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DO CARMO, WANEISSA P.D.; BORNATOWSKI, HUGO; OLIVEIRA, ELTON C.;  
FÁVARO, LUÍS L.

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## Diet of the chola guitarfish, *Rhinobatos percellens* (Rhinobatidae), in the Paranaguá Estuarine complex

WANESSA P.D. DO CARMO<sup>1</sup>, HUGO BORNATOWSKI<sup>2</sup>,  
ELTON C. OLIVEIRA<sup>1,3</sup> and LUÍS L. FÁVARO<sup>1</sup>

<sup>1</sup>Universidade Federal do Paraná, Setor de Ciências Biológicas,  
Laboratório de Reprodução e Comunidade de Peixes, Departamento de Biologia Celular,  
Centro Politécnico, s/n, Jardim das Américas, Caixa Postal 19031, 81531-990 Curitiba, PR, Brasil

<sup>2</sup>Instituto de Pesca, Divisão de Pesca Marítima, Avenida Bartolomeu de Gusmão, 192,  
Ponta da Praia, 11030-906 Santos, SP, Brasil

<sup>3</sup>Universidade Tecnológica Federal do Paraná, Campus Dois Vizinhos,  
Estrada para Boa Esperança, Km 04, 85660-000 Dois Vizinhos, PR, Brasil

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### ABSTRACT

The chola guitarfish, *Rhinobatos percellens*, is one of the most-captured batoids on the Brazilian coast, and an important predator of benthic community. Stomachs from *R. percellens* were sampled in the Paranaguá estuarine complex (March/2006 to March/2007 and October/2008 to September/2009). The stomachs obtained were used for describing the diet of *R. percellens*, and verifying if there are seasonal and ontogenetic differences in their feeding into the estuarine area. The general analysis showed a specialized diet with a predominance of three species in food contents: *Leptochela serratorbita*, Caridea remains and *Ogyrides alphaerostris*. Ontogenetic and seasonal analysis did not reveal significant differences in the food consumption. These data reveal that *R. percellens* is a specialist predator of *L. serratorbita*, and this food component is consumed by all size classes.

**Key words:** Elasmobranchii, Rhinobatidae, diet, specialist, Caridea, estuary.

### INTRODUCTION

Predators and mesopredators can have a fundamental influence on the structure and function of marine communities. Elasmobranchs can affect populations of invertebrates and fishes, representing an important role in the transfer of energy among marine trophic levels (Heithaus et al. 2008, Ferretti et al. 2010). The study of the diet and feeding habits of fishes, through the analysis of stomach contents, has been standard practice for understanding basic biological aspects and the establishment of trophic

relationships in an ecosystem (Gerking 1994, Braga et al. 2012, Bornatowski et al. 2014a).

Batoid fish regularly occupy intermediate trophic levels (trophic level <4.0) in their communities (e.g. Navia et al. 2007, Vaudo and Heithaus 2011, Bornatowski et al. 2014b). Therefore, batoids can be considered mesopredators that provide an important link between top predators and lower trophic levels in the marine ecosystem, and play an important role in marine ecosystem dynamics (Vaudo and Heithaus 2011).

*Rhinobatos percellens* (Walbaum, 1792) is a benthic species that can reach up to 100 cm total

Correspondence to: Wanessa P.D. do Carmo  
E-mail: [wancarmo@yahoo.com.br](mailto:wancarmo@yahoo.com.br)

length. It is found in coastal waters up to 110 meters deep (McEachran and Carvalho 2002), from the Gulf of Mexico to northern Argentina (Figueiredo 1977). It is one of the most-captured rays on the Brazilian coast (Chaves and Robert 2003, Costa and Chaves 2006, Bornatowski et al. 2009), and an important component of the coastal benthic ecosystem and fisheries around the world, as others rhinobatids (Blanco-Parra et al. 2012). Studies show that the species has been overexploited by fisheries, particularly on the Brazilian continental shelf (De-Franco et al. 2010). Meneses et al. (2005) report that *R. percellens* has no commercial value in some states of northeastern Brazil, while Costa and Chaves (2006) and Bornatowski et al. (2010) report commercialization in the south. Based on preliminary investigations in the Paranaguá Estuarine Complex (a subtropical estuary in southern Brazil), *R. percellens* of several size classes can be found throughout the year (E.C. Oliveira and L.F. Fávaro, unpublished data).

