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Diet and reproductive aspects of the endangered butterfly ray *Gymnura altavela* raising the discussion of a possible nursery area in a highly impacted environment

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Identification of critical habitat is a well-recognized essential component of sustainable resource management. Nursery grounds are considered critical habitats (Medeiros et al., 2015; Rangel et al., 2018). These sites can be used by many species, separated in space and time, and it is important to understand why and how they are used, especially now, in view of the rapid decline of aquatic populations and the increase of coastal pollution (Simpfendorfer and Milward, 1993; Heithaus, 2007; Rosenfelder et al., 2012). An area used as a nursery would necessarily harbor a larger proportion of juveniles for recruitment to adult populations, on average, than would other habitats (Gunter, 1967; Beck et al., 2001). Beck et al. (2001) also emphasized the importance of these juveniles surviving and increasing in number. Generally, suitable nurseries for elasmobranchs are shallow areas with a large food supply and low predation pressure, where gravid females bear their offspring or lay their eggs and the juveniles pass their first weeks, months or years (Castro, 1993). However, the presence of an abundance of juveniles does not by itself mean that an area actually exercises this role. Heupel et al. (2007) suggested a group of criteria to identify an elasmobranch nursery area. Nursery grounds are normally located in shallow coastal areas such as estuaries (*e.g.* Gadig et al., 2002; Medeiros et al., 2015; Rangel et al., 2018).

These fishes have been under strong fishing pressure, and their biological vulnerability makes their management and conservation a delicate and complex matter (Dulvy et al., 2014; 2017). Authors have already cited this group of cartilaginous fish as a challenge and priority in conservation (Dulvy et al., 2017). The butterfly ray *Gymnura altavela* (Linnaeus, 1758) (Myliobatiformes: Gymnuridae) is abundant in important estuary in Rio de Janeiro, southeastern Brazil (Silva-Junior et al., 2016; Paiva et al., 2018). According to the International Union for Conservation of Nature, this specie is vulnerable worldwide, with little biological information available for the southeastern Atlantic (Vooren et al., 2007). *Gymnura altavela* inhabits shallow marine and brackish waters, and is widely distributed along the western and eastern Atlantic Ocean and the Mediterranean and Black seas (Weigmann, 2016). In Brazil, this ray is critically threatened, its presence is confirmed only for the south-southeastern region, where it is under strong pressure from fishing, with a drastic decline in the catch (Vooren, 2007; ICMbio, 2014), as well as from high levels of contaminants (Rosenfelder et al., 2012).

This study aimed to analyze reproductive aspects and the nursery conditions for the butterfly ray, according to the criteria proposed by Beck et al. (2001) and Heupel et al. (2007) in eutrophic tropical estuary and also generate information about the population aspects, diet composition and reproductive biology of this endangered and little-known specie.

Guanabara Bay (22°41'–22°03'S; 043°16'–043°01'W) is an estuarine ecosystem, semi-enclosed, with semi-diurnal tides, tropical climate and strong marine influence. The estuary is located in South America, southeastern Brazil, in the metropolitan region of Rio de Janeiro state (Figure 1). From the bar at the bay mouth (lower estuary) to the innermost areas (upper estuary) is a natural and anthropic hydrologic gradient, which is driven both by rainfall and by the discharge of untreated domestic and industrial pollution that impact the water quality, distribution of sediments and biota (Silva-Junior et al., 2016).

