

## Use of a species-rich and degraded tropical estuary by Elasmobranchs

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Conservation of elasmobranch populations is a priority, as 31% of shark and ray species have already gone extinct and 64 threatened species need new or more stringent protections, including improvement in fisheries management. One of the main reasons for this critical condition is that many species inhabit coastal areas, including estuaries, and are directly affected by the degradation of their habitat (Martin, 2005; Davidson et al., 2016, Rodrigues et al., 2018). Estuaries are among the most resilient ecosystems and provide adequate conditions for nursery, feeding, growth, sheltering and breeding of elasmobranch populations (Maes et al., 2004; Farrugia et al., 2001), but many estuarine systems worldwide are heavily polluted. This study aimed to contribute to the knowledge of elasmobranchs in an impacted tropical estuary in southeastern Brazil, Guanabara Bay, Rio de Janeiro, determining the time of year and the sites with the highest incidence of these animals.

From the mouth (lower estuary) to the innermost areas (upper estuary) of Guanabara Bay a natural and anthropic hydrological gradient exists, which is driven by discharge from domestic and industrial pollution, affecting the quality of water, sediments and biota (Silva-Junior et al., 2016). The entrance of the bay (area 4) has a strong marine influence on the hydrological and sedimentary characteristics, with high salinities and fine sediments. In the central channel (area 3), where depths of 30 meters are found, hydrodynamics is intensified by the influence of a salt wedge associated with tidal currents. The upper part of the estuary (areas 1 and 2), mainly on the Northwest and West sections where depths are low and the circulation strict, the less saline and eutrophic waters are found. The Northeast (area 2) and East regions are less polluted thanks to their proximity with the conservation unit of the Guapimirim basin. However, a strong population growth has been recorded around those areas in recent decades

(Ribeiro and Kjerfve, 2002; Silva-Junior et al., 2016). The dry season occurs in winter (June to August) when the water column is more homogeneous, and the rainy season is in summer (December to March), when vertical stratification is common (Valentin et al. 1999; Figueiredo Jr and Fernandez, 2012).

Sampling areas were determined with the aim to depict the above-mentioned environmental conditions (Figure 1). Detailed information on each sampling site is available in Silva-Junior et al. (2016) and sediment data are available in Corrêa and Vianna (2016). Sampling was conducted (IBAMA/Brazilian Institute of Environment and Water Resources - Permit No. 055, 12/05/2005) in two phases. The first (Phase I) consisted of biweekly surveys from July 2005 through June 2007, in five areas, with two tows per area, covering 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) sampling scheme was performed monthly from November 2012 through March 2015 in Areas 3 and 5 only, with three tows each (Figure 1). Samples were collected using the same trawl net and the same fishing vessel, operating at an average trawling speed of 1.5 knots. The net employed was 7-m long with a 14-m ground rope, and the mesh size was 18 mm between adjacent knots. This methodology collected an assortment of demersal animals. The fish caught were sacrificed by placing them on ice and then transported to the laboratory, where they were frozen. Elasmobranchs were identified with appropriate literature (e.g. Gomes et al., 2010). Each specimen was measured (total length -  $L_T$  for Rhinobatidae, and disc width -  $W_D$  for Dasyatidae and Gymnuridae) in centimeters (cm) and their total mass measured as grams (g). The sex and stage of maturity (juveniles or adults) for males were based on shape and rigidity of clasper.

