

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

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24

25 Abstract

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

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

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43

44 1. Introduction

45

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

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

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

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

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

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

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

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

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

118

119 2. Materials and Methods

120

121 2.1 Study area

122 The Rio de Janeiro state (RJ) coast, in southeastern Brazil, is under high anthropogenic
123 pressure because it is an important urban and industrial center for Brazil (Molisani *et al.*
124 2007; Baptista-Neto *et al.* 2013). Despite the anthropogenic disturbance, RJ ranks in third

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

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

140

141 2.2 Sampling

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

150 30 cm) or adults (≥ 30 cm). In addition, a third size class was also used for the larger adult
151 fish (> 60 cm) caught in the outer part of GB, since they exceed the theoretical maximum size
152 from population I (Vazzoler 1971).

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

160 Fishes were separated into groups according to sampling season (S: summer, W: winter),
161 sampling year (2013 or 2014), and size class (S: < 30 cm, M: 30-60 cm, L: > 60 cm) and the
162 number of specimens (n) in each group are presented in Table 1.

163

164 2.3 Stable isotope measurements

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

175 sulfide (IAEA-S-1; $\delta^{34}\text{S} = -0.3\text{‰}$) were used as certified reference materials for measurement
176 of stable isotope ratios of carbon, nitrogen and sulfur, respectively. All these reference
177 materials (International Atomic Energy Agency, Vienna, Austria) are calibrated against the
178 international references Vienna Pee Dee Belemnite (for carbon), Atmospheric Air (for
179 nitrogen) and Vienna Canyon Diablo Troilite (for sulfur). Standard deviations on multi-batch
180 replicate measurements of internal lab standards (Sulfanilic acid and animal muscle tissue)
181 analyzed interspersed with samples (one replicate of each standard every 15 analyses) were
182 0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and 0.4‰ for $\delta^{34}\text{S}$ respectively.

183

184 2.4 Statistical analyses and data processing

185 Fishes were grouped according to sampling location, season, year and size class (see Table
186 1). Inter-group differences in carbon, nitrogen and sulfur stable isotope ratios were
187 investigated through multiple comparisons. Since Shapiro-Wilk normality tests revealed that
188 several datasets did not follow a Gaussian distribution, non-parametric procedures were
189 applied, *i.e.* Mann-Whitney U test when 2 groups were compared and Kruskal-Wallis one-
190 way analysis of variance followed by Dunn's post hoc test when 3 groups were compared. All
191 the tests were performed using the statistical software GraphPad Prism 5.0.

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

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

212

213 3. Results

214

215 3.1 Stable isotopes ratios of carbon, nitrogen, sulfur in whitemouth croaker.

216

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

225 The $\delta^{15}\text{N}$ data for whitemouth croaker in Guanabara Bay indicated that small and medium
226 fish were ^{15}N -enriched in summer compared to winter 2014. $\delta^{15}\text{N}$ values for small fish in
227 summer 2014 were significantly higher than in winter 2014 (Mann-Whitney test, $p < 0.05$;
228 Fig. 2B) The same pattern of ^{15}N enrichment in summer was also observed for the medium
229 size class when comparing the three sampling periods in Guanabara Bay (Kruskal-Wallis, $p <$
230 0.05), however, the $\delta^{15}\text{N}$ values were significantly higher in summer 2013 ($16.6 \pm 0.6\text{‰}$) than
231 in summer 2014 ($14 \pm 0.8\text{‰}$) (Dunn's test; $p < 0.05$; Fig. 2B). Comparisons between the
232 different size classes within the same sampling period demonstrated that the smallest
233 whitemouth croaker were significantly ^{15}N -depleted in comparison to medium and large
234 specimens (winter: Dunn's test, $p < 0.05$; summer: Mann-Whitney test, $p < 0.05$; Fig.2B).
235 Examination of the sulfur isotope data demonstrated that fish from summer 2014 presented
236 significantly higher $\delta^{34}\text{S}$ values than those from winter 2014. This pattern was observed both
237 for small (summer 2014: $14.5 \pm 1.2\text{‰}$; winter 2014: $13.5 \pm 1.1\text{‰}$; Mann-Whitney test, $p <$
238 0.05 ; Fig. 2C) and medium size classes (summer 2013: $14.5 \pm 0.6 \text{‰}$; winter 2014: $13.8 \pm$
239 2‰ ; summer 2014: $15 \pm 0.8 \text{‰}$; Dunn's test, $p < 0.05$; Fig. 2C). Although the large fish were
240 only sampled in winter 2014, they showed significantly higher $\delta^{34}\text{S}$ values compared to small
241 and medium size classes from the same sampling season (Dunn's test, $p < 0.05$; Fig. 2C).