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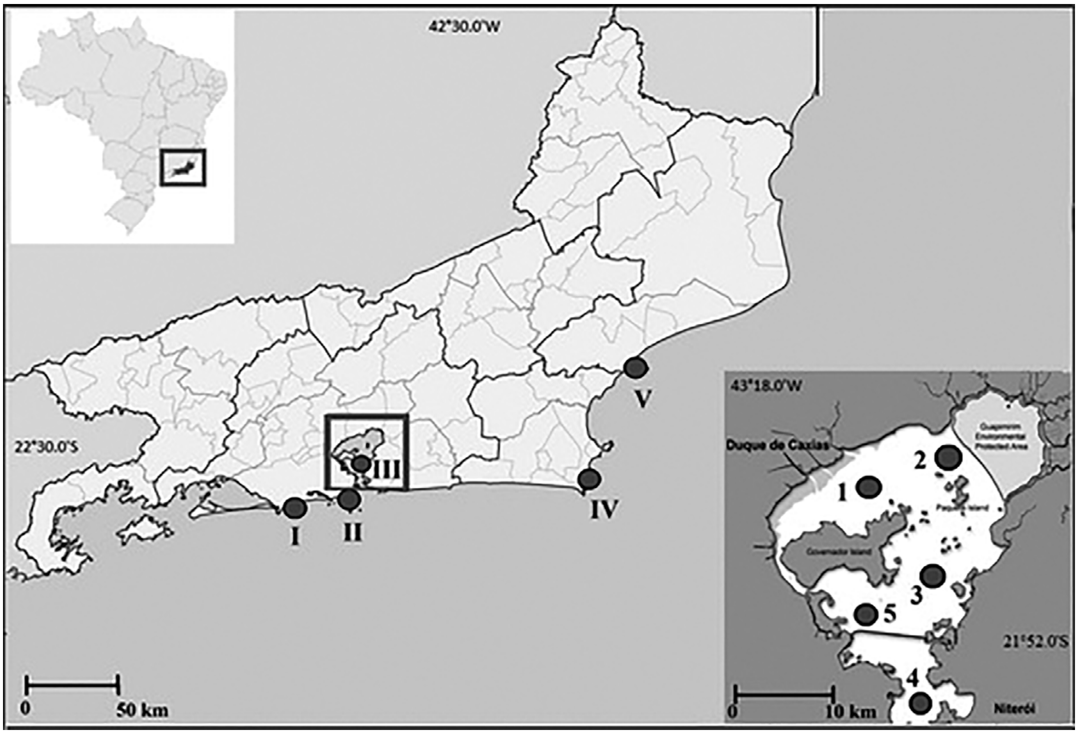

Figure 1. Map of estuary of Guanabara Bay and Rio de Janeiro state, Brazil, southwestern Atlantic, showing locations of samples taken from 2013 through 2014. I - Recreio dos Bandeirantes beach, II - Copacabana Beach, III - Guanabara Bay (taken from July 2005 through June 2007 and from November 2012 through March 2015, 1 - Duque de Caxias, 2 - Paquetá Island, 3 - Central Channel, 4 - Botafogo/Jurujuba, 5 - Governador Island and 6 - Copacabana Beach), IV - Cabo Frio city and V - Macaé city (Abreu, 2006, modified).

The biological sampling was conducted by means of scientific collections (IBAMA/Brazilian Institute of Environment and Water Resources – Permit No. 055, 12/05/2005) in Guanabara Bay, in two phases, targeting demersal fish. The first (Phase I) consisted of biweekly collections from July 2005 through June 2007, in five areas, with two tows per area, along the entire estuarine gradient. Areas 1 and 2 were in the upper estuary, Area 5 in the middle, and Areas 3 and 4 in the lower estuary. The second (Phase II) consisted of samples taken monthly from November 2012 through March 2015 in only Areas 3 and 5, with three tows per area (Figure 1). Samples were collected using the same trawl net and the same fishing vessel in all collections, operating at an average trawling speed of 1.5knots. The net employed was 7m long with a 14-m groundrope, and the mesh size was 18mm. *Gymnura altavela* caught were killed by cooling and then transported on ice to the laboratory, where they were frozen. The specimens was measured (disc width - W_D) in centimeters (cm) and the total mass in grams (g) was obtained. The sex and stage of maturity (juveniles or adults) were obtained externally, by the shape and rigidity of the clasper. Females were classified by the condition of the gonads, after dissection using a ventral incision (Capapé et al., 1979).

The density and biomass of *G. altavela* per area trawled (A_T) were calculated using the equation: $A_T = D*h*X_2$, where D is the distance traveled by the net in kilometers (km), h is the length of the headrope (km) and X_2 is a constant that represents the fraction of the length of the headrope, referring to the width of the trajectory swept by the net mouth (Sparre and Venema, 1997). The distance traveled (D) corresponds to the velocity of the tow (1.5knots=2.78km/h^{-1}) multiplied by the length of time taken by the trawl (0.5h). The headrope measured 0.015km and the value of the constant X_2 was 0.5 (Barletta et al., 2005). The catch per unit area trawled was used to calculate the density (ind km^{-2}) and biomass (g km^{-2}), dividing the number and biomass of the individuals caught in each trawl by the area swept (A_T) (Sparre and Venema, 1997). The individuals sampled were grouped by bimesters and collection localities, in order to identify possible temporal or spatial patterns of occurrence. The numerical differences between juveniles and adults, males and females, and by size class ($W_{D,}$ cm) were tested using Chi-square (χ^2) (Zar, 1999) Additional individuals of *G. altavela* were obtained from artisanal fishermen, at different locations on the coast of Rio de Janeiro state with different types of fishing gear and it is not possible to standardize the effort (Figure 1).