There are few studies about feeding ecology of *R. percellens*, and all of them have been carried out on the continental shelf (e.g. Shibuya et al. 2005, Bornatowski et al. 2010, Grijalba-Bendeck et al. 2012). The lack of studies of *R. percellens* in estuaries and the lack of data about feeding biology of elasmobranchs in these environments in Brazil are factors that triggered this work, which showed that *R. percellens* use the estuary for feeding purpose. The aims of the present study were to: (i) describe the feeding of *R. percellens* in the Paranaguá Bay; (ii) assess ontogenetic and seasonal variations in diet; and iii) verify if this specie is a generalist or a specialist feeder.

## MATERIALS AND METHODS

### STUDY AREA

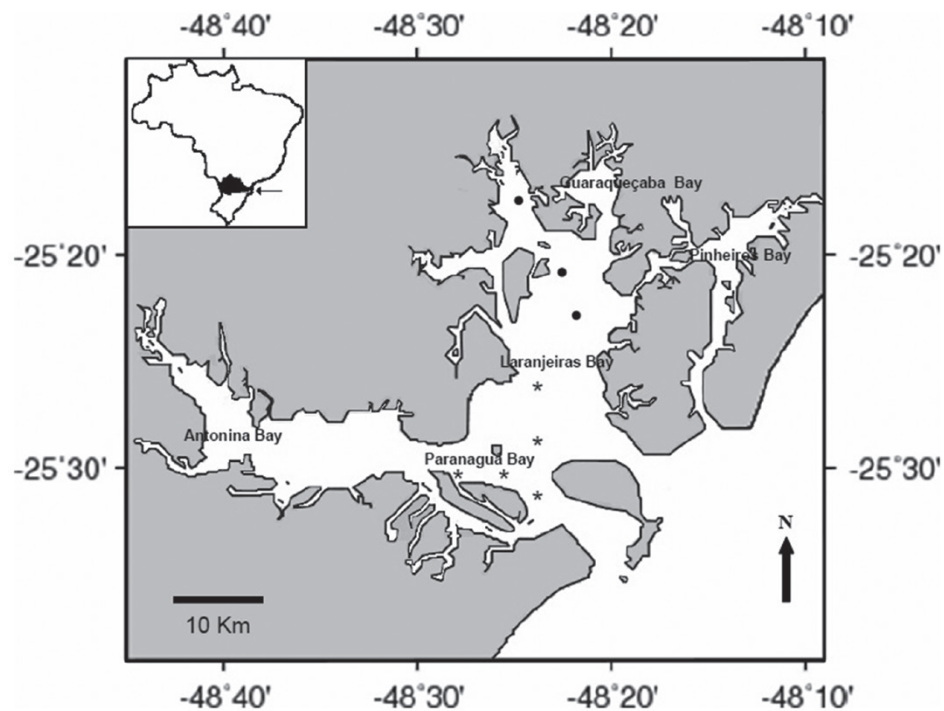
The Paranaguá Complex (between 25° 15' S – 48° 45' W and 25° 35' S – 48° 10' W), covering 551.8 km<sup>2</sup> (Noernberg et al. 2004), is the largest estuary of the Brazilian Southern coast (Bigarella et al. 1978),

formed by Guaraqueçaba, Pinheiros, Laranjeiras, Paranaguá and Antonina bays. It is connected to the ocean by narrow channels (relative to the total extent of the estuary, but sufficient to allow great influence from the adjacent ocean) (Fig. 1). The inland areas, with truly estuarine characteristics, are less than 5 m deep, while the regions with greater oceanic influence are up to 30 m deep (Iapardes 2001). The bottom sediment in the funnel zone is composed of fine sand and silt, while in the lower zone, it is composed of well-sorted fine to very fine sand (Soares et al. 1997). The tidal regime is semi-diurnal with diurnal inequalities. Mean neap and spring tidal heights are 1.3 and 1.7 m respectively at the bay mouth, and 2.0 and 2.7 m at Antonina Bay (Marone and Jamiyanaa 1997). Mean freshwater inputs of 41 m<sup>3</sup> s<sup>-1</sup> (1997 winter) and 182 m<sup>3</sup> s<sup>-1</sup> (1998 summer) were quantified for the drainage area upstream of the Paranaguá Harbour (Mantovanelli 1999).

