in 54.22% of model solutions (Fig. 4B). Differences in niche size between size classes
285 were observed for winter 2014, as medium fish had a larger ellipse than small and large ones
286 for both carbon and nitrogen and carbon and sulfur data in over 99.80% of model solutions. In
287 addition, small fish in winter 2014 had a larger isotopic niche than large fish in 97.24% of
288 model runs built using carbon and nitrogen data (Fig. 4A), but this trend was not observed in
289 carbon and sulfur niches (only 54.22% of model solutions). Finally, the converse pattern to that
290 observed in winter 2014 was found in summer 2014 with no intersize classes differences in
291 niche size observed, as SEA of small and medium fish differed in only 50.75% and 71.35% of
292 model solutions for carbon and nitrogen and carbon and sulfur data, respectively (Fig. 4).

293

294 Discussion

295

296 According to Herzka (2005), the isotopic composition of an individual can provide a record of
297 its migration history, constituting also a valuable tool to distinguish subpopulations of the same

298 species that vary in their patterns of habitat use and migration. Overall, comparisons of $\delta^{13}\text{C}$,
299 $\delta^{34}\text{S}$, and $\delta^{15}\text{N}$ data between size classes highlighted that the whitemouth croaker,
300 *Micropogonias furnieri*, displays size-related habitat segregation in Guanabara Bay (GB).
301 The whitemouth croaker is a partial spawner that reproduces year-round (Isaac-Nahum and
302 Vazzoler 1987; Vazzoler 1991), and juvenile whitemouth croakers are regularly present in GB
303 throughout the whole year (Andrade-Tubino *et al.* 2009). Studies on whitemouth croaker
304 distribution have shown that juveniles (small: < 30 cm) are mostly present inside the estuaries
305 (Andrade-Tubino *et al.* 2009; Mulato *et al.* 2015). Thus, the ^{13}C -enriched values presented by
306 juvenile whitemouth croaker (Fig. 2A) may be the result of feeding mostly inside GB.
307 Dissimilarities in $\delta^{13}\text{C}$ values are usually associated with differences in energy sources between
308 distinct food webs with benthic and inshore trophic chains typically ^{13}C enriched compared to
309 pelagic and offshore ones (Peterson and Fry 1987; Herzka 2005). Stable isotope studies
310 focusing on GB are scarce, particularly regarding potential whitemouth croaker prey items and
311 (or) other fish species. Nevertheless, previous studies focusing on the isotopic composition of
312 particulate organic matter have found ^{13}C -enriched particulate organic matter in GB compared
313 to coastal areas (Kalas *et al.* 2009; Martins *et al.* 2016). In addition, more ^{13}C -enriched values
314 are expected to occur in eutrophic estuaries with a predominance of autochthonous organic
315 matter, such as GB (Kalas *et al.* 2009). Moreover, a previous study on the trophic relationships
316 among organisms in GB, focusing on the feeding habits of Guiana dolphins, *Sotalia guianensis*,
317 has found ^{13}C -depleted values in marine fish, *i.e.*, *Paralichthys brasiliensis* ($\delta^{13}\text{C} = -17.8 \pm$
318 0.7 ‰ in winter, $-16.7 \pm 0.4 \text{ ‰}$ in summer) and *Porichthys porosissimus* ($\delta^{13}\text{C} = -18.9 \pm 0.5$
319 ‰ in winter, $-17.6 \pm 0.5 \text{ ‰}$ in summer) compared to benthic invertebrates (*Litopenaeus*
320 *schmitti*; $\delta^{13}\text{C} = -15.6 \pm 0.6 \text{ ‰}$ in winter, $-15.2 \pm 0.2 \text{ ‰}$ in summer) (Bisi *et al.* 2012). The
321 abovementioned marine fish species feed on zoobenthos and use GB opportunistically as a
322 nursery area (Elliott *et al.* 2007; Silva Jr. *et al.* 2016). Thus, according to Bisi *et al.* (2012), the

323 primary carbon source for these fishes is probably from a neritic food web outside GB. Here,
324 $\delta^{13}\text{C}$ of small juvenile fishes was comparable to one of the benthic invertebrates sampled by
325 Bisi *et al.* (2012), while adult whitemouth croakers were more ^{13}C -depleted (Fig. 2A). This
326 could indicate that small fishes mostly feed in the inner part of the bay, while adult fishes
327 gradually start to use coastal shelf areas as feeding habitats.

