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

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Abstract

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

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

1. Introduction

The Sciaenidae, popularly known as croakers or drums due to the sound they produce using muscles associated with the swim bladder, are a widely distributed fish family throughout the world (Nelson, 2006). Croakers constitute commercially important resources in the Western Atlantic Ocean, and are the major fishery resource in Brazil, as they represent 22% of marine and 9% of freshwater fish landings (Chao *et al*., 2015). The whitemouth croaker, *Micropogonias furnieri* (Desmarest, 1823) (Perciformes, Sciaenidae) is widely distributed in coastal waters of the Western Atlantic Ocean, occurring from the Yucatán Peninsula (Mexico, 28º N) to the Gulf of San Matías (Argentina, 41º S) (Isaac, 1988; Vazzoler, 1991). However, despite its wide latitudinal distribution, this species becomes abundant from Rio de Janeiro state at 23º S latitude southwards to the shelf of Uruguay (Vazzoler, 1991). In Brazil, it constitutes one of the main demersal resources with an average annual catch of 42,000 metric tons between 2000 and 2015 (Chao *et al*., 2015; FAO, 2017). In addition, the species also represents one of the most important fishery resources in coastal demersal fisheries in Uruguay and Argentina (Vasconcellos and Haimovici, 2006; FAO, 2017).

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

Several aspects of the whitemouth croaker’s life history have been well studied in population II, including its food and feeding habits, age and growth, reproductive biology and length distribution (Muelbert and Weiss, 1991; Macchi *et al*., 2003; Albuquerque *et al*., 2009, 2010, 2012; Olsson *et al.*, 2013; Costa *et al*., 2014). Studies on the movement patterns of population II have shown that seasonal migrations by adults to open water following the displacement of the convergence between the Brazilian and Falkland currents, with higher densities between 31º and 35ºS in the summer and between 27º and 31ºS in winter (Isaac, 1988). In addition to these seasonal migrations, in temperate regions of Brazil (population II), whitemouth croaker displays ontogenetic habitat shifts being estuarine-dependent during early life stages, while adults can spawn in the estuaries or in shallow coastal areas (Mendoza-Carranza and Vieira, 2008; Albuquerque *et al*., 2012; Costa *et al*., 2014). Most fish species undertake movement patterns during their life cycle and defining these ontogenetic movements plays a key role in understanding their ecology, which is important for both conservation and exploitation management purposes. Although knowledge has been generated on whitemouth croaker biology in Brazilian waters (Population II), information is still scarce for population I, whose distribution includes Rio de Janeiro state (RJ) (Isaac, 1988; Vazzoler, 1991).

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

Stable isotope ratios (SIR) of light biogenic elements have proved to be valuable tools for the study of trophic relationships in marine ecosystems, as well as providing insights into animal migration (Hobson, 1999; Das *et al*., 2000; Kolasinski *et al*., 2009; Botto *et al*., 2011; MacKenzie *et al*., 2011; Bisi *et al*. 2012, 2013). SIR serve as natural tags, as their natural distribution reflect and integrate the history of physical and metabolic processes of the environment (Peterson and Fry, 1987; Herzka, 2005). The isotopic ratios of carbon (typically expressed as $\delta^{13}C$) are used to establish the sources of organic matter that support food webs (McCutchan *et al.*, 2003). The fractionation of carbon isotopes during photosynthesis by the producers of the food web helps to identify food sources originating from different systems, *i.e.* terrestrial versus marine, coastal versus oceanic, or benthic versus pelagic (Peterson and Fry, 1987; Boutton, 1991). Also, sulfur isotope ratios ($\delta^{34}S$ values) can be used as a second tracer of organic matter sources that is independent of the carbon isotopic distribution (Connolly *et al.*, 2004). Nitrogen stable isotope ratio ($\delta^{15}N$ values) can also be used to trace organic matter sources, but are more commonly applied to provide information on the position occupied by a species in a trophic web, as nitrogen isotopes show predictable stepwise increases in values from prey to consumer (DeNiro and Epstein, 1981).

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

2. Materials and Methods

2.1 Study area

The Rio de Janeiro state (RJ) coast, in southeastern Brazil, is under high anthropogenic pressure because it is an important urban and industrial center for Brazil (Molisani *et al.*, 2007; Baptista-Neto *et al.*, 2013). Despite the anthropogenic disturbance, RJ ranks in third place amongst Brazilian marine fisheries, with the whitemouth croaker constituting one of the most important target species in the state (FIPERJ, 2015).

The Guanabara Bay (GB) (22º03′ to 22º41′ S / 43º01′ to 43º16′ W; Fig. 1– IBGE, 2016), located within the metropolitan area of RJ, has remarkable ecological, social and economical importance for the Brazilian southeastern region. Despite the impact of diverse anthropogenic activities, including input of sewage and industrial waste in its drainage basin, GB is one of the most species-rich tropical estuarine ecosystems on the Brazilian coast and continues to sustain important fisheries and a large number of fishermen (Jablonski *et al*., 2006; Dorneles *et al*., 2013; Silva-Jr *et al*., 2016). GB has a total surface area of 384 km^2 extending a maximum distance of 28 km on its east-west axis and 30 km on its north-south axis, with depths ranging from less than a meter in marginal regions to more than 50 meters in the central channel. The bay holds about two billion cubic meters of water, with water volume maintained by the inflow from 91 rivers and channels, in addition to the exchange with the Atlantic Ocean through the narrow bay entrance in semi-diurnal tide cycles with an amplitude of up to 1.4 meters (Kjerfve *et al*., 1997; Catanzaro *et al*., 2004).