The climate is Mesothermal Humid Subtropical (Cfa), with hot summers and a dry season that is not well defined (Maack 1981). Lana et al. (2001) reported a rainy period during the summer and a dry period during the winter. For the region, average annual precipitation is 2500 mm and average humidity is 85% (Lana et al. 2001). The salinity inside the estuary, on the sites where samples were obtained, varied from 25 to 31 (Oliveira and Fávaro 2011), and outside the estuary it was 34 (SEMA 2006).

### DATA COLLECTION

The animals were collected every month (March 2006 - March 2007 and October 2008 - September 2009) at eight different collection points distributed along the north-south axis of the Paranaguá Estuarine Complex. The specimens were caught by trawl carried out for ten minutes at each sampling site. The trawl net had the following dimensions: 8 m mouth, 7 m cod end, 1.5 cm mesh size, knot-to-knot and otter boards weighing approximately 8 kg. All procedures were carried out according to



**Figure 1** - Paranaguá Estuarine Complex. The dots (•) indicate the collection sites where specimens were not obtained and the asterisks (\*) indicate the sites where *Rhinobatos percellens* specimens were obtained.

the international practices for animal use and care under the control of an internal committee of the Universidade Federal do Paraná, Brazil.

After collecting, specimens were anesthetized with benzocaine, and had their spinal cord quickly sectioned, before being transported to the laboratory. Sex and morphometric data (total length - TL - in millimeters, total weight in grams) were determined. The digestive tract was removed, and the degree of fullness (*DF*) was determined macroscopically, according to the classes: *DF* 1 = 0 (empty stomach), *DF* 2 = 1-25%, *DF* 3 = 26-50%, *DF* 4 = 51-75%, *DF* 5 = 76-100%. Stomach contents were examined under a stereoscopic microscope. The identification of food items was done to the lowest possible taxonomic level.

On this study, the pattern used for season was: Spring, from October to December; Summer, from January to March; Autumn, from April to June and Winter from July to September.

#### DATA ANALYSIS

Frequency of occurrence (%), numerical (%) and weight (%) were obtained to compute the Index of Relative Importance (*IRI*). Frequency of occurrence (%*FO*), numerical (%*N*) and weight (%*W*), were represented by the respective formulas: %*FO* =  $(ei / E) \times 100$ , where %*FO* = percent frequency of occurrence of the sampled item, *ei* = quantity of stomachs containing item *i*, *E* = total number of stomachs containing prey, %*N* =  $(ni / N) \times 100$ , where %*N* = percent numerical frequency of the sampled item, *ni* = number of items *i* ingested, *N* = total number of ingested items, %*W* =  $(wi / W) \times 100$ , where %*W* = percent weight frequency of the sampled item, *wi* = weight of item *i*, *W* = total weight of the sampled items (Hyslop 1980, Hacunda 1981). For stomach contents analysis, only 56 stomachs (of 62) were used, and stomachs with material completely digested or an empty stomach were excluded from the analysis.

To assess the importance of various prey taxa in the *R. percellens* diets, the Index of Relative Importance (IRI, Pinkas et al. 1971, modified by Hacunda 1981) was calculated:  $IRI = (\%N + \%W) * \%FO$ . The values of IRI were standardized in percentage values according to Cortés (1997).