328 $\delta^{15}\text{N}$ values have been used in studies in marine food webs, with special focus on trophic level
329 assessment (Das *et al.* 2000; Boecklen *et al.* 2011; Botto *et al.* 2011; Bisi *et al.* 2012, 2013;
330 Middelburg 2014). Studies on stomach contents analysis have shown whitemouth croaker to
331 feed on a wide range of taxonomic groups, with ingested prey varying in accordance to species
332 availability in each area (Mendoza-Carranza and Vieira 2008; Morasche *et al.* 2010; Denadai
333 *et al.* 2015). In addition, ontogenetic diet shifts between life stages are observed, with juveniles
334 commonly feeding on polychaetes and shrimps, while crustaceans, polychaetes, molluscs, and
335 small fish are the most frequent prey consumed by adult whitemouth croaker (Vazzoler 1991;
336 Figueiredo and Vieira 2005; Morasche *et al.* 2010; Olsson *et al.* 2013). Therefore, the lower
337 $\delta^{15}\text{N}$ values observed in the smallest (< 30 cm) whitemouth croaker in the present study could
338 be linked to ontogenetic changes in trophic position and feeding habits, thus corroborating the
339 earlier studies, which were based on stomach content analysis.

340 There is usually only a small isotopic fractionation for sulfur with changes in trophic level
341 (Peterson and Fry 1987; McCutchan *et al.* 2003; Herzka 2005). However, producers that
342 predominantly utilize seawater sulfates (*e.g.*, phytoplankton approximately +20‰ $\delta^{34}\text{S}$) tend
343 to be ^{34}S -enriched, while those organisms that use sulfate available from precipitation (*e.g.*,
344 upland plants approximately +2 to +8‰ $\delta^{34}\text{S}$) or other sources formed by bacterial sulfate
345 reduction in anaerobic sediments (approximately -24‰ $\delta^{34}\text{S}$) show lower $\delta^{34}\text{S}$ values (Peterson
346 and Howarth 1987; Thode 1991; Connolly *et al.* 2004). The $\delta^{34}\text{S}$ values recorded in the largest
347 (> 60 cm) whitemouth croaker sampled might indicate that they feed less inside GB and instead

348 rely on prey living in continental shelf waters (*i.e.*, where sulfur cycling in the water column
349 also determines the sulfur isotopic composition of primary producers). Interestingly, in winter
350 2014 (*i.e.*, the only season in which all size classes could be sampled), standard ellipses (Fig.
351 3) showed partial overlap between isotopic niches of small and medium fish as well as between
352 niches of medium and large fish; however, little to no overlap was observed between isotopic
353 niches of the small and large fishes. Caution must be exercised when interpreting isotopic niche
354 parameters of large fishes, as this group's sample size was small, which can generate
355 uncertainty in some ellipse-based metrics (Syvaranta *et al.* 2013). Nevertheless, this finding is
356 consistent with an ontogenetic movement pattern related to feeding, where juveniles feed
357 mostly inside the estuaries and large adults feed mostly in continental shelf waters, with
358 medium-sized adults forming a transition group that feeds in both habitats, which is supported
359 by the much larger isotopic niche observed for medium fish in winter 2014 (Fig. 4). Although
360 it is generally used as a proxy of the trophic niche, the isotopic niche actually reflects variability
361 in stable isotope ratios caused by both biotic (related to consumed resources) and
362 scenopoetic (related to habitat) factors (Newsome *et al.* 2007; Flaherty and Ben-David 2010).
363 In this context, a fish group that feeds over multiple habitats is expected to have a large isotopic
364 niche. This spatial distribution, in which the smaller individuals remain in the inner part of the
365 estuary and larger ones concentrate themselves in the outer part of the bay, could indicate an
366 ontogenetic movement from the estuary to the adjacent continental shelf waters as fish grow
367 (Vicentini and Araújo 2003). Our isotopic data corroborate the investigation conducted on
368 whitemouth croaker from Sepetiba Bay (an estuary close to GB), which concluded that juvenile
369 fish remain in the shallower parts of the bay before moving to continental shelf waters as they
370 grow (Costa and Araújo 2003). A diagram summarising the hypothesised whitemouth croaker
371 habitat shift in GB over the fish growth can be found in Fig. 5.