242

243 3.2 Stable isotope ellipses - relative positions

244 SIBER results suggested that the core isotopic niche of medium whitemouth croakers
245 sampled in summer 2013 was markedly separated from any other group when looking at C &
246 N ellipses (Fig. 3A, black ellipse), but was strongly overlapping with most groups when
247 considering the C and S ellipses (Fig. 3B). When compared with C and S ellipses associated
248 with the same size class (*i.e* medium fish), the summer 2013 ellipse was completely included
249 within the winter 2014 ellipse (Fig. 3B, green ellipse), and overlapping for 0.72‰^2 (*i.e.* 79%

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

271

272 3.3 Standard ellipse areas

273 Areas of the standard ellipses associated with each fish group varied widely, with SEA_c
274 values ranging from 0.57 ‰^2 (C & N ellipse of medium fishes from winter 2014; Fig. 3A,

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

292

293 4. Discussion

294

295 According to Herzka (2005), the isotopic composition of an individual can provide a record
296 of its migration history, constituting also a valuable tool to distinguish subpopulations of the
297 same species that vary in their patterns of habitat use and migration. Overall, comparisons of
298 $\delta^{13}\text{C}$, $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$ data between size classes highlighted that the whitemouth croaker
299 (*Micropogonias furnieri*) displays size-related habitat segregation in Guanabara Bay (GB).

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

325 whitemouth croakers were more ^{13}C -depleted (Fig. 2A). This could indicate that small fishes
326 mostly feed in the inner part of the bay, while adult fishes gradually start to use coastal shelf
327 areas as feeding habitats.

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

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

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

373 The isotopic niche width of medium-sized fish from GB showed a strong seasonal pattern, as
374 ellipses for this size class were much bigger in winter 2014 than in either summer 2013 or

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

400 However, regardless of the relative importance of trophic- and habitat-related factors,
401 changes in the SACW influence on the estuary are likely to explain much of the observed
402 seasonal differences.

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

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

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

445

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Table 1: Number of specimens (n) from Guanabara Bay analysed for size class (S: < 30 cm; M: 30 – 60 cm and L: > 60 cm) and period.

Size class (cm)	Period		
	Summer/13	Winter/14	Summer/14
S (< 30)	-	65	47
M (30 - 60)	18	43	28
L (> 60)	-	6	-
Total	18	114	75

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728 **Figure captions**

729

730 **Figure 1:** Map of Guanabara Bay, in Rio de Janeiro (RJ) state, Brazil. The circled red dot in
731 the bottom right insert shows the position of Guanabara Bay in Brazil.

732

733 **Figure 2:** $\delta^{13}\text{C}$ (A), $\delta^{15}\text{N}$ (B) and $\delta^{34}\text{S}$ (C) of whitemouth croakers from Guanabara Bay.
734 Error bars are the full range of the data, box limits are the upper and lower quartiles, solid
735 bars are medians, and crosses are means. Each fish group code includes the sampling season
736 (S: summer, W: winter), sampling year (2013 or 2014), and the fish size class (S: < 30 cm,
737 M: 30-60 cm, L: > 60 cm).

738

739 **Figure 3:** Isotopic niches of whitemouth croakers from Guanabara Bay built using carbon
740 and nitrogen (A) and carbon and sulfur (B) data. Symbols are individual measurements, and
741 solid lines represent the bivariate standard ellipses associated to each fish group. Group codes
742 includes the sampling season (S: summer, W: winter), sampling year (2013 or 2014), and the
743 fish size class (S: < 30 cm, M: 30-60 cm, L: > 60 cm).