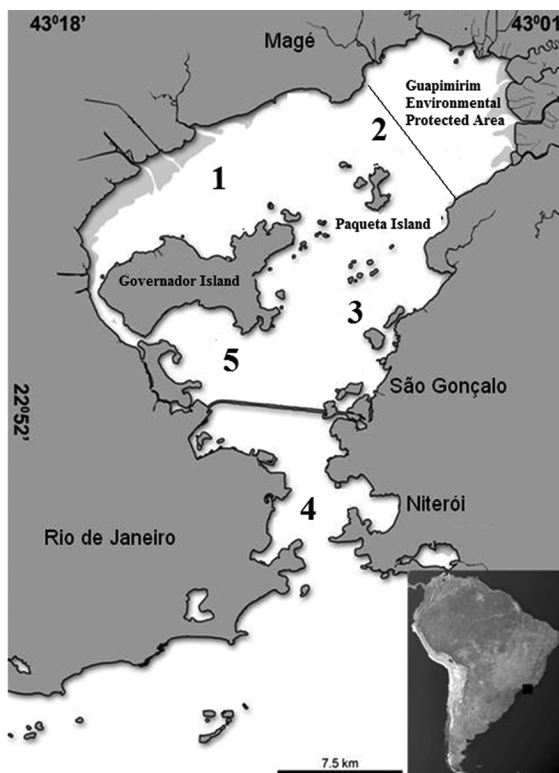
A cumulative species-occurrence curve was used to determine whether the sampling effort was representative of the elasmobranch species inventory in the bay. The density and biomass of elasmobranchs per area trawled ( $A_T$ )

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**Figure 1.** Guanabara Bay showing locations of samples taken from July 2005 through June 2007 (Phase I) and from November 2012 through March 2015 (Phase II - only points 3 and 5), Rio de Janeiro, Brazil, southwestern Atlantic. 1- Duque de Caxias, 2- Paqueta Island, 3- Central Channel, 4- Botafogo/Jurujuba, 5- Governador Island.

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 head rope (km) and  $X_2$  is a constant that represents the fraction of the length of the head rope ( $h X_2$ ), 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 ( $1.5 \text{ knots} = 2.78 \text{ km h}^{-1}$ ) multiplied by the length of time taken by the trawl (0.5 h). The head rope measured 0.015 km 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 ( $\text{ind km}^{-2}$ ) and biomass ( $\text{g km}^{-2}$ ), dividing the number and biomass of the individuals caught in each trawl by the area swept ( $A_T$ ). The use of the analysis by area is necessary from the moment that different sampling efforts were carried out in the different phases, allowing to compare the catches. The individuals sampled were grouped by time and site, in order to identify possible temporal or spatial patterns of occurrence.

To determine spatial and temporal relationships in the composition and abundance of the species, nonparametric

multivariate analyses were employed, using the program PRIMER version 6.0 (Clarke and Gorley, 2006). The similarity matrix between sampling areas and time (Phase I) was constructed using the Bray-Curtis coefficient, after applying the square root-transformation. Based on the matrix, a cluster analysis was performed by the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method. An ordination analysis (for the sampling areas in phase I) was used to determine whether the species composition varied along the estuary, through nMDS (non-metric multidimensional scaling) (Zar, 1999).

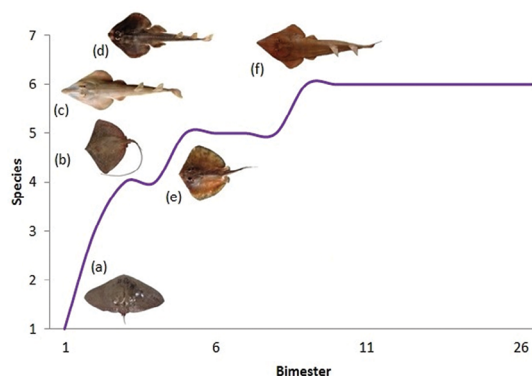
Ninety-seven elasmobranchs were caught (Table 1), 69 in the first and 28 in the second phase. Six species belonging to three families, Dasyatidae, Gymnuridae and Rhinobatidae were recorded in phase I, the butterfly ray *G. altavela* ( $n=38$ ), lesser guitarfish *Zapteryx brevirostris* (Müller and Henle, 1841) ( $n=17$ ), stingray *Dasyatis hypostigma* (Santos and Carvalho, 2004) ( $n=7$ ), Brazilian guitarfish *Pseudobatos horkelii* (Müller and Henle, 1841) ( $n=4$ ), southern guitarfish *Pseudobatos percellens* (Walbaum, 1792) ( $n=2$ ) and longnose stingray *Hypanus guttatus* (Bloch and Schneider, 1801) ( $n=1$ ). In phase II, only *G. altavela* ( $n=26$ ) and *H. guttatus* ( $n=2$ ) occurred. Analysis of the cumulative catch indicated that the sampling effort was sufficient to carry out the inventory of demersal elasmobranchs in the estuary, and no additional species were recorded after the ninth bimester (Figure 2). The effort and the spatial coverage were reduced in Phase II, this could be related to the absence of new catches after the ninth bimester.