372 The isotopic niche width of medium-sized fish from GB showed a strong seasonal pattern, as
373 ellipses for this size class were much larger in winter 2014 than in either summer 2013 or
374 summer 2014 (Fig. 4). This suggests that medium-sized fish exploit a greater range of food
375 resources in winter than in summer. Differences in isotopic composition between winter and
376 summer fish in this size class may be linked to changes in local food availability or seasonal
377 changes in food preferences or that fish move to feed elsewhere (Vizzini and Mazzola 2003).
378 Studies have shown that during the spring-summer, GB is under the influence of the South
379 Atlantic Central Water (SACW) (Silva Jr. *et al.* 2016) and this nutrient-enriched water mass is
380 known to influence the richness, diversity, and abundance of organisms (DeLeo and Pires-
381 Vanin 2006; Soares-Gomes and Pires-Vanin 2003; Bonecker *et al.* 2014). In addition, Silva Jr.
382 *et al.* (2016) have shown that demersal ichthyofauna richness increases in GB during the
383 summer. Besides, a study on feeding ecology of whitemouth croaker in a coastal area in close
384 proximity to the mouth of GB by Morasche *et al.* (2010) observed the lowest variety of food
385 items to occur in winter. Therefore, one would expect whitemouth croaker to occupy a smaller
386 isotopic niche in winter than in summer, which is in direct contradiction with our results. In
387 this context, it is unlikely that differences in food items alone drive the observed niche shift
388 patterns, and habitat-related variability probably plays a major role. One possible explanation
389 would be that in winter, GB does not offer a favourable trophic environment for medium-sized
390 fish, as prey density and diversity are low. Therefore, these fish may forage over a wider range
391 of habitats, explaining their high isotopic niche size in winter. Conversely, in summer, prey
392 species are abundant inside the estuary and therefore, medium-sized whitemouth croaker might
393 restrict their feeding habitat to feeding mostly inside the bay, which would explain the
394 comparatively smaller summer isotopic niche. Interestingly, a seasonal shift in niche width was
395 much less marked in small fish, where a difference could only be seen for one of the set of
396 ellipses (*i.e.*, winter 2014) (Fig. 4). Since small fish likely feed inside the estuary all year long,

397 this might be another indicator that habitat use could be a major driver of isotopic niche width
398 in the studied population. However, regardless of the relative importance of trophic- and
399 habitat-related factors, changes in the SACW influence on the estuary are likely to explain
400 much of the observed seasonal differences.