The possibility that Guanabara Bay functions as a nursery ground for *G. altavela* was examined based on the criteria of Beck et al. (2001), (i) as to the presence or absence of potential predators on the juveniles, and (ii) if abundant food is available, favoring growth. We also considered the criteria proposed by Heupel et al. (2007), including (iii) density, that is, if juvenile individuals were found in higher numbers in this area than in nearby areas; and (iv) fidelity, if the habitat is used by juveniles in successive years, while other locations are not. Only wthe requirement to determine if the same individuals tend to return frequently to the locale could not be evaluated, because another sampling method would be needed.

To determine the diet composition of *G. altavela*, the feeding biology was studied using the specimens caught in the trawls, in Guanabara Bay, these rays were caught in September 2013, February-March 2014 and August 2017. The study of stomach contents of *G. altavela* was supplemented with specimens obtained by artisanal fishing along the coast of the Rio de Janeiro state, with a gill net, between May and October 2013. The stomachs were fixed in 10% formol and after were preserved in 70% ethanol. Subsequently, were opened and their contents analyzed with the aid of a stereoscopic microscope, and the items identified to the lowest possible taxonomic level and weighed to the nearest 0.1g. Teleost otoliths found were measured in millimeters, and identified based on the shape and the groove of the ventral otolith, according to Corrêa and Vianna (1992-1993), and consultation in the otolith collection of the Laboratório de Mamíferos Aquáticos/ UERJ, according to Melo et al. (2010).

In the analysis of the stomach contents, the frequency of occurrence ($\%F_O$) and the Alimentary Index ($\%I_{AI}$) were calculated in order to evaluate the importance of each item relative to the total mass of all items (changing one of the variables to percentage mass – $\%M$) with the equation: $\%I_{AI1}=[(\%F_{O1}*\%M_1)/\Sigma(\%F_{OT}*\%M_T)]*100$ (see Viana et al., 2017). The otolith measurements were used in the equation $L_T=a(L_O)^b$ where L_T is the total length, *a* (23.4308) and *b* (0.975) are constants, and L_O is the otolith length (mm), thus obtaining the size of the fish ingested (Melo et al., 2010). These data were used to determine the linear relationship (y=*a*x+*b*) between the width of the disc of the ray and the length of the prey.

Using the larger sample number, the adjusted length-mass relationship (LMR) was calculated by the equation $M_{T} = a\ W_D{}^b$, where M_T is the total mass (g), W_D the disc width (cm), and parameters *a* and *b* are estimated based on the least-squares method, after log-transforming the values of M_T and W_D. The relationship between the disc width (W_D, cm) and total length ($L_{T,}$ cm) was obtained by $W_D=aL_T+b$, since the disc width as well as the total length can be used as a unit. To compare the sizes between sexes, a graph of the sex ratio with 10-cm class intervals was constructed and the values tested for each class by X^2 (Zar, 1999). The size at first sexual maturity (L_{50}) of the females was calculated, by the equation $p=1/1+e^{[-r(L-L50)]}$, where *p*=proportion of adult individuals; r=rate of increase, L=length class and L_{50} the size at which 50% of individuals are mature (King, 2007). It was not possible to obtain L_{50} for males, due to the small number of intermediate-sized adult males, which are necessary for a good fit of the equation.

In Guanabara Bay, 64 individuals of *G. altavela* were captured, 80% juveniles, 66% males and 34% females. In the first phase, the locations with the most occurrences and higher biomassa was Duque de Caxias - upper estuary (13, 89 ind km^{-2}, 30128 g km^{-2}), and the period of highest incidence was January-February 2007. In phase II the rays were found only at Governador Island (13,76 ind km^{-2}, 33485 g km^{-2}), with the peak of occurrence in November-December 2012 (Figures 2 and 3). The proportions of both males (X^2=9.7, df=1, p<0.01) and juveniles (X^2=35.2, df=1, p<0.01) were significantly higher. The W_D ranged from 30.3–108.0cm and the total individual mass from 350–11,000g.