For the ontogenetic analysis, specimens were divided into four size classes from: 1) 15.0 to 30.9 cm, 2) 31.0 to 45.9 cm, 3) 46.0 to 60.9 cm and 4) 61.0 to 75 cm. To test ontogenetic variation in diets and seasons, a similarity matrix was calculated using Bray-Curtis similarity coefficient, produced from percentage values of the IRI of each food item. Diet similarity was analyzed with Non-Metric Multidimensional Scaling Analysis (NMDS) and analyse of similarity (ANOSIM) were used to test the null hypothesis of no differences in the diet composition between factors. Analyses of percentage of similarity (SIMPER) were used to estimate the contribution of each prey category to ontogenetic variation in diets. The analyses were performed using PRIMER v6 software (Clarke and Gorley 2006). The NMDS analysis was selected over other ordination methods because it makes fewer assumptions about the nature of the data, allows the use of any distance measure of the samples, and also seeks to preserve the distance relationships among the samples in the low-dimensional ordination space (Clarke 1993). A cluster analysis was produced using the Bray-Curtis measure to verify similarity between seasons. Seasons were defined as: Spring (October to December), Summer (January to March), Autumn (April to June), Winter (July to September).

Diet niche breadth was estimated using Levin's ( $B_i$ ):  $B_i = 1/\sum P_j^2$ , where  $P_j$  is the fraction by weight of each food in the diet ( $\sum P_j = 1$ ) (Krebs 1999). The values were standardized ( $B_A$ ) so that it ranged from 0 to 1 by using the equation  $B_A = (B_i - 1)/(N - 1)$ , where N was the number of classes (Krebs 1999). Low values indicate diets dominated by few preys items (specialist predators) while higher values indicate generalist diets.

## RESULTS

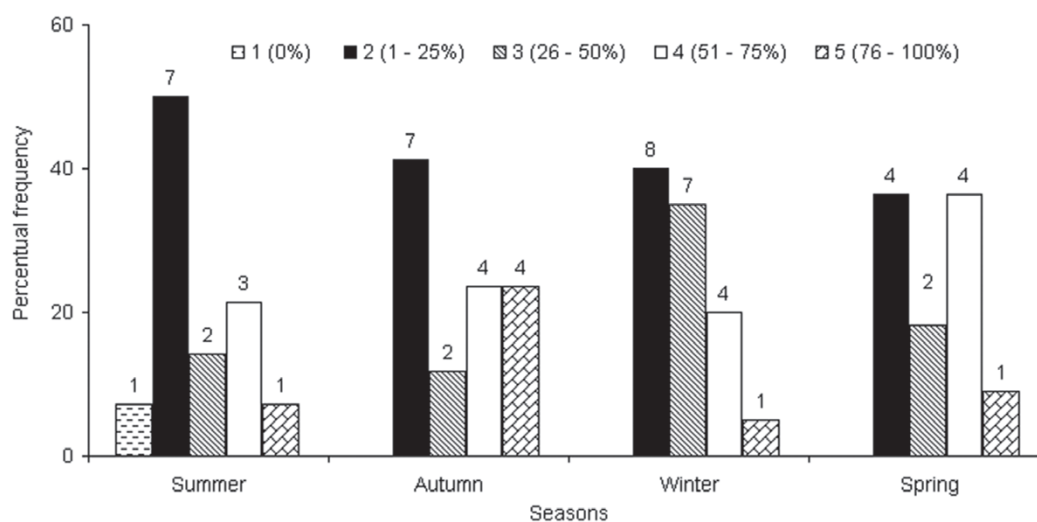
The 62 specimens of *R. percellens* caught, ranged from 17.2 cm TL to 72.6 cm TL. Regarding the degree of fullness ( $DF$ ) for the 62 specimens analyzed in the study, the results are shown in Table I. Seasonal analysis of the  $DF$  showed only empty stomach ( $DF = 1$ ) was observed during the summer. Individuals with stomach contents were found in all seasons, with different degrees of fullness. Stomachs with  $DF = 2$  (containing little food) predominated, followed by those with  $DF = 4$  (almost full) (Table I and Fig. 2).

**TABLE I**  
Degree of Fullness ( $DF$ ) for the 62 specimens analyzed in the study period.

Degree Fullness	n	%
1	1	1.6
2	26	41.9
3	13	21.0
4	15	24.2
5	7	11.3

*Rhinobatos percellens* had a specialized diet ( $B_A = 0.06$ ), and according to %IRI, the main food items were: *Leptochela serratorbita* Bate, 1888 (combclaw shrimp), *Ogyrides alphaerostris* (Kingsley, 1880) (estuarine long eye shrimp) and Caridea remains (including *L. serratorbita* and *O. alphaerostris*) (Table II). In relation to frequency of occurrence (%), Caridea remains and *L. serratorbita* were most frequently observed, respectively in that order, followed by Brachyura remains.