2.2 Sampling

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

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

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

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

2.3 Stable isotope measurements

Dorsal white muscle samples were oven-dried over 3 days to constant weight at 60°C before being ground into a homogeneous powder using mortar and pestle. Circa 4 mg of dry, powdered material were used for stable isotope analysis. Measurements of stable isotope ratios were performed via continuous flow - elemental analysis - isotope ratio mass spectrometry (CF-EA-IRMS) at the Laboratory for Oceanology, University of Liege (Belgium), using a vario MICRO cube C-N-S elemental analyzer (Elementar Analysensysteme GMBH, Hanau, Germany) coupled to an IsoPrime100 isotope ratio mass spectrometer (Isoprime, Cheadle, United Kingdom). Isotopic ratios were expressed using the widespread delta (δ) notation (Coplen, 2011). Sucrose (IAEA-C-6; $\delta^{13}C = -10.8 \pm 0.47‰$; mean ± SD), ammonium sulfate (IAEA-N-2; $\delta^{15}N = 0.40 \pm 0.20‰$; mean ± SD) and silver sulfide (IAEA-S-1; $\delta^{34}S$ = -0.30‰) were used as certified reference materials for measurement of stable isotope ratios of carbon, nitrogen and sulfur, respectively. All these reference materials (International Atomic Energy Agency, Vienna, Austria) are calibrated against the international references Vienna Pee Dee Belemnite (for carbon), Atmospheric Air (for nitrogen) and Vienna Canyon Diablo Troilite (for sulfur). Standard deviations on multi-batch replicate measurements of internal lab standards (Sulfanilic acid and animal muscle tissue) analyzed interspersed with samples (one replicate of each standard every 15 analyses) were 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ and 0.4‰ for $\delta^{34}S$ respectively.

2.4 Statistical analyses and data processing

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

For fish groups with $n \geq 6$, ecological niches were explored using the SIBER (Stable Isotope Bayesian Ellipses in R) method (Jackson *et al*., 2011). This approach involves the use of standard ellipses (bivariate equivalent of standard deviation; Jackson *et al*., 2011) to define isotopic niches, *i.e.* the space occupied by an animal population in a bivariate isotopic space. Since variation in the isotopic composition of animals (*i.e.* position of points in the isotopic space) is driven by both consumed prey items (Jackson *et al*., 2011; Layman and Allgeier, 2012) and habitat use (Flaherty and Ben-David, 2010), this isotopic niche can be used as a proxy of the realized ecological niche. Size and position of ellipses carry complementary information about animal ecology. A larger ellipse suggest that an animal population commonly uses more trophic and habitat resources. Overlap between ellipses associated with different populations suggests that these populations partly exploit the same food and/or habitat resources. The bigger the overlap, the more resources are shared by the two populations. Here, SIBER 2.0.3 was run in the R 3.2.2 statistical environment (R Core Team, 2015). Two separate sets of ellipses were constructed: one using $\delta^{13}C$ and $\delta^{15}N$ data, and another using $\delta^{13}C$ and $\delta^{34}S$ data. The areas of all ellipses were estimated using the SEA_c correction for small sample sizes, as outlined in Jackson *et al*. (2011). The areas of these ellipses were also estimated using Bayesian modelling (SEA_B, 10^6 iterations), and direct inter-group pairwise comparisons of SEA_B were performed. Model solutions were presented using credibility intervals of probability density function distributions. Pairwise comparisons were considered meaningful when probability of occurrence exceeded 95%.

3. Results

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

There were no significant differences in $\delta^{13}C$ values between winter and summer 2014 for the small size class (*Mann-Whitney* test, $p > 0.05$; Fig. 2A) or for the medium size class between all three sampling periods (*Kruskal-Wallis*, $p > 0.05$; Fig. 2A). As individuals from the large class were only sampled in winter 2014, interseasonal comparisons between sampling periods were not possible for this group. Significant differences in $\delta^{13}C$ values were found between size classes in Guanabara Bay with less ^{13}C-depleted values recorded for small whitemouth croaker in winter 2014 (-15.2 ± 0.89 ‰; Dunn's test, $p < 0.05$) and summer 2014 (-15.3 ± 1.00‰; *Mann-Whitney* test, $p < 0.05$) (Fig.2A).

The $\delta^{15}N$ data for whitemouth croaker in Guanabara Bay indicated that small and medium fish were ^{15}N-enriched in summer compared to winter 2014. $\delta^{15}N$ values for small fish in summer 2014 were significantly higher than in winter 2014(*Mann-Whitney* test, $p < 0.05$; Fig. 2B) The same pattern of ^{15}N enrichment in summer was also observed for the medium size class when comparing the three sampling periods in Guanabara Bay (*Kruskal-Wallis*, $p < 0.05$), however, the $\delta^{15}N$ values were significantly higher in summer 2013 (16.6 ± 0.65‰) than in summer 2014 (14.0 ± 0.77‰) (Dunn's test; $p < 0.05$; Fig. 2B). Comparisons between the different size classes within the same sampling period demonstrated that the smallest whitemouth croaker were significantly ^{15}N-depleted in comparison to medium and large specimens (winter: Dunn's test, $p < 0.05$; summer: *Mann-Whitney* test, $p < 0.05$; Fig.2B).