Seasonally, in Phase I, the density was highest at the end of summer and beginning of autumn (March-April), in both 2006 and 2007 ( $30.9 \text{ ind km}^{-2}$ ); and the biomass was highest in March-April 2006 ( $39,052.4 \text{ g km}^{-2}$ ). *Zapteryx brevirostris* and *G. altavela* were the main contributors in these periods in both years. In phase II, highest density ( $63.7 \text{ ind km}^{-2}$ ) and biomass ( $213,706.3 \text{ g km}^{-2}$ ) were recorded in late spring (November-December 2012) (Table 2). Including both phases, 73% of the elasmobranchs were caught in the November-April period. The cluster analysis for Phase I (Figure 3) showed that temporally the samples formed two main groups according the abundance: (1 - blue) when 6 or more individuals were caught, mostly from November through April; and (2 - red) when fewer specimens were sampled, between May and October.

Spatially, in the first phase of the study, most elasmobranchs were caught in the lower estuary (63.7%),

**Table 1.** Species of elasmobranchs caught and recorded in Guanabara Bay, Brazil, southwestern Atlantic. Status of the species in the IUCN Red List in the world, and in Brazil (ICMbio, 2014), total number (N), disc width (WD) or total length (LT\*), mass, relative frequency (%) (Phase I, July 2005 - June 2007 and Phase II, November 2012 - March 2015). DD - Data deficient, VU - Vulnerable, NT - Near threatened, CR - Critically endangered.

Species	IUCN Red list	ICMbio	N	%	$W_D/L_T$ (cm)		Mass (g)		Record
					Min.	Máx.	Min.	Máx.	
<i>Dasyatis hypostigma</i>	DD	-	7	7.2	15.3	32.8	109	1347	captured
<i>Gymnura altavela</i>	VU	CR	64	66	30.3	108.0	181	11000	captured
<i>Hypanus guttatus</i>	DD	-	3	3.1	18.5	20.8	160	270	captured
<i>Pseudobatos horkelii</i> *	CR	CR	4	4.1	24.0	115.0	44	7200	captured
<i>Pseudobatos percellens</i> *	NT	-	2	2.1	31.6	93.2	101	3263	captured
<i>Zapteryx brevirostris</i> *	VU	VU	17	17.5	14.0	48.6	160	906	captured
TOTAL			97	100					
<i>Rhinoptera bonasus</i>									literature
<i>Pristis perotteti</i>									literature
<i>Sphyrna tiburo</i>									literature
<i>Sphyrna zygaena</i>									literature
<i>Aetobatus narinari</i>									probability
<i>Atlantoraja castelnaui</i>									probability
<i>Narcine brasiliensis</i>									probability



**Figure 2.** Cumulative sampling curve of elasmobranchs caught bimonthly (Phase I: July 2005 - June 2007 and Phase II: November 2012 - March 2015) in Guanabara Bay, southwestern Atlantic. (a) *Gymnura altavela*, (b) *Dasyatis hypostigma*, (c) *Pseudobatos horkelii*, (d) *Zapteryx brevirostris*, (e) *Hypanus guttatus* (f) *Pseudobatos percellens*.

with 80% of the catches in area 4, which had the highest diversity and 83% of the species. *Zapteryx brevirostris* dominated in this area, comprising 48% of the catches. The highest density was also observed in area 4 (33.7 ind km<sup>-2</sup>), and the lowest in area 2 (2 ind km<sup>-2</sup>). In the second phase, elasmobranchs were found only in the middle estuary (area 5), with a density of 29.6 ind km<sup>-2</sup>. In the lower estuary (area 3) no elasmobranchs were sampled (Figure 4a). Cluster analysis of the catch in Phase I showed that area 4 was highly distinct from the other

sampling locations. This result was related to the higher elasmobranch abundance in area 4, in addition to the occurrence of *Z. brevirostris*, which was restricted to this area. In fact, most elasmobranch species were recorded in area 4. Other similar groups were areas 5 and 1, probably related to the presence of *G. altavela* (Figure 4b).