The size classes for the NMDS analysis ranged from: 1) 15.0 to 30.9 cm (n=16), 2) 31.0 to 45.9 cm (n=7), 3) 46.0 to 60.9 cm (n=23) and 4) 61.0 to 75.0 cm (n=16), carried out using Bray-Curtis similarity, did not result in separation of size classes (Fig. 3). Using ANOSIM, no significant difference was detected between class sizes ( $R_{\text{global}} = -0.014$ ,  $p = 0.635$ ). *Leptochela serratorbita* was the predominant food component in all size classes.



**Figure 2** - Distribution of the seasonal percentage frequency of the degree of fullness of *R. percellens*. The numbers over the bars indicate the absolute frequency.

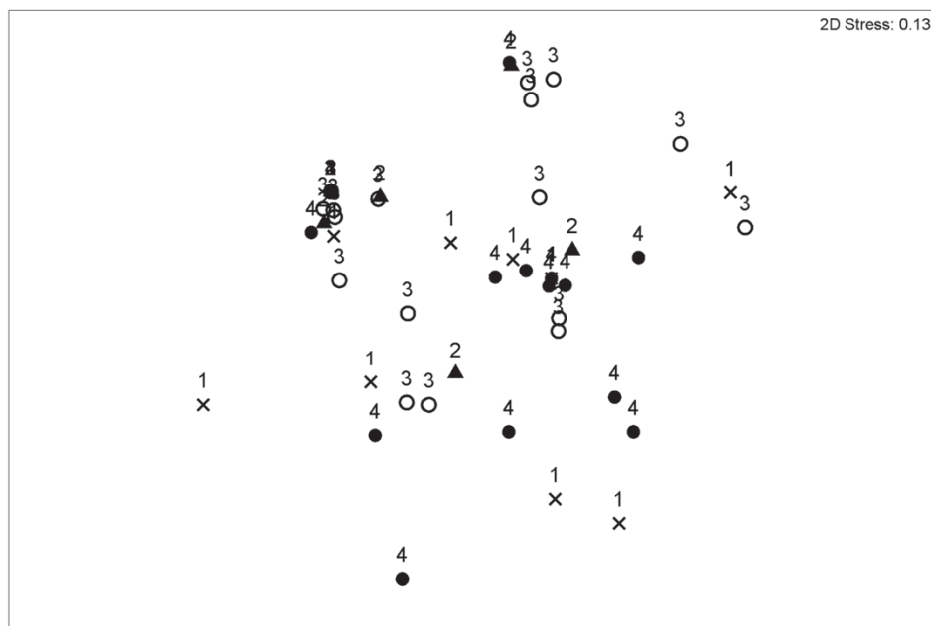
**TABLE II**  
Percentage frequency of number of specimens (%N), percentage frequency of occurrence (%FO), percentage frequency of weight (%W) and percentage of the Index of Relative Importance (%IRI) of the food items consumed by *Rhinobatos percellens* in the Paranaguá Estuarine Complex.