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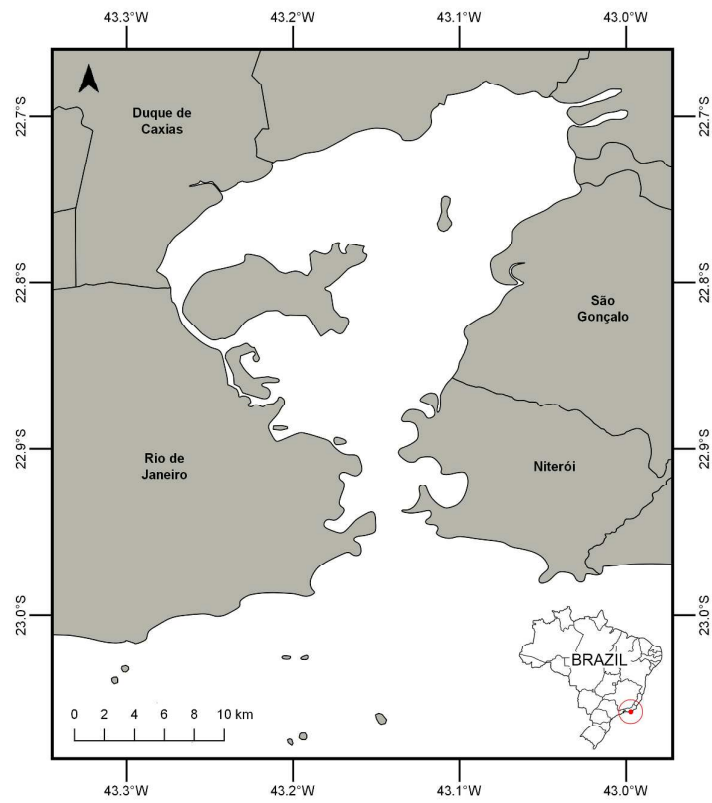
745 **Figure 4:** Boxplots of model-estimated bivariate standard area (SEA_B) for ellipses built using
746 carbon and nitrogen (A) and carbon and sulfur (B) stable isotope ratios. Dark, median and
747 light grey boxes are respectively the 50%, 75% and 95% credibility intervals of the
748 probability of density function distributions of the model solutions, and black dots are the
749 modes of these distributions. Red dots represent the standard ellipse areas computed using a
750 frequentist algorithm adapted for small sample sizes (SEA_C). Each fish group code includes
751 the sampling season (S: summer, W: winter), sampling year (2013 or 2014), and the fish size
752 class (S: < 30 cm, M: 30-60 cm, L: > 60 cm).

753

754 **Figure 5:** Diagram summarising the hypothesised whitemouth croaker habitat shift in
755 Guanabara Bay over the fish growth. Three regions are indicated: (A) inner bay; (B) entrance
756 of the bay; (C) continental shelf waters.

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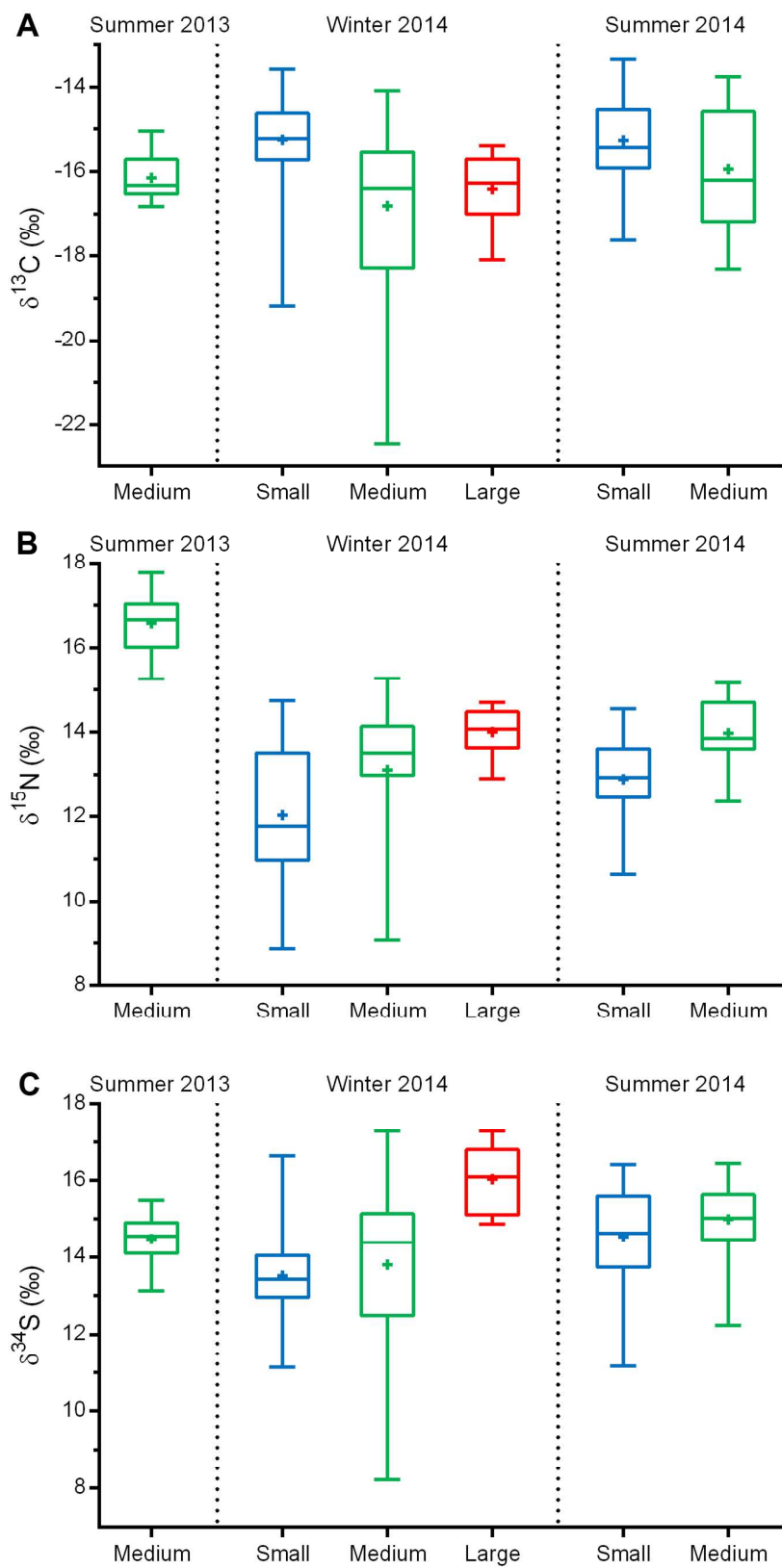
759 **Figure 1**