Examination of the sulfur isotope data demonstrated that fish from summer 2014 presented significantly higher $\delta^{34}S$ values than those from winter 2014. This pattern was observed both for small (summer 2014: 14.5 ± 1.17‰; winter 2014: 13.5 ± 1.05‰; *Mann-Whitney* test, $p < 0.05$; Fig. 2C) and medium size classes (summer 2013: 14.5 ± 0.60 ‰; winter 2014: 13.8 ± 1.97‰; summer 2014: 15.0 ± 0.85 ‰; Dunn's test, $p < 0.05$; Fig. 2C). Although the large fish were only sampled in winter 2014, they showed significantly higher $\delta^{34}S$ values compared to small and medium size classes from the same sampling season (Dunn's test, $p < 0.05$; Fig. 2C).

3.2 Stable isotope ellipses - relative positions

SIBER results suggested that the core isotopic niche of medium whitemouth croakers sampled in summer 2013 was markedly separated from any other group when looking at C & N ellipses (Fig. 3A, black ellipse), but was strongly overlapping with most groups when considering the C and S ellipses (Fig. 3B). When compared with C and S ellipses associated with the same size class (*i.e* medium fish), the summer 2013 ellipse was completely included within the winter 2014 ellipse (Fig. 3B, green ellipse), and overlapping for 0.72 ‰^{2} (*i.e.* 79% of its area) with summer 2014 (Fig. 3B, blue ellipse). The overlap between medium fish sampled in winter and summer 2014 was considerable for both C & N (1.42 ‰^{2}, *i.e.* 53% of the smallest ellipse area; Fig. 3A, green and blue ellipses) and C & S (2.45 ‰^{2}, *i.e.* 63% of the smallest ellipse area; Fig. 3B, green and blue ellipses) core isotopic niches. The C & N ellipses for the small size class in winter (Fig. 3A, red ellipse) and summer 2014 (Fig. 3A, grey ellipse) were strongly overlapping (2.69 ‰^{2}, *i.e.* 78% of the smallest ellipse area). Overlap between isotopic niches for small fishes between seasons was also present, to a lesser extent, for C & S ellipses (overlap: 1.40 ‰^{2}, *i.e.* 43% of the smallest ellipse area; Fig. 3B, red and grey ellipses). In winter 2014, both C & N (Fig. 3A) and C & S (Fig. 3B) ellipses of small (red ellipses) and medium (green ellipses) fish showed considerable overlap (1.87 ‰^{2} or 40% of the smallest ellipse area for C & N and 1.92 ‰^{2} or 59% of the smallest ellipse area for C & S respectively; Fig. 3). This trend seemed less strong in summer 2014, where overlap between small (grey ellipses) and medium (blue ellipses) fish was more moderate (0.58 ‰^{2} or 22% of the smallest ellipse area for C & N and 1.76 ‰^{2} or 46% of the smallest ellipse area for C & S respectively; Fig. 3). In winter 2014, overlap between isotopic niches of small (red ellipses) and large (orange ellipses) fish were very low for C & N ellipses (0.08 ‰^{2} or 4% of the smallest ellipse area; Fig. 3A) and nil for C & S ellipses (Fig. 3B) respectively. Finally, in the same season, ellipses associated with medium (green) and large (orange) fish were strongly overlapping when looking at C & N data (1.72 ‰^{2} or 82% of the smallest ellipse area; Fig. 3A), but much more separated when looking at C & S data (overlap: 0.59 ‰^{2} or 21% of the smallest ellipse area; Fig. 3B).

3.3 Standard ellipse areas

Areas of the standard ellipses associated with each fish group varied widely, with SEA_c values ranging from 0.57 ‰^{2} (C & N ellipse of medium fishes from winter 2014; Fig. 3A, Fig. 4A) to 11.15 ‰^{2} (C & S ellipse of medium fishes from winter 2014; Fig. 3B, Fig. 4B). Medium fishes from winter 2014 had the widest isotopic niche by far, with a larger niche than any other group in nearly all model solutions (99.80% of model solutions for C & N data, > 99.99% of model solutions for C & S data; Fig. 4). Interestingly, the model suggested that the isotopic niche of medium fishes sampled in summer was bigger in 2014 than in 2013 for both C & N and C & S data (> 99.99% of model solutions in each case; Fig. 4). The C and N isotopic niche of small fishes was larger in winter 2014 than in summer 2014 in 99.81% of model solutions (Fig. 4A), but this trend was not seen in C and S niches, as the winter ellipse was only bigger in 54.22% of model solutions (Fig. 4B). Differences in niche size between size classes were observed for winter 2014, as medium fish had a bigger ellipse than small and large ones for both C & N and C & S data in over 99.80% of model solutions. In addition, small fish in winter 2014, had a larger isotopic niche than large fish in 97.24% of model runs built using C & N data (Fig. 4A), but this trend was not observed in C & S niches (only 54.22% of model solutions). Finally, the converse pattern to that observed in winter 2014, was found in summer 2014 with no inter-size classes differences in niche size observed, as SEA of small and medium fish differed in only 50.75% and 71.35% of model solutions for C & N and C & S data, respectively (Fig. 4).