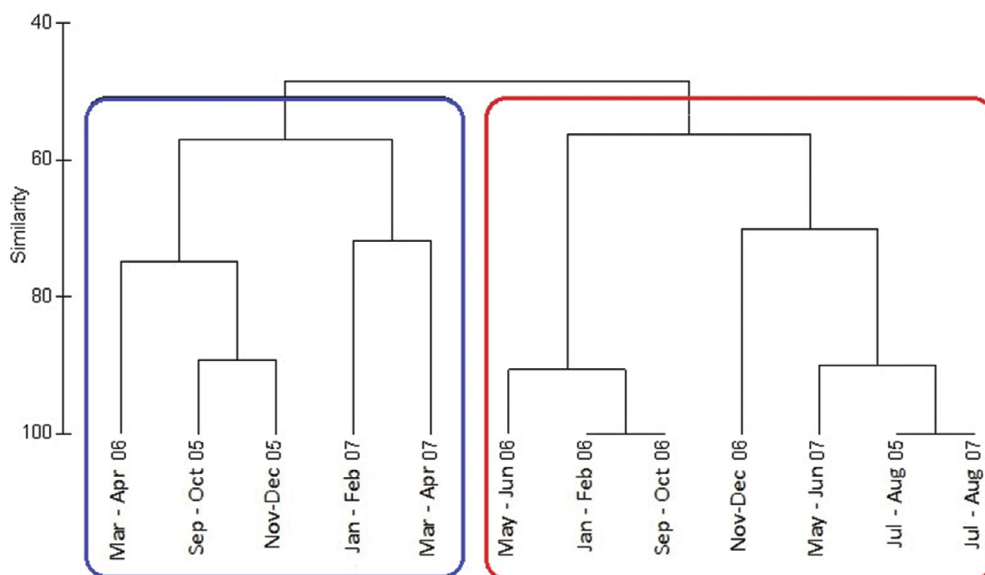
The conservation of elasmobranchs is important as these fish have long life cycles and play important roles at high trophic levels (Abilhoa et al., 2007; Dulvy et al., 2017; Gonçalves-Silva and Vianna, 2018). Elasmobranch bycatch in fisheries focused on crustaceans and teleosts is a major problem, which in most cases is not documented in fishery statistics (Stevens et al., 2000; Francis et al., 2001; Rodrigues et al., 2018). In addition, ray fisheries have always been neglected in Brazil and ray meat consumption remains frequently unnoticed as consumers are not entirely aware of their fish diet composition (Bornatowski et al., 2017). Two out of six species sampled in this study (*D. hypostigma* and *H. guttatus*) are deficiently studied and the remainder species are in a threatened state, with the most serious being *P. horkelii*, classified as critically endangered in the IUCN red list (IUCN, 2018). In Brazil *G. altavela*, *P. horkelii* and *Z. brevirostris* are endangered species ICMbio (2014) (Table 1), and Rosenfelder et al. (2012) have reported *G. altavela* and *Z. brevirostris*, from Guanabara Bay, with a high degree of contamination.

**Table 2.** Species, number of individuals of elasmobranchs (N), density (ind km<sup>-2</sup>) and biomass (g km<sup>-2</sup>) per area trawled sampled in Guanabara Bay, Brazil, southwestern Atlantic (Phase I\*, July 2005 - June 2007 and Phase II, November 2012 - March 2015). *H. gut*, *Hypanus guttatus*; *D. hyp*, *D. hypostigma*; *G. alt*, *Gymnura altavela*; *P. hor*, *Pseudobatos horkelii*; *P. per*, *P. percellens*; *Z. bre*, *Zapteryx brevirostris*.