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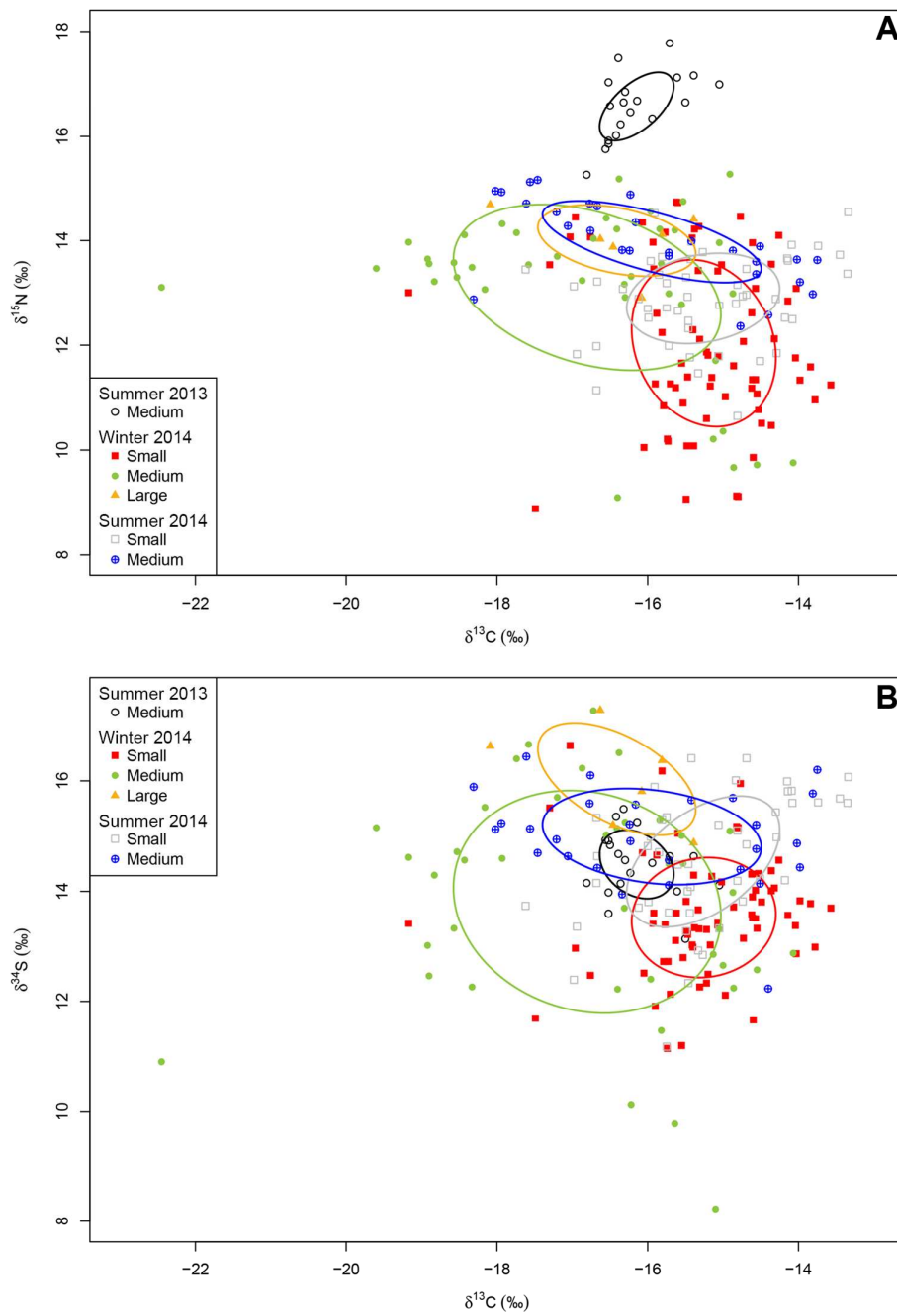
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Figure 2