4. Discussion

According to Herzka (2005), the isotopic composition of an individual can provide a record of its migration history, constituting also a valuable tool to distinguish subpopulations of the same species that vary in their patterns of habitat use and migration. Overall, comparisons of

$\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ data between size classes highlighted that the whitemouth croaker (*Micropogonas furnieri*) displays size-related habitat segregation in Guanabara Bay (GB). The whitemouth croaker is a partial spawner that reproduces year-round (Isaac-Nahum and Vazzoler, 1987; Vazzoler, 1991), and juvenile whitemouth croakers are regularly present in GB throughout the whole year (Andrade-Tubino *et al.*, 2009). Studies on whitemouth croaker distribution have shown that juveniles (S: < 30 cm) are mostly present inside the estuaries (Andrade-Tubino *et al.*, 2009; Mulato *et al.*, 2015). Thus, the ^{13}C-enriched values presented by juvenile whitemouth croaker (Fig. 2A) may be the result of feeding mostly inside GB. Dissimilarities in $\delta^{13}C$ values are usually associated with differences in energy sources between distinct food webs with benthic and inshore trophic chains typically ^{13}C-enriched compared to pelagic and offshore ones (Peterson and Fry, 1987; Herzka, 2005). Stable isotope studies focusing on Guanabara Bay are scarce, particularly regarding potential whitemouth croaker prey items and/or other fish species. Nevertheless, previous studies focusing on the isotopic composition of the particulate organic matter (POM) have found ^{13}C-enriched POM in Guanabara bay, compared to coastal areas (Kalas *et al.*, 2009; Martins *et al.*, 2016). In addition, more ^{13}C-enriched values are expected to occur in eutrophic estuaries with a predominance of autochthonous organic matter, such as Guanabara Bay (Kalas *et al.*, 2009). Moreover a previous study on the trophic relationships among organisms in GB, focusing on the feeding habits of Guiana dolphins (*Sotalia guianensis*), has found ^{13}C-depleted values in marine fish, *i.e. Paralonchurus brasiliensis* ($\delta^{13}C = -17.8 \pm 0.7$ ‰ in winter, -16.7 ± 0.4 ‰ in summer) and *Porichthys porosissimus* ($\delta^{13}C = -18.9 \pm 0.5$ ‰ in winter, -17.6 ± 0.5 ‰ in summer), compared with benthic invertebrates (*Litopenaeus schmitt;* $\delta^{13}C = -15.6 \pm 0,6$ ‰ in winter, -15.2 ± 0.2 ‰ in summer) (Bisi *et al.*, 2012). The abovementioned marine fish species feed on zoobenthos and use GB opportunistically as a nursery area (Elliot *et al.*, 2007; Silva-Jr *et al.*, 2016). Thus, according to Bisi *et al.* (2012), the primary carbon source for these fishes are probably from a neritic food web outside GB. Here, $\delta^{13}C$ of small juvenile fishes was comparable with one of the benthic invertebrates sampled by Bisi *et al.* (2012), while adult whitemouth croakers were more ^{13}C-depleted (Fig. 2A). This could indicate that small fishes mostly feed in the inner part of the bay, while adult fishes gradually start to use coastal shelf areas as feeding habitats.

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

There is usually only a small isotopic fractionation for S with changes in trophic level (Peterson and Fry 1987; McCutchan *et al*. 2003; Herzka, 2005). However, producers that predominantly utilize seawater sulfates (*e.g.* phytoplankton ~ +20‰ $\delta^{34}S$) tend to be ^{34}S-enriched, while those organisms that use sulfate available from precipitation (*e.g* upland plants ~ + 2 to + 8‰ $\delta^{34}S$) or other sources formed by bacterial sulfate reduction in anaerobic sediments (~ -24‰ $\delta^{34}S$) show lower $\delta^{34}S$ values (Peterson and Howarth, 1987; Thode, 1991; Connolly *et al*., 2004). The $\delta^{34}S$ values recorded in the largest (≥ 60 cm) whitemouth croaker sampled might indicate that they feed less inside GB, and instead rely on prey living in continental shelf (CS) waters (*i.e.* where S cycling in water column also determines the S isotopic composition of primary producers). Interestingly, in winter 2014 (*i.e.* the only season in which all size classes could be sampled), standard ellipses (Fig. 3) showed partial overlap between isotopic niches of small and medium fish, as well as between niches of medium and large fish, however, little to no overlap was observed between isotopic niches of the small and large fishes. Caution has to be exercised when interpreting isotopic niche parameters of large fishes, as this group's sample size was small, which can generate uncertainty in some ellipse-based metrics (Syvaranta *et al*. 2013). Nevertheless, this finding is consistent with an ontogenetic movement pattern related to feeding, where juveniles feed mostly inside the estuaries and large adults feed mostly in CS waters, with medium-sized adults forming a transition group that feeds in both habitats, which is supported by the much larger isotopic niche observed for medium fish in winter 2014 (Fig. 4). Although it is generally used as a proxy of the trophic niche, the isotopic niche actually reflects variability in stable isotope ratios caused by both bionomic (related to consumed resources) and scenopoetic (related to habitat) factors (Newsome *et al*., 2007; Flaherty and Ben-David 2010). In this context, a fish group that feeds over multiple habitats is expected to have a large isotopic niche. This spatial distribution, in which the smaller individuals remain in the inner part of the estuary and larger ones concentrate themselves in the outer part of the bay, could indicate an ontogenetic movement from the estuary to the adjacent continental shelf (CS) waters as fish grow (Vicentini and Araújo 2003). Our isotopic data corroborate the investigation conducted on whitemouth croaker from Sepetiba Bay (an estuary close to Guanabara Bay), which concluded that juvenile fish remain in the shallower parts of the bay, before moving to CS waters as they grow (Costa and Araújo, 2003). A diagram summarising the hypothesised whitemouth croaker habitat shift in GB over the fish growth can be found in Fig. 5.