401 In addition to seasonal differences in isotopic niche size, differences in standard ellipse position
402 and area were observed between medium-sized whitemouth croakers sampled in GB in summer
403 2013 and 2014. These trends were largely linked to differences in $\delta^{15}\text{N}$ values (Fig. 2), which
404 showed a 2.6‰ shift between the two years. Such a shift might provide evidence for feeding
405 at different trophic levels for the same species and environment in two consecutive summers,
406 since the $\delta^{15}\text{N}$ shift between diet and consumer is usually assumed to be from +2.6‰ to +3.4‰
407 (McCutchan *et al.* 2003). This apparent trophic level shift could be explained by the species
408 richness increase driven by the higher SACW influence on GB in 2013. Using the temperature-
409 salinity characteristics, Emery and Meinck (1986) suggested that the SACW is a water body
410 with temperatures between 5 and 18 °C and salinity varying from 34.3 to 35.8. However,
411 Miranda (1985) suggested temperatures between 8 and 20 °C for the SACW in southeastern
412 Brazil. Despite the lack of consensus regarding the temperature, the water temperature in GB
413 was lower than 18 °C in summer 2013 (November 2013 to February 2014), while this thermal
414 pattern was not repeated in the following summer (data obtained from the Hydrobiology
415 Laboratory - Guanabara Bay monitoring - Federal University of Rio de Janeiro 2016),
416 corroborating the hypothesis of a stronger SACW influence in summer 2013. It is known that
417 isotopic changes do not immediately follow diet alterations (Peterson and Fry 1987); however,
418 recent experimental work has found $\delta^{15}\text{N}$ half-life estimates to be around 23.9 days and a
419 nitrogen trophic discrimination of $3.4\text{‰} \pm 0.4\text{‰}$ for estuarine whitemouth croaker
420 (Mont'Alverne *et al.* 2016), which suggests that the abovementioned stronger influence of the
421 SACW in November 2013 could be a valid explanation for the higher $\delta^{15}\text{N}$ values observed for

422 that summer (*i.e.*, December 2013 to March 2014). Moreover, the stronger influence of the
423 SACW in 2013 could also explain why the observed trend of seasonal niche size reduction in
424 summer was stronger in this year (Fig. 4). However, these results have to be taken with caution,
425 as baseline shifts in isotopic composition (*i.e.*, temporal and (or) spatial changes in isotopic
426 composition of producers at the base of the food webs, which might be reflected in higher
427 consumers through cascading effects) could have a strong influence on measured values,
428 complicating data interpretation (Boecklen *et al.* 2011).

429 Stable isotope ratios of carbon, nitrogen, and sulfur constitute valuable tools that can be applied
430 to provide more information regarding the ontogenetic movements of whitemouth croaker.
431 Changes in body size imply changes in many ecological features, such as diet, trophic status,
432 and dispersal ability, which in turn may have consequences on the food web structure and (or)
433 dynamics. The $\delta^{13}\text{C}$, $\delta^{34}\text{S}$, and $\delta^{15}\text{N}$ values generated by the present study support evidence that
434 juvenile and adult whitemouth croakers in GB occupy different niches, with juveniles feeding
435 mostly in estuaries and inshore bays and adults appearing to feed in multiple habitats located
436 both inshore and in continental shelf waters, with marked seasonal trends. This suggests that
437 adult and juvenile whitemouth croakers should be treated as different components of the food
438 web for a better management of fisheries in Guanabara Bay, Rio de Janeiro state, Brazil.
439 Besides that, a better understanding of the habitats that serve as nurseries for juveniles and the
440 factors that drive the estuarine dependence will provide more information for conservation,
441 management, and rehabilitation of estuarine habitats to sustain the coastal fisheries of these
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443

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Table 1: Number of whitemouth croaker, *Micropogonias furnieri*, specimens from Guanabara Bay analyzed for size class and period.

Size class (cm)	Period		
	Summer 2013	Winter 2014	Summer 2014
Small (< 30)	-	65	47
Medium (30 - 60)	18	43	28
Large (> 60)	-	6	-
Total	18	114	75

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722

723 **Figure captions**

724

725 **Figure 1:** Map of Guanabara Bay in Rio de Janeiro state, Brazil. The circled red dot in the
726 bottom right insert shows the position of Guanabara Bay in Brazil.

727

728 **Figure 2:** (A) $\delta^{13}\text{C}$, (B) $\delta^{15}\text{N}$, and (C) $\delta^{34}\text{S}$ of whitemouth croakers, *Micropogonias furnieri*,
729 from Guanabara Bay. Error bars are the full range of the data, box limits are the upper and
730 lower quartiles, solid bars are medians, and crosses are means. Fish size classes: small, <30
731 cm; medium, 30–60 cm; large, >60 cm.