Taxonomic group	%N	%FO	%W	%IRI	Food items	%N	%FO	%W	%IRI
Brachyura	9	87.6	33.4	8.4	<i>Callinectes ornatus</i>	0.7	8.9	8.7	1.2
					<i>Callinectes</i> sp.	2.8	16.1	13.3	3.7
					<i>Hepatus pudibundus</i>	5.0	14.3	5.6	2.2
					Ocypodidae	0.1	1.8	0.9	<0.01
					<i>Panopeus</i> sp.	0.3	3.6	0.3	<0.01
					Brachyura's remains	<0.1	23.2	1.7	0.6
					Portunidae's remains	<0.1	17.9	2.8	0.7
					<i>Uca</i> sp.	0.1	1.8	0.1	<0.01
Dendrobranchiata	2.8	26.9	13.2	1.3	<i>Acetes</i> sp.	0.1	1.8	<0.1	<0.01
					<i>Litopenaeus schmitti</i>	0.4	3.6	9.5	0.5
					<i>Lucifer</i> sp.	0.1	1.8	<0.1	<0.01
					<i>Pleoticus muelleri</i>	1.4	12.5	2.5	0.7
					<i>Rimapenaeus constrictus</i>	0.7	5.4	1.0	0.1
					<i>Sicyonia dorsalis</i>	0.1	1.8	0.2	<0.01
Pleocyemata	75.9	185.7	36.8	86.6	<i>Alpheus heterochaelis</i>	0.1	3.6	0.5	<0.01
					<i>Exhippolysmata oplophoroides</i>	0.1	1.8	<0.1	<0.01
					<i>Leptochela serratorbita</i>	37.5	64.3	10.1	44.1
					<i>Ogyrides alphaerostris</i>	34.3	10.7	2.7	5.7
					<i>Palaemonetes</i> sp.	0.7	7.1	0.1	0.1
					Caridea's remains	3.1	96.4	23.3	36.7
					Thalassinidea	0.1	1.8	0.1	<0.01
					Unidentified	0.4	1.8	0.1	<0.01
Amphipoda	0.5	3.6	0.2	0	Gammaridae	0.1	1.8	0.1	<0.01
Mysidaceae	0.1	1.8	0.1	<0.01	Mysidaceae	0.1	1.8	0.1	<0.01
Mollusca	7.6	3.6	0.2	0.4	Bivalve	7.6	3.6	0.2	0.4
Polychaeta	<0.1	1.8	0.1	<0.01	Polychaeta	<0.1	1.8	0.1	<0.01



TABLE II (continuation)

Taxonomic group	%N	%FO	%W	%IRI	Food items	%N	%FO	%W	%IRI
Teleostei	3.7	23.2	15.6	3	<i>Pomadasys corvinaeformis</i>	0.1	1.8	9.2	0.2
					<i>Synodus foetens</i>	0.1	1.8	0.1	<0.01
					Teleostei's remains	3.5	19.6	6.3	2.8



**Figure 3** - MDS of diet of different size classes of *R. percellens*, based on Bray-Curtis similarity of transformed IRI values. Four classes range from: 1) 15 to 30 cm (n=16), 2) 31 to 45 cm (n=7), 3) 46 to 60 cm (n=23) and 4) 61 to 75 cm (n=16).

**TABLE III**  
Percentage of the seasonal IRI of the food items of *R. percellens*  
in the Paranaguá Estuarine Complex. Sum = summer, Aut =  
autumn, Win = winter and Spr = spring.

Taxonomic group	Food items	Sum	Aut	Win	Spr
Brachyura	<i>C. ornatus</i>	2.7	0.4	2.0	0.9
	<i>Callinectes</i> sp.	9.1	10.8	1.5	
	<i>H. pudibundus</i>	18.1	0.6	0.5	1.4
	Ocypodidae	1.1			
	<i>Panopeus</i> sp.				0.3
	Brachyura's remains	0.6	0.5	0.3	
	Portunidae's remains	3.5	0.3		4.5
	<i>Uca</i> sp.	0.4			
Dendrobranchiata	<i>Acetes</i> sp.			<0.01	
	<i>L. schmitti</i>		1.3	1.1	
	<i>Lucifer</i> sp.	0.3			
	<i>P. muelleri</i>	4.7	0.6	0.2	
	<i>R. constrictus</i>		0.1	0.1	0.8
	<i>S. dorsalis</i>	0.5			

TABLE III (continuation)

Taxonomic group	Food items	Sum	Aut	Win	Spr
Amphipoda	Unidentified				0.2
	Gammaridae				0.1
Mysidaceae	Mysidaceae				<0.01
Pleocyemata	<i>A. heterochaelis</i>			<0.01	0.3
	<i>E. oplophoroides</i>				0.1
	<i>L. serratorbita</i>	24.0	52.1	66.0	17.8
	<i>O. alphaerostris</i>	0.3	1.0		23.0
	<i>Palaemonetes</i> sp.	0.3	0.2	0.1	0.1
	Caridea's remains	28.4	27.0	26.0	44.3
	Thalassinidea				0.1
Mollusca	Bivalve		1.4	0.6	
Polychaeta	Polychaetas				<0.01
Teleostei	<i>P. corvinaeformis</i>		2.3		
	Teleostei's remains	6.0	1.4	1.4	6.3
	<i>S. foetens</i>			0.1	