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

In addition to seasonal differences in isotopic niche size, differences in standard ellipse position and area were observed between medium-sized whitemouth croakers sampled in GB in summer 2013 and 2014. These trends were largely linked to differences in $\delta^{15}N$ values (Fig. 2), which showed a 2.6‰-shift between the two years. Such a shift might provide evidence for feeding at different trophic levels for the same species and environment in two consecutive summers since the $\delta^{15}N$ shift between diet and consumer is usually assumed to be from +2.6 to +3.4‰ (McCutchan *et al.* 2003). This apparent trophic level shift could be explained by the species-richness increase driven by the higher SACW influence on Guanabara Bay in 2013. Using the temperature-salinity characteristics, Emery and Meinck (1986) suggested that the SACW is a water body with temperatures between 5 and 18 °C, and salinity varying from 34.3 to 35.8 S. However, Miranda (1985) suggests temperatures between 8 and 20 °C for the SACW in Southeastern Brazil. Despite the lack of consensus regarding the temperature, the water temperature in GB was lower than 18 °C in summer 2013 (November 2013 to February 2014), while this thermal pattern was not repeated in the following summer (data obtained from the Hydrobiology laboratory - Federal University of Rio de Janeiro), corroborating the hypothesis of a stronger SACW influence in summer 2013. It is known that isotopic changes do not immediately follow diet alterations (Peterson and Fry, 1987); however, recent experimental work has found $\delta^{15}N$ half-life estimates to be around 23.9 days and trophic discrimination of 3.42 ± 0.42 $\delta^{15}N$ (‰) for estuarine whitemouth croaker (Mont'Alverne *et al.*, 2016), which suggests that the abovementioned stronger influence of the SACW in November 2013 could be a valid explanation for the higher δ^{15}N values observed for that summer (*i.e.* December 2013 to March 2014). Moreover, the stronger influence of the SACW in 2013 could also explain why the observed trend of seasonal niche size reduction in summer was stronger in this year (Fig. 4). However, these results have to be taken with caution, as baseline shifts in isotopic composition (*i.e.* temporal and/or spatial changes in isotopic composition of producers at the base of the food webs, which might be reflected in higher consumers through cascading effects) could have a strong influence on measured values, complicating data interpretation (Boecklen *et al*., 2011).

5. Conclusions

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

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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

Figure captions

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

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

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

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

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

Figure 1

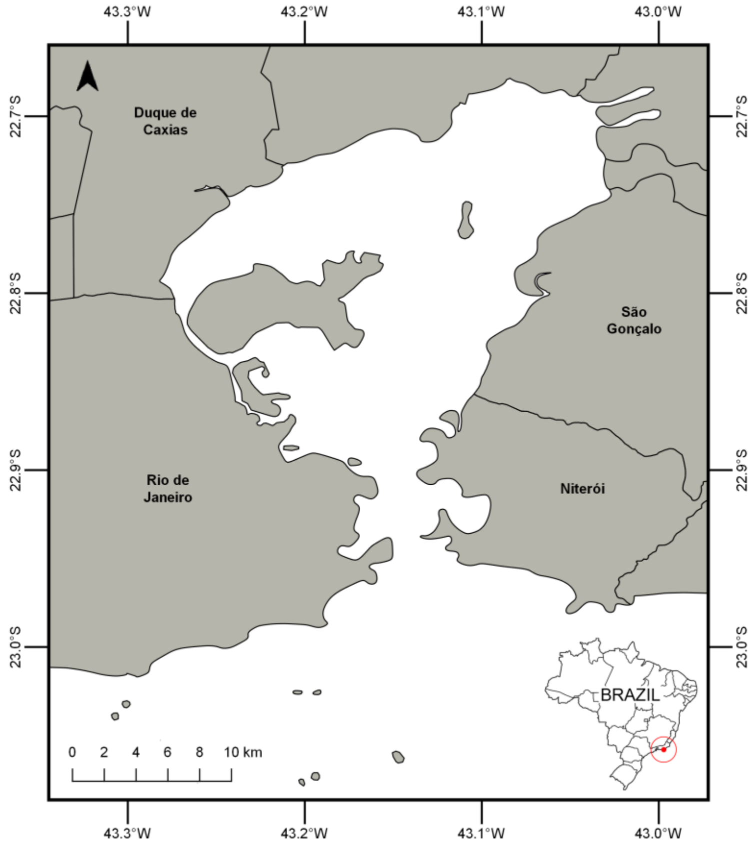

43.3°W
43.2°W
43.1°W
43.0°W
22.7°S
22.8°S
22.9°S
23.0°S
Duque de Caxias
São Gonçalo
Rio de Janeiro
Niterói
BRAZIL
0
2
4
6
8
10 km