732

733 **Figure 3:** Isotopic niches of whitemouth croakers, *Micropogonias furnieri*, from Guanabara
734 Bay built using (A) carbon and nitrogen and (B) carbon and sulfur data. Symbols are individual
735 measurements and solid lines represent the bivariate standard ellipses associated with each fish
736 group. Fish size classes: small, <30 cm; medium, 30–60 cm; large, >60 cm.

737

738 **Figure 4:** Boxplots of model-estimated bivariate standard area (SEAB) for ellipses built using
739 (A) carbon and nitrogen and (B) carbon and sulfur stable isotope ratios. Dark, medium, and
740 light grey boxes are, respectively, the 50%, 75%, and 95% credibility intervals of the
741 probability of density function distributions of the model solutions, and black dots are the
742 modes of these distributions. Red dots represent the standard ellipse areas computed using a
743 frequentist algorithm adapted for small sample sizes (SEAC). Fish size classes: small, <30 cm;
744 medium, 30–60 cm; large, >60 cm.

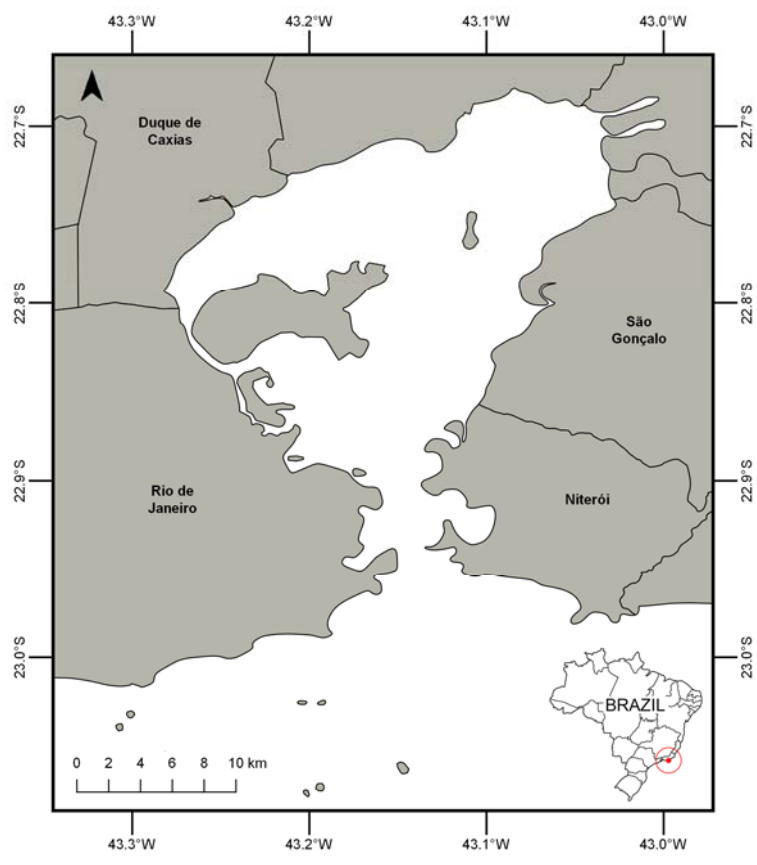
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746 **Figure 5:** Diagram summarising the hypothesized whitemouth croaker, *Micropogonias*
747 *furnieri*, habitat shift in Guanabara Bay over the fish growth. Three regions are indicated: A,
748 inner bay; B, entrance of the bay; C, continental shelf waters.