Seasonal analysis of food item occurrence verified that the 2 main prey of *R. percellens* occurred in all seasons (Table III). Seasonal analysis of Bray-Curtis similarity, carried out on 29 food items, using the percentage of the Index of Relative Importance (%IRI), showed greater similarity between autumn and winter. Spring had the lowest similarity to the others seasons in both analyses (Fig. 4).

#### DISCUSSION

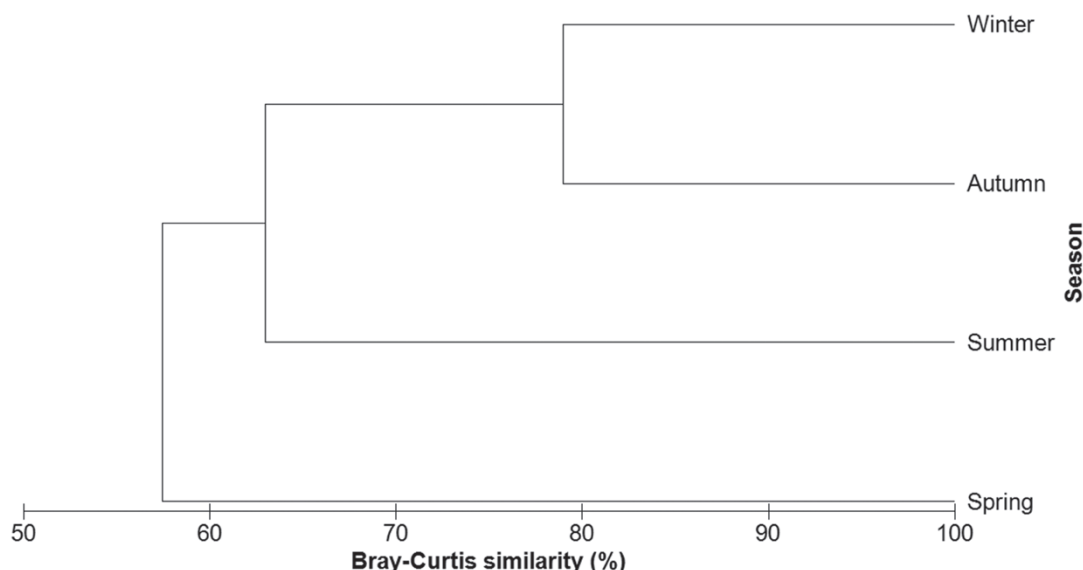
The occurrence of the species was verified through the catches in the estuarine areas of Laranjeiras and Paranaguá bays, which have a greater marine influence, according to the environmental analysis carried out by Lana et al. (2001). The sample size is low because the study occurred in an estuary, where the abundance of this species is in fact low (Fávaro and Oliveira *personal communication*), and also contrasts with the very low numbers or absence of any species of elasmobranch in estuarine studies of Lenanton (1977), Loneragan et al. (1989), Potter et al. (1983) and Potter and Hyndes (1994). According to Espinoza et al. (2011), the patterns of site fidelity and inter-annual use remain uncertain for many coastal elasmobranchs

due to the lack of quantitative behavioral data. The specimens of *R. percellens* caught in the estuary were smaller (< 73.0 cm - TL) than the specimens obtained by Bornatowski et al. (2010), who used gillnets on the continental shelf in Southern Brazil (between the coasts of Paraná and Santa Catarina). The difference in length could be related to the fishing method, associated with environment characteristics (estuary x continental shelf).

The only specimen with an empty stomach was caught during the summer and was the smallest individual in the study (172 mm TL). This total length was close to size-at-birth reported in the literature (Rocha and Gadig 2013). Therefore, the specimen with empty stomach could be explained by the fact that this individual still had a yolk reserve that could supply all necessities during the first days of life, according to the description of Brazilian Guitarfish *Rhinobatos horkelii* Müller and Henle, 1841 carried out by Rosângela Lessa (*personal communication*).