Figure 2

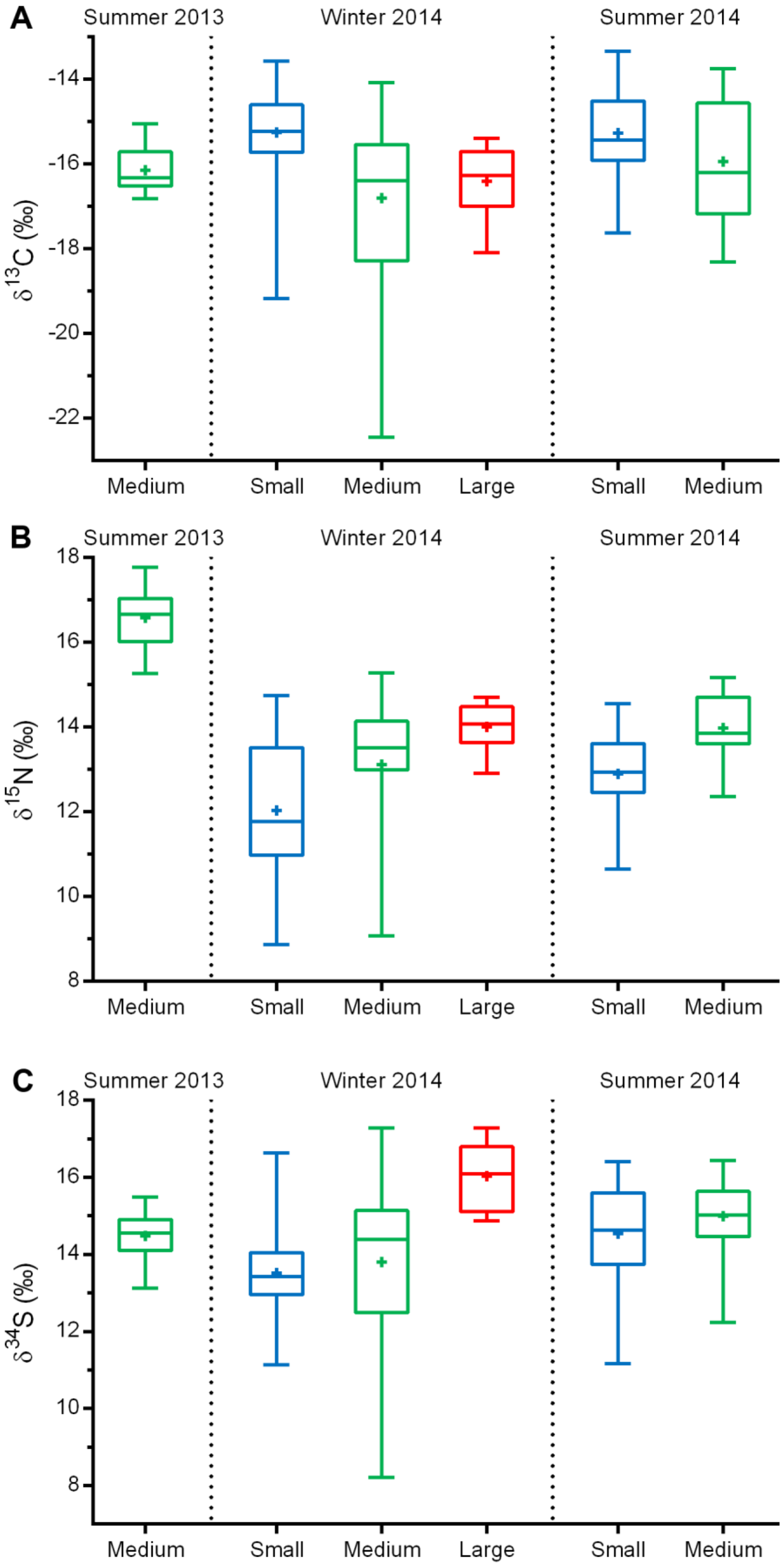

A
Summer 2013
Winter 2014
Summer 2014
δ^{13}C (‰)
-14
-16
-18
-20
-22
Medium
Small
Medium
Large
Small
Medium
B
Summer 2013
Winter 2014
Summer 2014
δ^{15}N (‰)
18
16
14
12
10
8
Medium
Small
Medium
Large
Small
Medium
C
Summer 2013
Winter 2014
Summer 2014
δ^{34}S (‰)
18
16
14
12
10
8
Medium
Small
Medium
Large
Small
Medium