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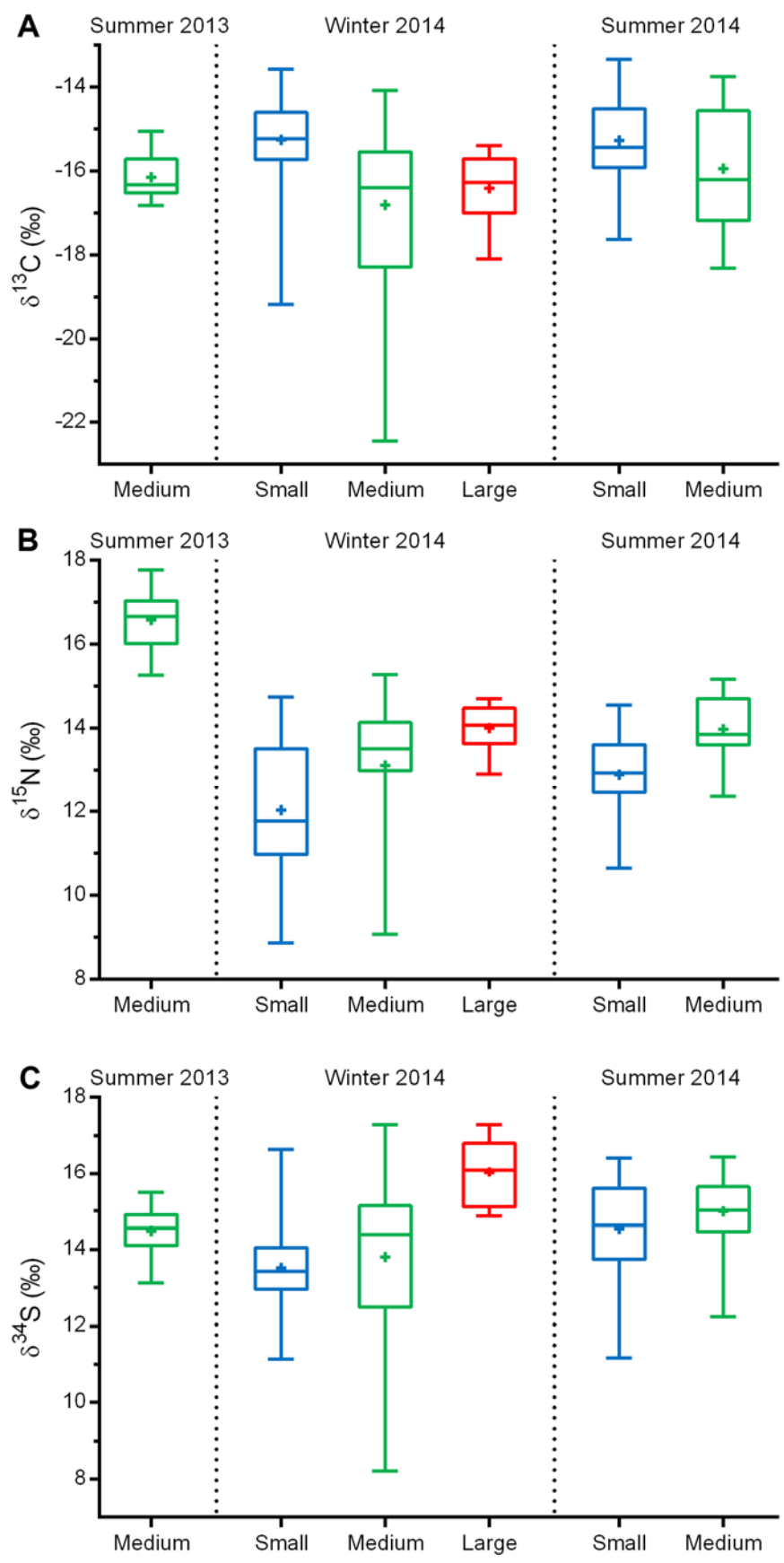
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751 **Figure 1**