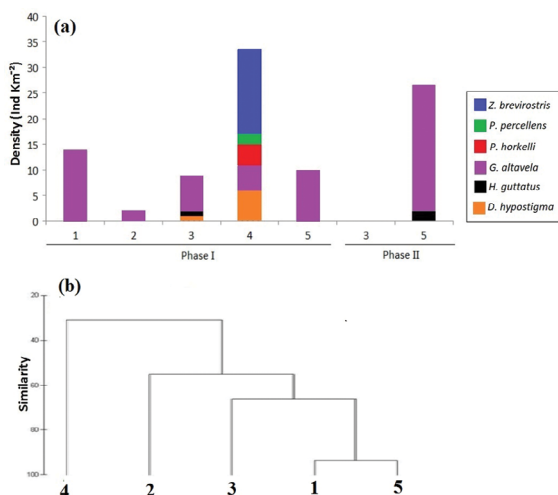
		<i>H. gut</i>	<i>D. hyp</i>	<i>G. alt</i>	<i>P. hor</i>	<i>P. per</i>	<i>Z. bre</i>	<i>N</i>	Ind km <sup>-2</sup>	g km <sup>-2</sup>
2005*	Jul - Aug			3				3	7.1	5481.0
	Sep - Oct		2	3	1			6	14.3	26547.6
	Nov - Dec		2	3	1		1	7	16.7	6378.6
2006*	Jan - Feb			1			1	2	4.8	2395.2
	Mar - Apr	1	3	4	1		4	13	30.9	39052.4
	May - Jun			2			1	3	7.2	13223.8
	Jul - Aug			3				3	7.1	25285.7
	Sep - Oct			1			1	2	4.8	8740.5
	Nov - Dec			4		1		5	11.9	19111.9
2007*	Jan - Feb			7			3	10	23.8	25247.6
	Mar - Apr			5	1	1	6	13	30.9	32447.6
	May - Jun			2				2	4.8	14750.0
2012	Nov - Dec			8				8	63.7	213706.3
2013	Jan - Fev			4				4	31.8	83968.3
	Mar - Apr			1				1	7.9	6349.2
	May - Jun	1		3				4	31.8	55952.4
	Jul - Aug	1		1				2	15.9	15000.0
	Sep - Oct			1				1	7.9	4761.9
	Nov-Dec			1				1	7.9	5396.8
2014	Jan-Fev							0	-	-
	Mar-Apr			2				2	15.9	44285.7
	May-Jun							0	-	-
	Jul-Aug							0	-	-
	Sep-Oct							0	-	-
	Nov-Dec			2				2	15.9	92381.0
2015	Jan-Feb			2				2	15.9	13095.2
	Mar-Apr			1				1	7.9	5714.3

The review by Vianna et al. (2012) listed five species of elasmobranchs in Guanabara Bay. Four of those were caught in this study; the only exception was *Rhinoptera bonasus* (Mitchill, 1815). Vianna et al. (2012) compiled studies using different types of gear sampling throughout the water column. Probably *R. bonasus* was not captured in this study due to its benthopelagic habit (Froese and Pauly, 2018). In a publication of endangered species of

Rio de Janeiro, *Pristis perotteti* (Müller and Henle, 1841), *Sphyrna tiburo* (Linnaeus, 1758) and *Sphyrna zygaena* (Linnaeus, 1758) were reported for Guanabara Bay, but are possibly extinct locally nowadays (Buckup et al., 2000). However, *P. horkelii* and *P. percellens* were recorded here for the first time in the estuary. These species may be visitors in the estuary, since few individuals were caught during the study. In summary, seven species



**Figure 3.** Dendrogram (UPGMA, Bray-Curtis similarity coefficient) for elasmobranchs in Guanabara Bay, southwestern Atlantic (Phase I: July 2005 - June 2007). Two main clusters are delineated, based on bimonthly samples from the first phase of the study. Blue - bimesters when 6 or more individuals were caught. Red - when fewer specimens were sampled.