Figure 3

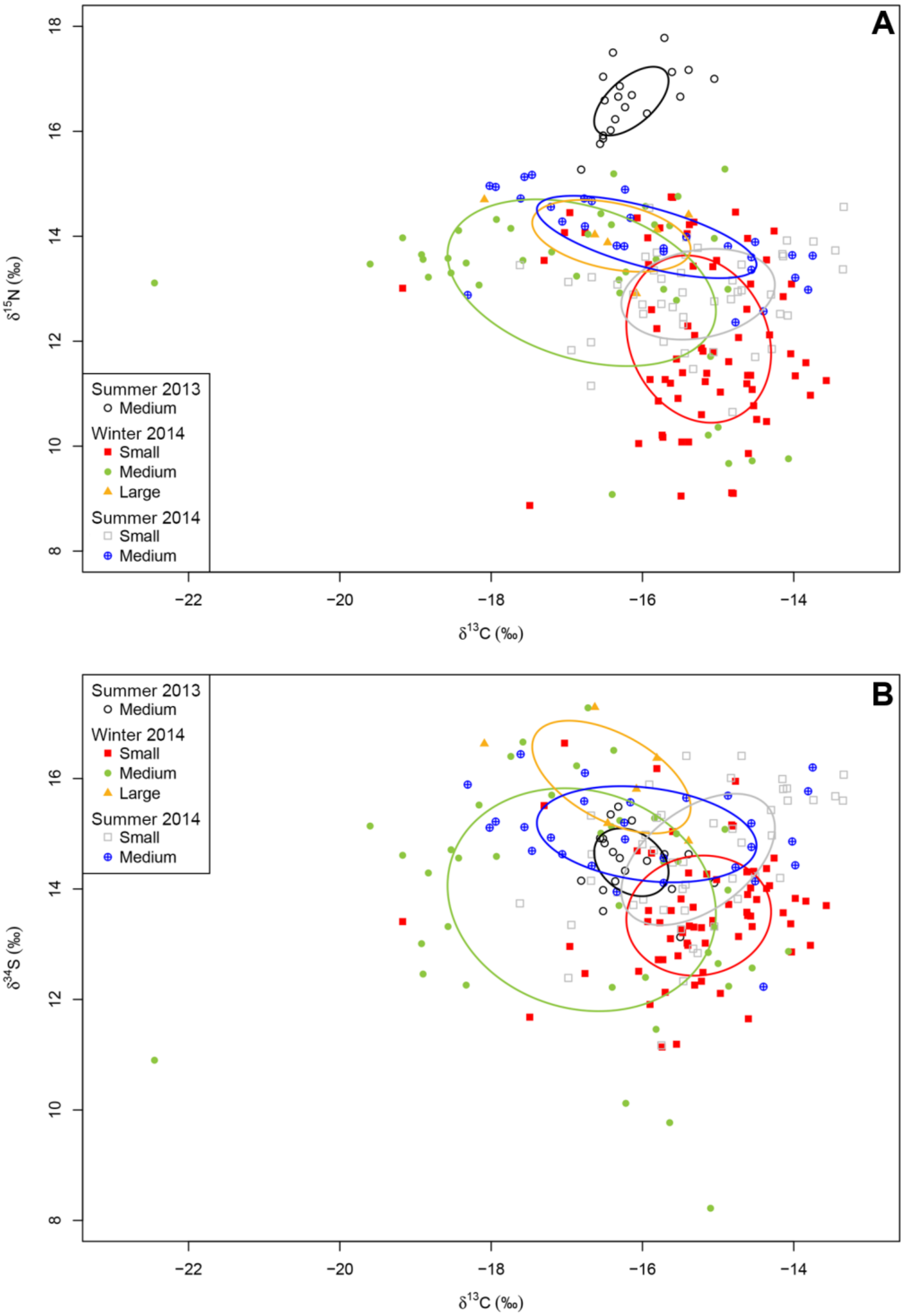

A
δ^{15}N (‰)
18
16
14
12
10
8
−22
−20
−18
−16
−14
δ^{13}C (‰)
Summer 2013
○ Medium
Winter 2014
■ Small
● Medium
▲ Large
Summer 2014
□ Small
⊕ Medium
B
δ^{34}S (‰)
16
14
12
10
8
−22
−20
−18
−16
−14
δ^{13}C (‰)
Summer 2013
○ Medium
Winter 2014
■ Small
● Medium
▲ Large
Summer 2014
□ Small
⊕ Medium