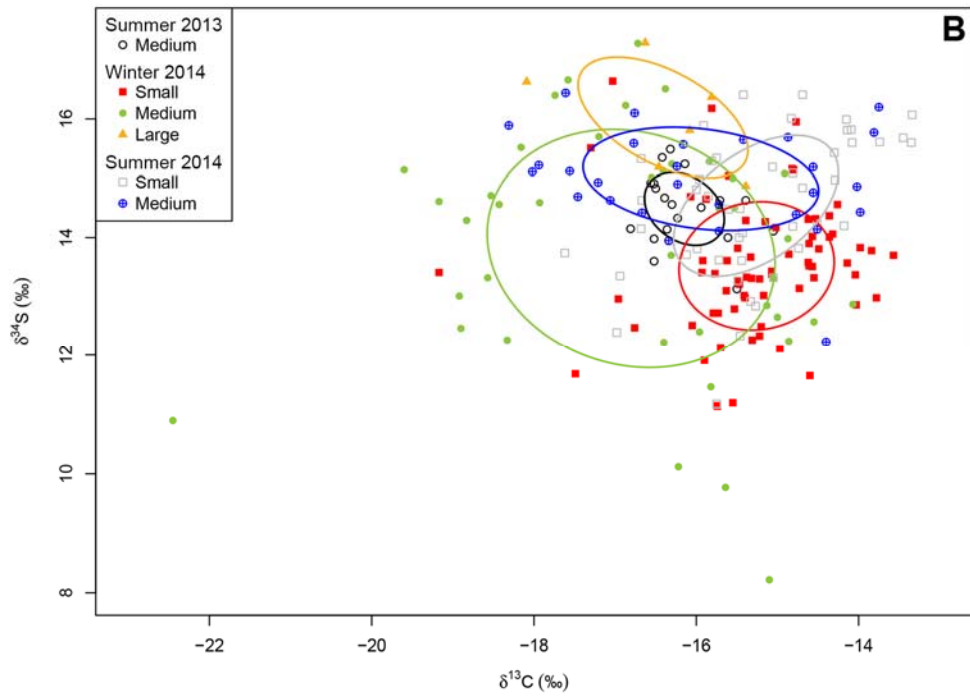
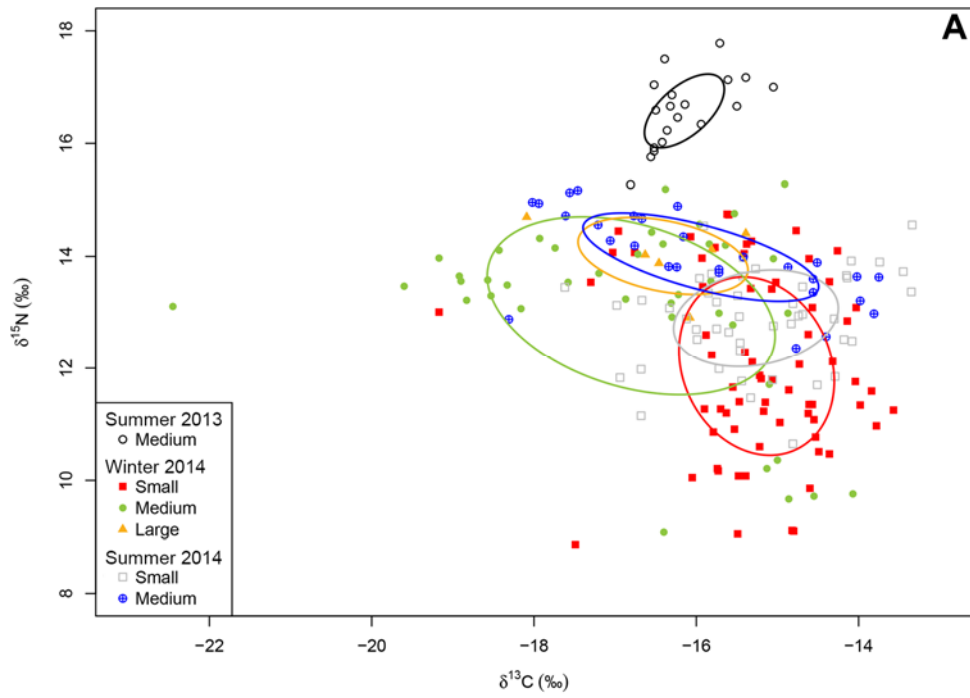