Gymnura altavela was present in all the sampling years (iv) and comprised 80% of the young individuals. The analysis of feeding biology (ii) was based on 25 stomachs of specimens from Guanabara Bay, 19 of them caught with a trawl and six with a drift net. Of these, seven were empty, and were excluded from the analysis. The ray is piscivorous and feeds mainly on the whitemouth croaker, *Micropogonias furnieri* (Desmarest, 1823) (55.6 F_O%, 51.4I_{AI}%) (Table 1). At coast of Rio de Janeiro, 47 *G. altavela* were use, 18 from Cabo Frio coast, 15 from Copacabana beach (adjacent to Guanabara Bay), four from Recreio dos Bandeirantes beach and one from Macaé coast. Of these, nine were empty, and were excluded from the analysis. The stomachs contained mainly teleost

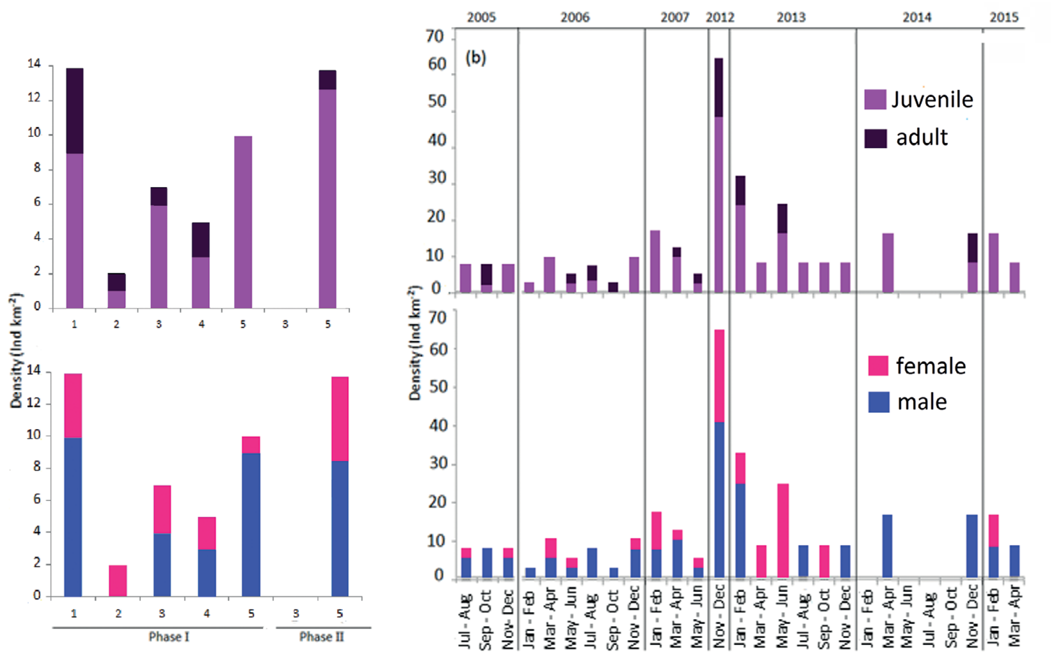

Figure 2. (a) Spatial distribution, 1- Duque de Caxias, 2- Paquetá Island, 3- Central Channel, 4- Botafogo/Jurujuba, 5- Governador Island; and (b) Temporal variation between adults and juveniles, males and females of *Gymnura altavela* (ind km^{-2}) in Guanabara Bay, southwestern Atlantic (July 2005 - June 2007 and November 2012 - March 2015).