Figure 4

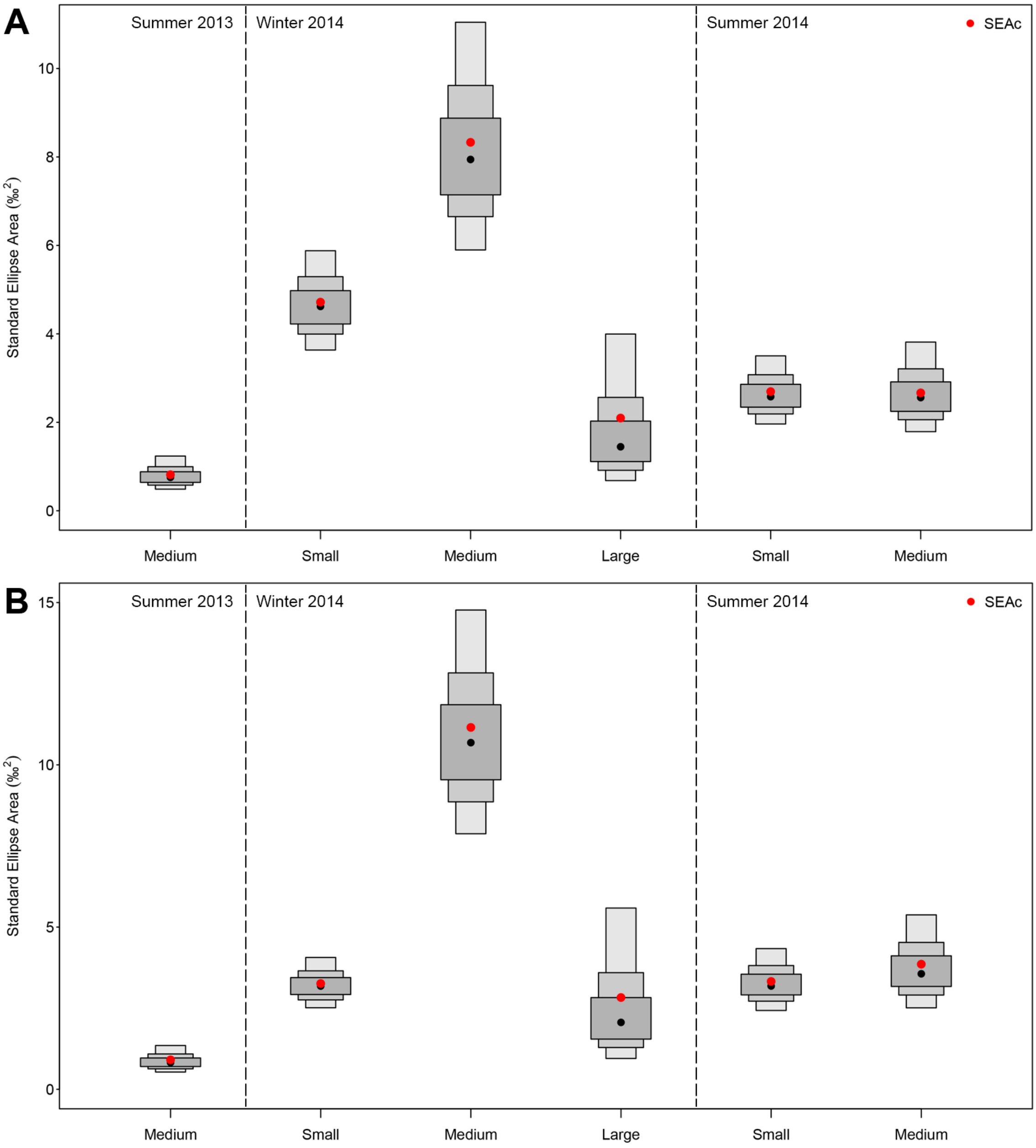
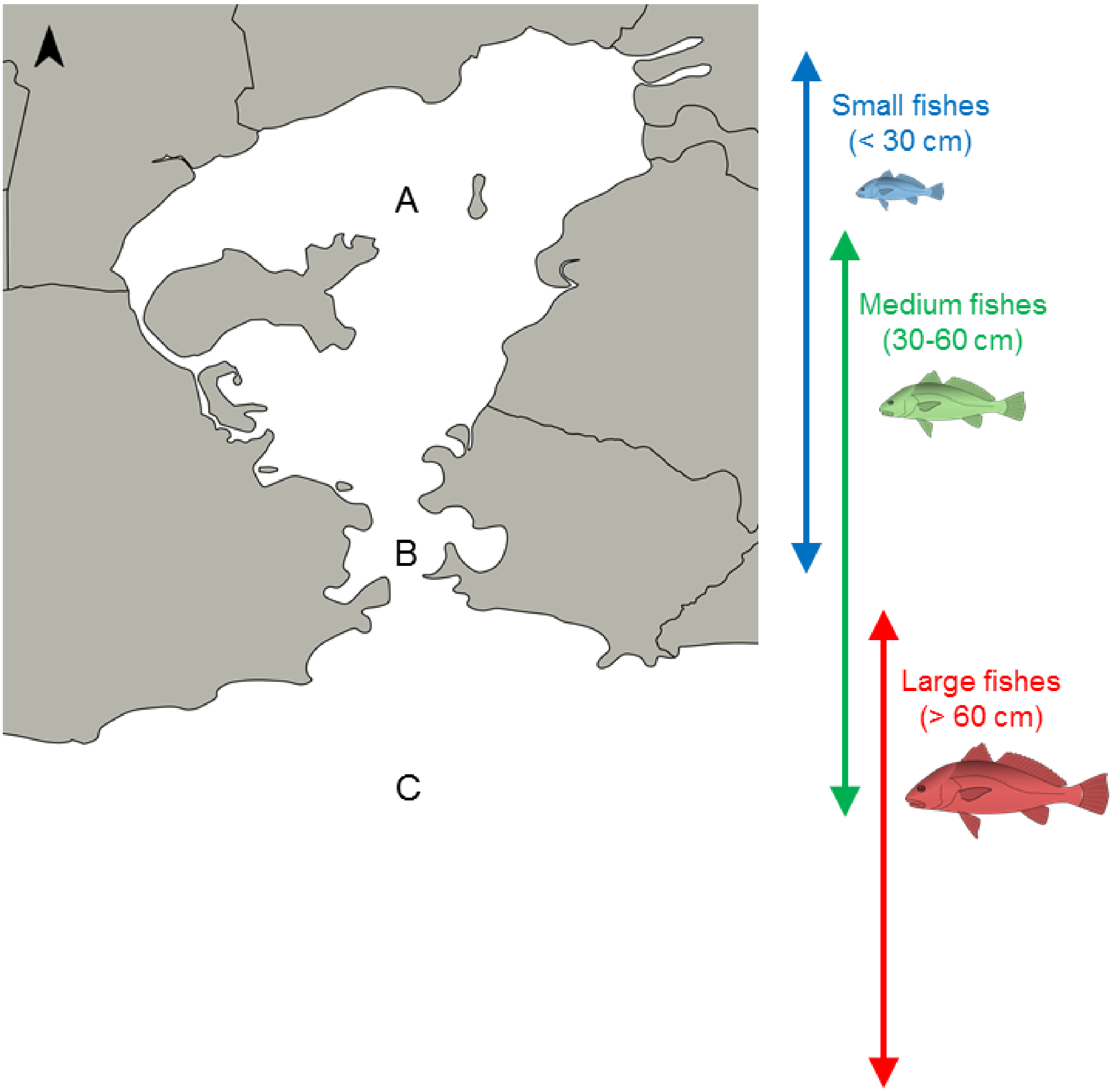

A
Summer 2013
Winter 2014
Summer 2014
SEAc
Standard Ellipse Area (‰²)
0
2
4
6
8
10
Medium
Small
Medium
Large
Small
Medium
B
Summer 2013
Winter 2014
Summer 2014
SEAc
Standard Ellipse Area (‰²)
0
5
10
15
Medium
Small
Medium
Large
Small
Medium

Figure 5


A
B
C
Small fishes
(< 30 cm)
Medium fishes
(30-60 cm)
Large fishes
(> 60 cm)