**Figure 4.** (a) Spatial distribution (ind km<sup>-2</sup>) of elasmobranch species in Guanabara Bay, southwestern Atlantic (Phase I: July 2005 - June 2007 and Phase II: November 2012 - March 2015). (b) Dendrogram (UPGMA, Bray-Curtis similarity coefficient) showing that area 4 was distinct from the other sampling locations, with areas 1 and 5 most similar according to the composition of rays in the estuary of Guanabara Bay, southwestern Atlantic (Phase I: July 2005 - June 2007). 1- Duque de Caxias, 2- Paquetá Island, 3- Central Channel, 4- Botafogo/Jurujuba, 5- Governador Island.

have been identified nowadays in GB, *G. altavela*, *H. guttatus*, *D. hypostigma*, *R. bonasus*, *P. horkelii*, *P. percellens* and *Z. brevirostris*. Other elasmobranch species may also occur, including *Aetobatus narinari* (Euphrasen, 1790), *Atlantoraja castelnaui* (Miranda-Ribeiro, 1907) and *Narcine brasiliensis* (Olfers, 1831), which have been

photographed in a coastal area adjacent to the bay (Ricardo Gomes, unpublished data).

Due to its location and extension, Brazil has both tropical (e.g. Chaves and Vendel, 2001; Viana et al., 2010; Dias et al., 2011; Soares et al., 2011) and subtropical estuaries (e.g. Chaves and Corrêa, 1998; Loebman and Vieira, 2005; Moura et al., 2012; Spach et al., 2013) (Table 3). So far, only the Paraguaçu River estuary (Reis-Filho et al., 2010) equals Guanabara Bay in elasmobranch species richness, while six species have been reported for Sepetiba Bay and Todos os Santos Bay (Soares et al., 2011; Dias et al., 2011). The locations with the highest elasmobranch richness in tropical estuaries are found in Thailand and Australia (Blaber et al., 1989; Blaber et al., 1995; Vidthayanon and Premcharoen, 2002). The more open the estuary is to the ocean, the higher the chance that elasmobranch species will occur, since most species are marine (Froese and Pauly, 2018). This is probably the case of the estuary in Thailand surveyed by Vidthayanon and Premcharoen (2002). Other variables may affect such comparison. Basílio et al. (2008) specifically targeted elasmobranchs; but many studies represented general ichthyofaunal surveys, and many did not use appropriate gear for catching demersal species, probably not efficiently sampling this group. The temporal series of collections and the sampling effort are fundamental variables. The longer the duration of a study, the greater the probability of generating new records, and therefore the rarefaction

**Table 3.** Survey of the works that obtained elasmobranch captures in Brazil and tropical estuaries in the world, with the fishing gear used and their respective references. Number of elasmobranchs species (N). BotT: Bottom trawl; BeaT: Beach trawl; OttT: Otter trawl; CasN: Cast net; GilN: Gill net; TraM: Manual trawl; FykN: Fyke net; BeaN: Beach seine net; BeamT: Beam trawl; SeiN: Seine net; StaN: Stake net; MesN: Mesh net; PurN: Purse seine net; BaiL: Baited trot line.

Estuary	Local (Coast)	N	Fishing gear	Reference
Sepetiba Bay	Brazil, Southeast	6	BotT	Araújo <i>et al.</i> ,1998
Todos os Santos Bay	Brazil, Northeast	6	more than 11	Soares <i>et al.</i> , 2011; Dias <i>et al.</i> , 2011
Curu River Estuary	Brazil, Northeast	5	CasN, GilN	Basilio <i>et al.</i> ,2008
Paraguaçu River Est.	Brazil, Northeast	7	TraM, GilN, CasN	Reis-Filho <i>et al.</i> ,2010
Caeté Estuary	Brazil, North	4	OttT	Barletta <i>et al.</i> , 1998; 2005
Pinheiros Bay	Brazil, South	4	FykN, BotT	Oliveira-Neto <i>et al.</i> ,2008; Schwarz <i>et al.</i> , 2006
Guaratuba Bay	Brazil, South	1	CasN, BotT, BeaT, GilN	Chaves & Corrêa, 1998; Chaves & Vendel, 2001
Vellar Estuary	India, Southeast	2	CasN	Murugan <i>et al.</i> ,2014
Embley Estuary	Australia, Northern	22	GilN, BeamT, StaN, MesN	Blaber <i>et al.</i> ,1989
Albatross Bay	Australia, Northern	17	GilN, SeiN, Trawl	Blaber <i>et al.</i> ,1995
Salomons estuary	Salomons Island	8	GilN, SeiN	Blaber & Milton, 1990
Gulf of Thailand	Thailand	30	Review	Vidthayanon & Premcharoen 2002
Gâmbia Estuary	Africa, West	4	PurN	Albaret <i>et al.</i> ,2004
Ebrié Lagoon	Africa, West	1	PurN	Ecoutin <i>et al.</i> ,2005
Tortuguero Estuary	Costa Rica	1	GilN, BaiL	Nordlie & Kelso, 1975