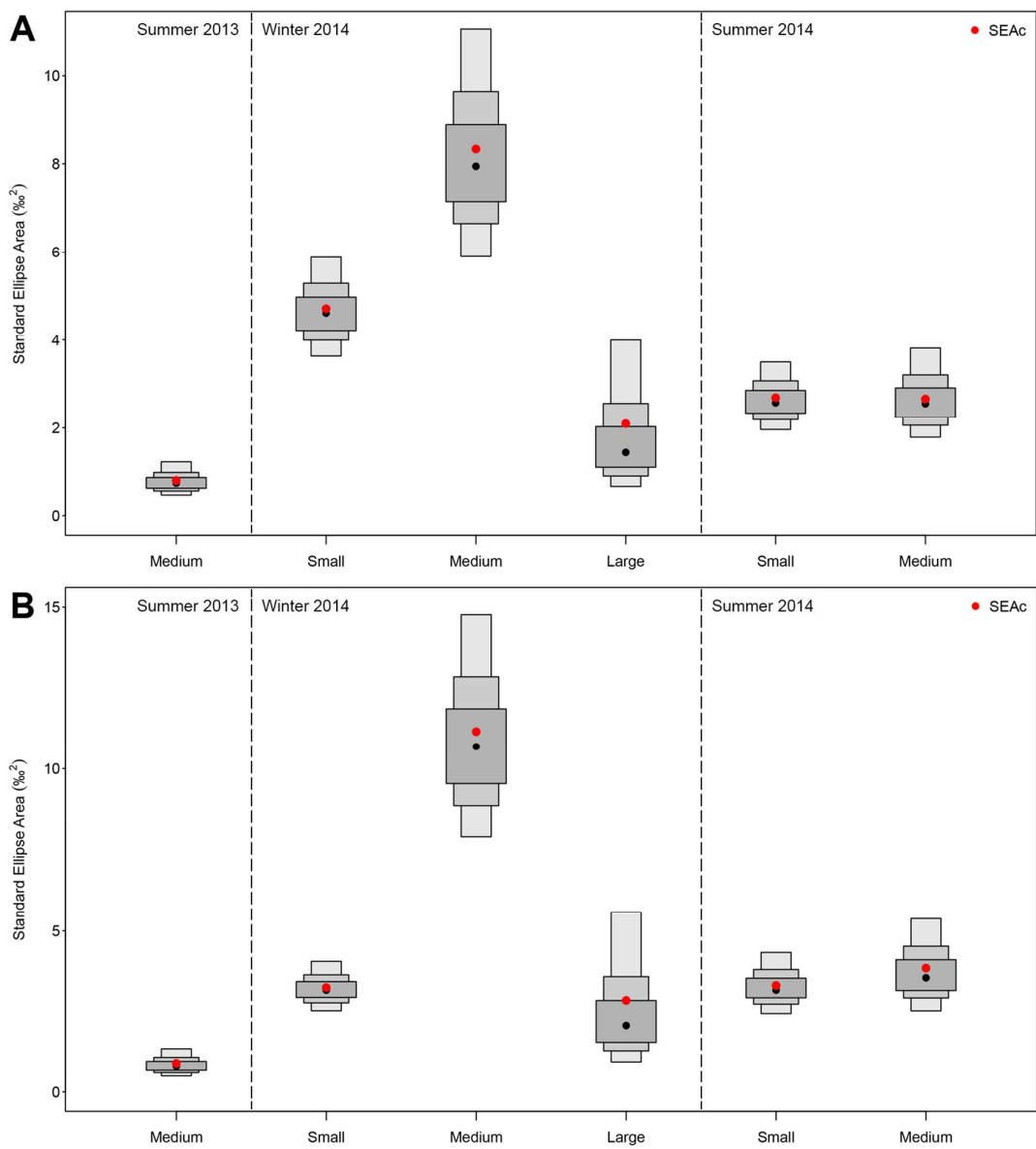
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764 **Figure 3**