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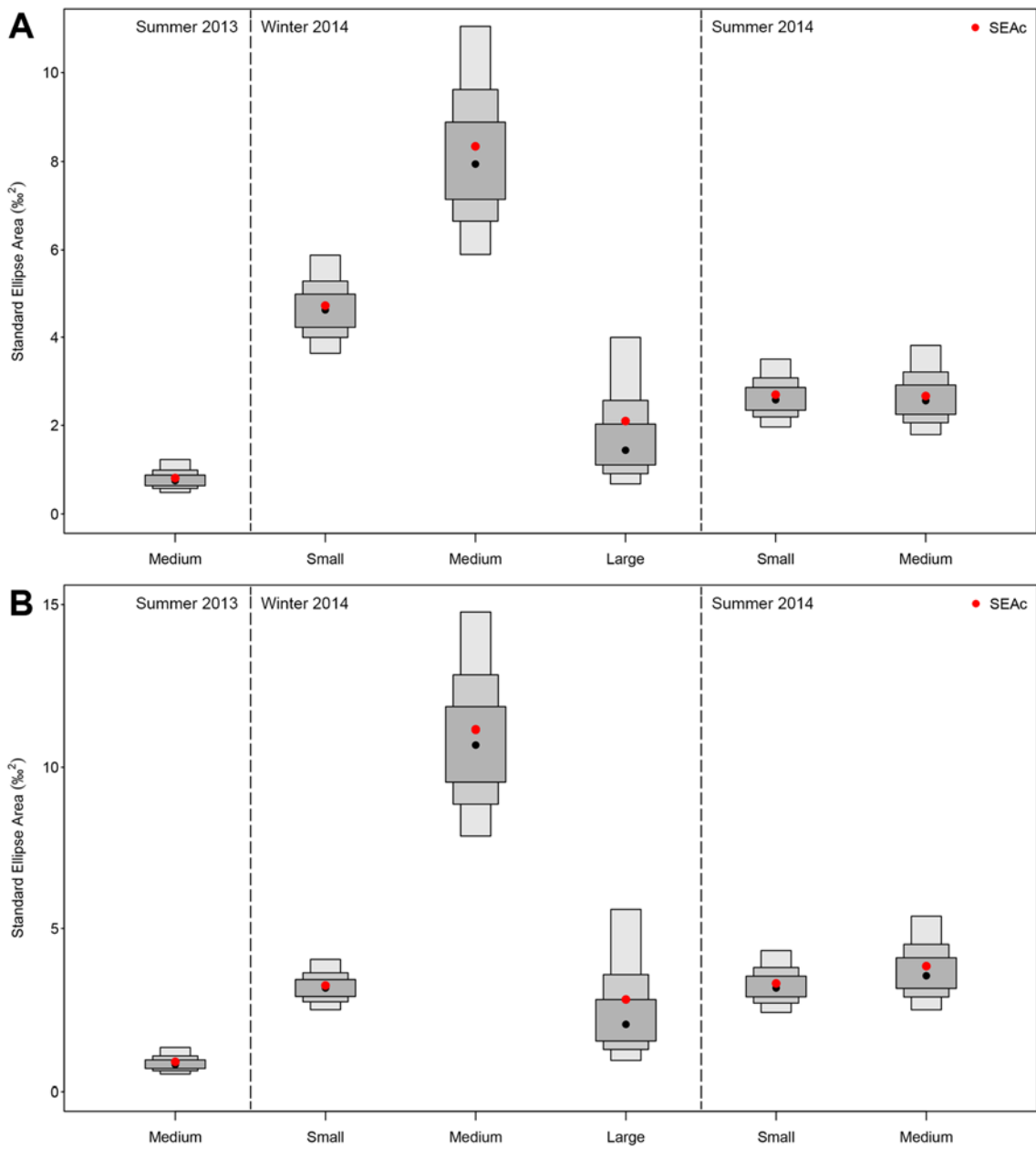
756 **Figure 3**



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759 **Figure 4**



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