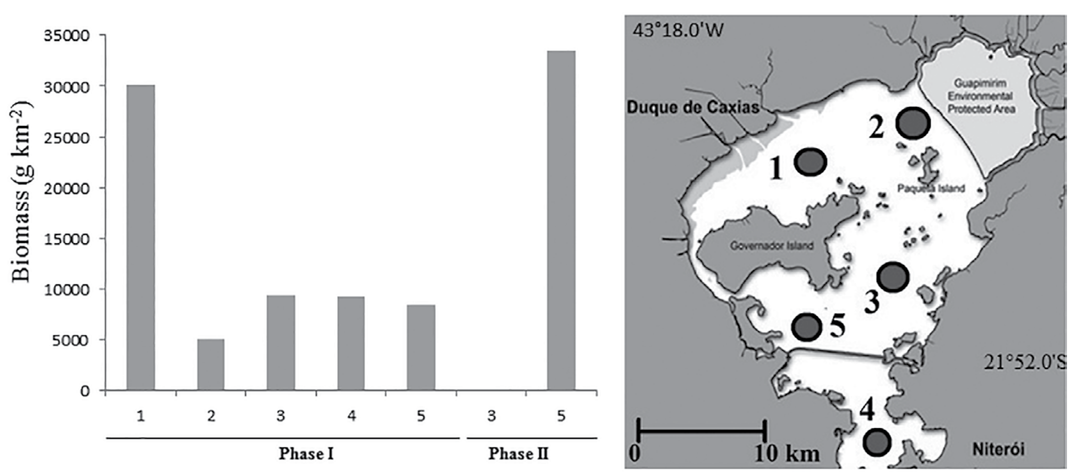

Figure 3. Spatial distribution 1- Duque de Caxias, 2- Paquetá Island, 3- Central Channel, 4- Botafogo/Jurujuba, 5- Governador Island of *Gymnura altavela* biomass (g km^{-2}) in Guanabara bay, southwestern Atlantic (July 2005 - June 2007 and November 2012 - March 2015.

fragments (71.0F_O%, 12.0I_{AI}%) (Table 1) following for whitemouth croaker (13.20F_O%, 12.0I_{AI}%). Two stomachs contained plastic material, one an entire drinking straw. A strong positive correlation (R^2=0.76) was found between the size of *G. altavela* and that of individuals of *M. furnieri* in the stomach contents, showing that larger rays tended to prey on larger whitemouth croakers (Figure 4).

Along the coast of Rio de Janeiro, 116 individuals of *G. altavela* were collected (Table 2). The relationships of length/mass and disc width/total length for males, females, and the sexes combined all showed high correlation coefficients (r^2>0.95), indicating a good fit (Table 3). In most size classes, males were more abundant in the smaller classes and females in the larger classes. However, this difference was only significant in the classes of 34.0–44.0cm (X^2=11.1; df =1; p<0.01), 44.1–54.0cm (X^2=6.9; df = 1; p<0.01) and 84.1–94.0cm (X^2=8.6; df =1; p<0.05), where males were more abundant. In class 94.1–144.0cm,

Table 1. Frequency of occurrence (%FO), percentage mass (%M) and alimentary index (%IAI) of prey items in the diet of *Gymnura altavela*, in Guanabara Bay and on the coast of Rio de Janeiro, Brazil, southwestern Atlantic.

Prey Items	Guanabara Bay (n=18)			Coast of Rio de Janeiro (n=38)		
	% F_O	% M	% I_{AI}	% F_O	% M	% I_{AI}
Teleosts						
Micropogonias furnieri	55.6	92.5	51.4	13.2	55.4	7.1
Cynoscion guatupuca	-	-	-	2.6	22.4	0.6
Ctenosciaena gracilicirrhus	-	-	-	2.6	1.7	0
Paralonchurus brasiliensis	-	-	-	2.6	2.2	0
Teleost fragments	22.2	4.2	0.9	71.0	17.2	12.0
Sciaenidae	5.6	1.8	0.1	-	-	-
Dorytheutis sp	-	-	-	6.7	0	0
Itens not identified	16.7	1.5	0.2	7.9	1.0	0

Table 2. Total number (N), size range (WD), fishing gear, average Biomassa (AV Biomass) and total biomass (T Biomass) for *Gymnura altavela* on the coast of Rio de Janeiro, southwestern Atlantic.

Location	N	Range DW (cm)	Gear	AV Biomass (g)	T Biomass (g)
Guanabara Bay	68	34.2 - 108.0	Trawl/Gill nets	2431	165284
Cabo Frio	22	60.2 - 143.0	Gill net	4731	11370
Copacabana	19	49.2 - 135.3	Gill net	5903	112160
Recreio dos Bandeirantes	6	45.1 - 78.5	Gill net	1895	104090
Macaé	1	76.1 - 115.8	Trawl	13150	13150

Table 3. (a) Parameters of the length–mass ($M_T = a\ WD^b$) and (b) disc width–total length ($W_D = a$ LT+b) relationships for *Gymnura altavela* on the coast of Rio de Janeiro, southwestern Atlantic.