Most elasmobranchs are exclusively marine, but some species have the ability to tolerate low salinities, thus being able to live in estuaries, rivers and lakes (Wosnick and Freire 2013). *Rhinobatos*





**Figure 4** - Cluster of the seasonal analysis of the Bray-Curtis similarity carried out for the 29 food items consumed by *R. percellens* in the Paranaguá Estuarine Complex.

*percellens* is sometimes caught in estuarine regions, and probably use this area to feeding. This fact is further supported by low catch in this paper and the high predominance of stomach fullness.

The food items found in this study pertained to diets of different species of the family Rhinobatidae. In general, decapod crustaceans tended to be the most important prey of rhinobatid species (e.g. Talent 1982, Kyne and Bennett 2002, White et al. 2004, Bornatowski et al. 2010, 2014b). The importance of Polychaeta and Amphipoda was reduced in *R. percellens* feeding, which agreed with the results obtained on the continental shelf for the same species (Bornatowski et al. 2010, 2014b). Other food items, such as the squid *Lolliguncula brevis* (Blainville, 1823), was one of the food items of *R. percellens* on the continental shelf of Southern Brazil (Bornatowski et al. 2010, 2014b), but it did not appear on this study in estuarine waters.

*Rhinobatos percellens* is considered a specialist feeder; even though it makes use of 29 food items, few are predominant. The relative abundance of a particular prey item in elasmobranchs' diets may be related to its abundance in the environment,

escape probability, foraging habitats or competition between predator species (Wetherbee and Cortés 2004, Heithaus 2004). The primary items found in the diet of the *R. percellens* (i.e. *L. serratorbita* and *O. alphaerostris*) are abundant crustaceans in estuarine areas (Calil 2005). Therefore, prey abundance appears to play an important role in determining the consistency of these items in all length classes and seasons. On the other hand, *R. percellens*, has a relatively larger mouth, allowing the consumption of elusive larger prey such as blue crabs and flatfishes (Dean et al. 2007). This fact was observed in the continental shelf by Bornatowski et al. (2010, 2014b), but not in the present study. So, we hypothesized that 1) *R. percellens* feed on the most abundant preys in the environment or, 2) large individuals >75 cm - TL (see Bornatowski et al. 2010, 2014b) feed on elusive larger preys.

In conclusion, our results showed that *R. percellens* is a specialist feeder, consuming predominantly *L. serratorbita*. Probably this species feeds on the most abundant prey in the environment, since this item, together with the Caridea remains (that include *L. serratorbita*

remains) was consumed by all classes and during all seasons. A hypothesis to be tested is the occupation of *R. percellens* between smaller (<70 cm TL) and larger individuals (>70 cm) in different environments: estuarine and continental shelf waters. These results are not determinants to the knowledge on the feeding habits of *R. percellens* in this area, because the sample size was very low, but these results are important as a first sample about feeding of *R. percellens* in this estuarine area.

#### ACKNOWLEDGMENTS

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#### RESUMO

A raia viola, *Rhinobatos percellens*, é um dos batoídeos mais capturados na costa brasileira, e é um importante predador da comunidade bêntica. Estômagos de *R. percellens* foram amostrados no complexo estuarino de Paranaguá (Março/2006 a Março/2007 e Outubro/2008 a Setembro/2009). Os estômagos obtidos foram utilizados para descrever a dieta de *R. percellens*, e verificar se há diferenças sazonais e ontogenéticas em sua alimentação nesta área estuarina. A análise geral mostrou uma dieta especializada com a predominância de três espécies em seu conteúdo alimentar: *Leptochela serratorbita*, restos de Caridea e *Ogyrides alphaerostris*. Análises ontogenéticas e sazonais não revelaram diferenças significantes no consumo de alimento. Estes dados revelam que *R. percellens* é um predador especialista em *L. serratorbita*, e este alimento é consumido por todas as classes de tamanho.

**Palavras-chave:** Elasmobranchii, Rhinobatidae, dieta, especialista, Caridea, estuário.

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