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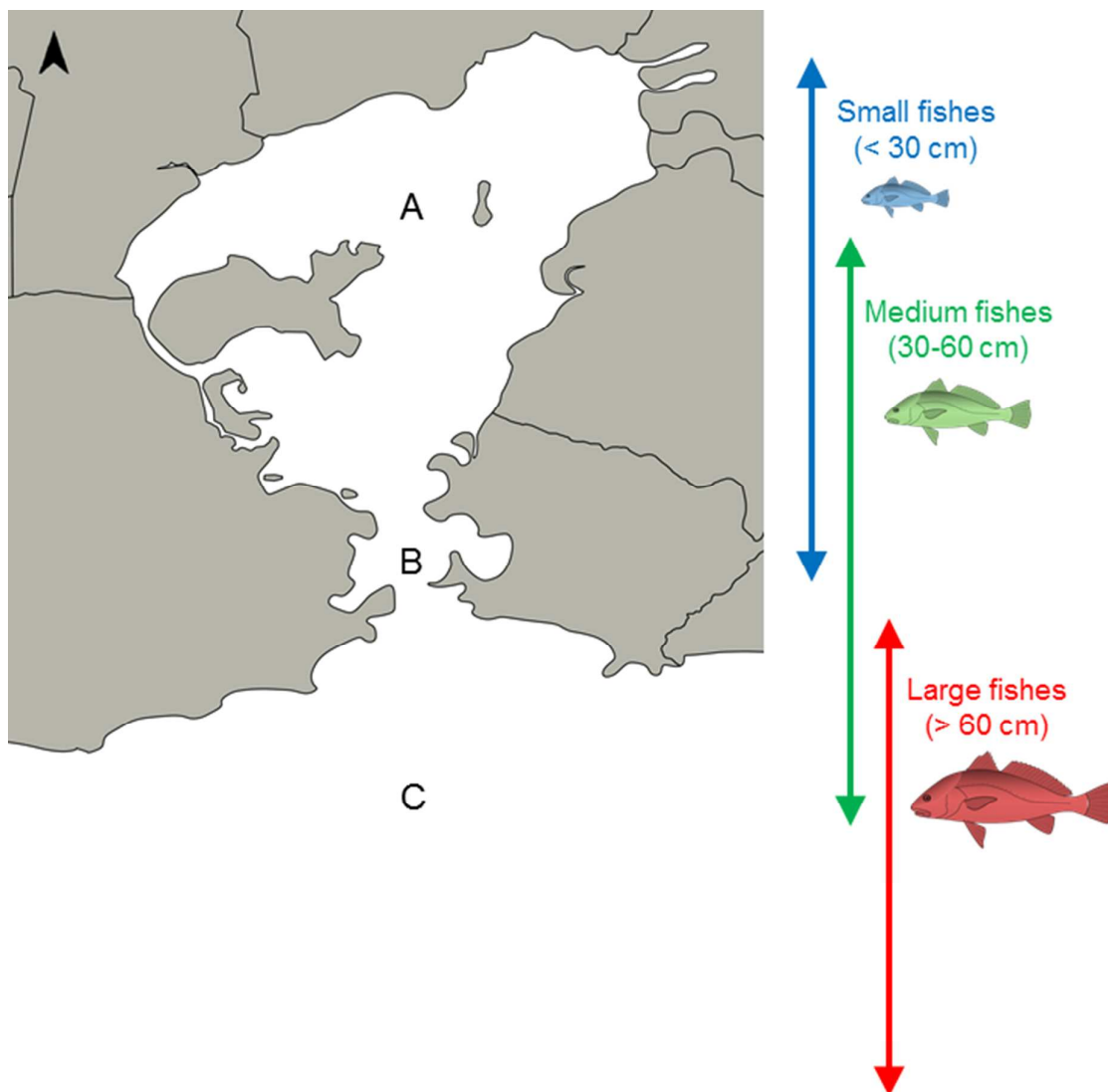
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767 **Figure 4**



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770 **Figure 5**

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