curve is important. In Guanabara Bay, the curve stabilized after 18 months of sampling. The longest studies lasted three years (Chaves and Corrêa, 1998; Basilio *et al.*, 2008) and the great majority for approximately one year.

Guanabara Bay is notable for its considerable richness of elasmobranch species compared to other tropical estuaries. This is probably explained by the influence from an annual low-intensity upwelling system in the adjacent coastal ocean. The cold SACW (South Atlantic Central Water) water mass upwells and the northeast winds cause this water mass to drift southwestward, reaching the bay, where the effect on the temperature and nutrients leads to enrichment of the marine biota (e.g. Valentin, 2001). Knowledge of the seasonal and spatial differences in environmental parameters is essential to understand the distribution of organisms in estuaries and forcing factors such as currents and precipitation are drivers of such dynamics (Elliot *et al.*, 2007). The SACW intrudes on the continental shelf in southeastern Brazil mainly between September and April, but the prevailing winds causing upwelling of this water mass are more frequent and stronger between late spring and summer (November to March). Arriving at Guanabara Bay, this oceanic water advances over the bottom, lowering the temperature and improving the water quality (Valentin, 1994). In parallel, during the rainy season (December to April) the monthly mean rainfall exceeds 100 mm, increasing the runoff from the bay's

4000 km<sup>2</sup> drainage basin. Salinities decrease and temperatures increase, with consequent thermohaline stratification of the water column (Silva-Junior *et al.*, 2016). Such seasonality helps to explain why the number of elasmobranch species caught increased from November to April. Notably, *Z. brevirostris* mostly occurred during the SACW influence.

Spatially, all the species except *G. altavela* occurred in the lower estuary, where 83% of individuals were caught. The richness at this point is explained by the more favorable environmental conditions, influenced by the ocean waters (Valentin *et al.*, 1999; Ribeiro and Kjerfve, 2002), and the higher diversity and abundance of the benthic fauna, including molluscs, crustaceans, polychaetes and small fish (Santi and Tavares, 2009; Neves *et al.*, 2013), which forms the base of the diet of demersal rays (Viana and Vianna, 2014; Viana *et al.*, 2017). *Zapteryx brevirostris* was caught only at the bay mouth, probably because of its preference for the marine habitat (Abilhoa *et al.*, 2007; Froese and Pauly, 2018). However, Wosnick and Freire (2013) showed that this species can be euryhaline, although they reached no conclusion as to its tolerance to low salinity, and few studies have reported this ray in estuarine environments.

*Gymnura altavela* was frequent and abundant in Guanabara Bay and its population was present mainly in the inner areas, which are more sheltered, with less calm

waters, although influenced by several rivers with a high degree of pollution (Silva-Junior et al., 2016). Gonçalves-Silva and Vianna (2018) have shown that this ray probably uses this estuary as nursery ground, mainly because of the large number of young individuals found. Guisande et al. (2013) reported that temperature and bathymetry were the variables with a higher contribution to the variance observed for species richness of batoids and sharks, while the negative effect of anthropogenic factors were also important. Such natural and anthropic influences are important in Guanabara bay.

In summary, elasmobranch species richness and abundance were high in Guanabara Bay, mainly from November to April and at the estuary mouth, influenced by water circulation and rainfall. Environmental degradation in Guanabara bay must be quickly reversed, given the importance of this estuary to endangered species of elasmobranchs.

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