(a)	Sex	N	W_D (cm)	M_T (g)	*a*	*b*	r²
	Both	116	34.2 - 143.0	329.7 - 25630.0	0.0064	3.0651	0.9837
	Female	52	39.1 - 143.0	509.3 - 25630.0	0.0068	3.0498	0.9890
	Male	64	34.2 - 108.0	329.7 - 11000.0	0.0059	3.0866	0.9782
(b)	**Sex**	**N**	**W_D (cm)**	**L_T (cm)**	***a***	***b***	**r²**
	Both	101	35.5 - 143.0	23.1 - 90.0	1.4788	2.1398	0.9640
	Female	47	39.1 - 143.0	26.5 - 90.0	1.5170	1.5642	0.9772
	Male	54	35.5 - 96.8	23.1 - 68.8	1.4041	4.3517	0.9515

only 11 individuals occurred, eight of which were females. The largest male caught had W_D of 108.0cm, and the largest female measured 143.0 cm, showing that females reach larger sizes than males. To estimate the size at first stage of maturation (L_{50}) the range of W_D of the females (N=59) analyzed was 39.1–143.0cm and the L_{50} was 71.7cm (Figure 5).

Gymnura altavela inhabits a highly eutrophic estuary off the coast of Rio de Janeiro, southeastern Brazil and their low numbers of captured individuals reflects their critically threatened status on the coast of Brazil (ICMbio, 2014). The butterfly ray population was present mainly in the inner areas, which are more sheltered, have calmer waters, and abundant whitemouth croakers (Mulato et al., 2015), this ray's preferred food. Juveniles predominated in the population (phase I, 79%; phase II, 81%) and these appear to prefer the inner areas; individuals in this stage of the life cycle seek shallow, sheltered locations that can provide more food (Mulato et al., 2015) and protection (Castro, 1993; Simpfendorfer and Milward, 1993). Juveniles of *G. altavela* were previously reported to enter lagoons and estuaries to find sufficient resources and to develop (*e.g* El Kamel et al., 2009). The catch was higher in the rainy months and during the incursion of the

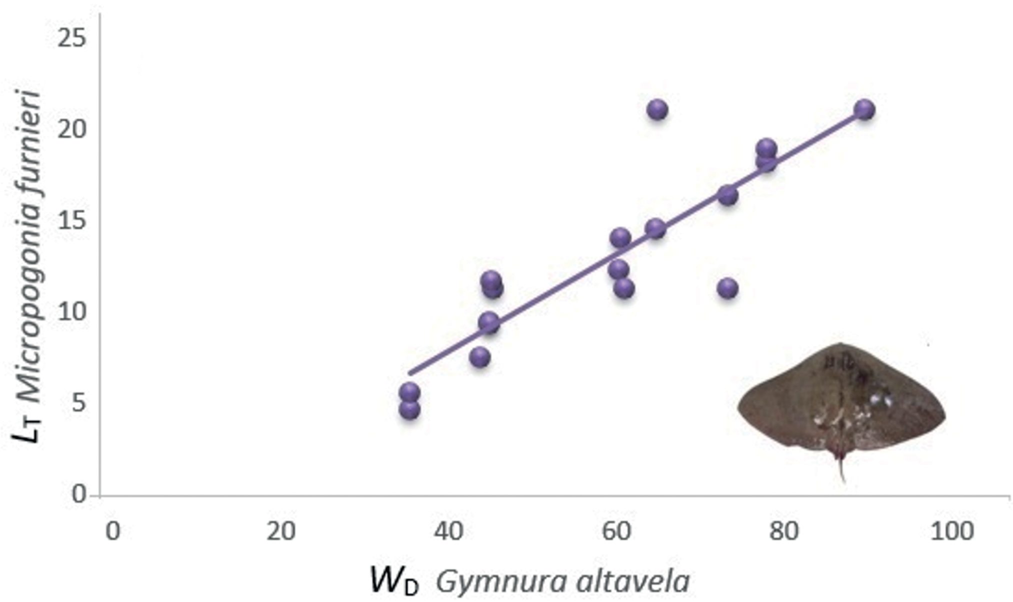

Figure 4. Correlation of *Gymnura altavela* disc width (WD, cm) and *Micropogonias furnieri* total length (LT, cm) from stomachs of *G. altavela* from Guanabara Bay and Copacabana Beach, southwestern Atlantic. LT = 0.2667WD -2.8586 R^2 = 0.7588.

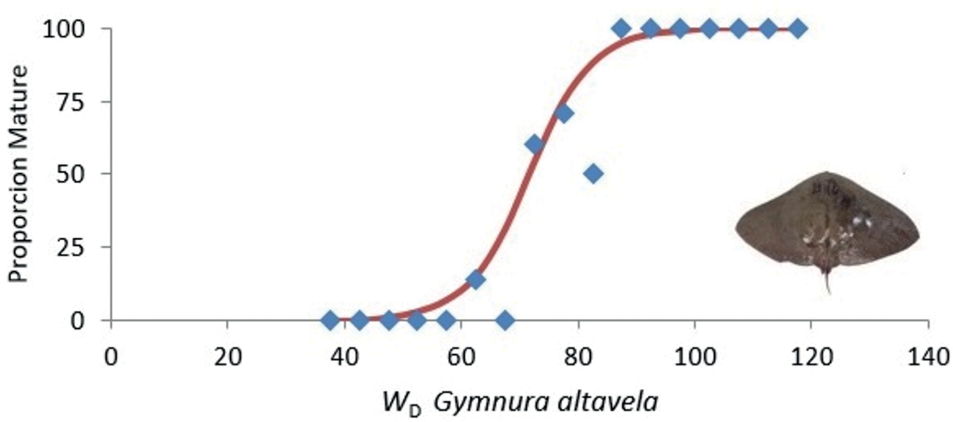

Figure 5. Graph of estimated size at first stage of maturation (L50) of females of *Gymnura altavela* (N = 59). The range of disc width (WD) analyzed was 39.1-143.0 cm, on the coast of Rio de Janeiro state, southwestern Atlantic.

SACW, the period when the water column is stratified (Valentin, 1994). However, although the butterfly ray is abundant in the bay, it is rare in nearby locations. At Itaipú just east of the bay mouth, Tubino et al. (2007) found only six individuals in two years of study. At Recreio dos Bandeirantes on the coast west of the bay, Silva-Junior et al. (2008) recorded only 19 individuals also in two years.

Bays are naturally favorable nursery environments, since they are more protected and shallow, with less competition, and access of larger organisms is restricted (Rountree and Able, 1996; Ebert, 2002; Heupel and Simpfendorfer, 2011). The 80% proportion of *G. altavela* juveniles in the bay calls attention to the nursery potential of this locale. Nursery grounds characteristically have a high density of juveniles (Beck et al., 2001); however, according to Heupel et al. (2007) this alone is insufficient to define a nursery ground, which should also have more juveniles than in other, nearby locales. The samples from Copacabana, Recreio dos Bandeirantes and Cabo Frio contained similar numbers of adults and juveniles. In one study conducted in a nearby part of the coast, only 26% of the individuals caught were juveniles (Silva-Junior et al., 2008). The small number of available studies impedes the analysis of this criterion; however, the present data and the small numbers of *G. altavela* found in other parts of the coast of the state of Rio de Janeiro make it clear that Guanabara Bay has a higher density of young individuals, conforming to the criterion of Heupel et al. (2007). The species was caught in every year of sampling, and juveniles failed to be recorded in only four bimesters, showing that *G. altavela* uses this area continuously, agreeing with another criterion of Heupel et al. (2007). The last requirement could not be analyzed; individuals were not marked and recaptured, and therefore we could not determine if the juveniles leave this estuary and then return. However, in tropical waters, individuals characteristically continue to reside on the nursery ground for several years, exploiting this protected and productive area (Castro, 1993; Ebert, 2002; Heupel et al., 2007). Great sharks *i.e. Galeocerdo cuvier*, *Carcharhinus plumbeus* or *Sphyrna lewini* are potential predators of *G. altavela* (Myers et al., 2007) and none of these large predators are found in the Guanabara Bay estuary (Silva-Junior et al., 2016). Showing that butterfly ray don't have any potential predator in this area.

The feeding study showed that *G. altavela* is piscivorous, confirming that the estuary offers an abundant food supply for this ray. The main food of *G. altavela* was the croaker, probably because it is one of the most abundant species in Guanabara Bay (*e.g.* Mulato et al., 2015; Silva-Junior et al., 2016). Teleosts were important in the diets of other members of *Gymnura. i.e*. *G. altavela* in the North Atlantic (Daiber and Booth, 1960) and Mediterranean Sea (Barría et al., 2015; Yemışken et al., 2017), *G. micrura* in northeastern Brazil (Yokota et al., 2013), and *G. australis* in the South Pacific (Jacobsen et al., 2009).

The present study showed that Guanabara Bay functions as a fundamental component of the conservation of rays in the southwestern Atlantic, since it is a potential nursery area for *G. altavela*, satisfying nearly the criteria proposed by Beck et al. (2001) and Heupel et al. (2007). The coast of Syria was also considered a potential nursery ground for this species, but this supposition was based on the large number of females relative to adult males and on the abundance of gravid females (Capapé et al., 1992; Alkusairy et al., 2014). *Gymnura altavela* in Guanabara Bay showed a different pattern, with a larger number of males, many juveniles, and no gravid females. At Copacabana, adjacent to the bay, most rays were adult females. We believe that these females bear their young on the coast and the juveniles enter the estuary to grow. In Brazil, few publications have treated the nursery grounds of elasmobranchs in estuaries (Gadig et al., 2002; Yokota and Lessa, 2006; Andrade et al., 2008; Bornatowski, 2008; Medeiros et al., 2015; Rangel et al., 2018). However, in other parts of the world, several studies, have demonstrated the importance of estuaries for this group (*e.g*. Rountree and Able, 1996; Heupel and Simpfendorfer, 2008; Froeschke et al., 2010; Heupel et al., 2010; Heupel and Simpfendorfer, 2011; Norton et al., 2012; Poulakis et al., 2013; Hoff, 2016).

Gymnura altavela, although critically threatened on the coast of Brazil (ICMbio, 2014), probably at risk worldwide, and vulnerable according to the International Union for Conservation of Nature, has been little studied (Vooren et al., 2007). Data for age, size, growth and reproduction are available only for the Atlantic Ocean (Daiber and Booth, 1960; Wigley et al., 2003); Mediterranean Sea (Capapé et al., 1992; Yeldan and Avsar, 2007; Psomadakis et al., 2008; El Kamel et al., 2009; Başusta et al., 2012; Alkusairy et al., 2014; Özbek et al., 2016), Adriatic Sea (Dulčić et al., 2003) and Aegean Sea (Filiz and Bilge, 2004). A latitudinal difference can affect the size, longevity, fecundity and maturation, as observed in studies with other elasmobranchs (*e.g.* Horie and Tanaka, 2002; Lombardi-Carlson et al., 2003). A larger size at sexual maturity in females than in males has also been found in other studies on *G. altavela* (Daiber and Booth, 1960; Capapé et al., 1992; Alkusairy et al., 2014). In the present study, we found the smallest maximum disc width in both sexes, compared to reports from the Mediterranean Sea and the North Atlantic (Daiber and Booth, 1960; Alkusairy et al., 2014; Özbek et al., 2016). This small size may be caused by latitudinal differences, or may reflect a population in decline, as seen for females measuring more than 200 cm, which were recorded in past decades (*e.g.* Daiber and Booth, 1960; Wigley et al., 2003) and are very probably now nonexistent. This interpretation is supported by recent results for the Syrian coast, where Alkusairy et al. (2014) found the smallest maximum sizes of individuals ever recorded in the Mediterranean Sea. For the size at first maturation of females, Capapé et al. (1992) obtained an L_{50} of 68 cm, slightly smaller than in this study.

The butterfly ray is endangered and effective management and conservation are needed. Identification of nurseries is an essential part of a recovery plan because they facilitate recruitment to the adult population. Norton (2012) based on the criteria of Heupel (2007), designated two estuaries in the United States as Critical Habitat for the endangered sawfish *Pristis pectinata*. Guanabara Bay can be considered a critical habitat for *G. altavela*, but problems such as the pollution of this bay must be rapidly reversed, particularly because the rays ingest plastic residues, as is not unknown for the group (Joyce et al., 2002), and they show high levels of contamination (Rosenfelder et al., 2012).

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