

Research Article

Dietary composition and trophic level of *Hypanus marianae* (Myliobatiformes: Dasyatidae), captured off Pernambuco coast, Brazil

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ABSTRACT. *Hypanus marianae*, a stingray endemic to Brazil, inhabits coastal coral reef areas occurring in the landings of artisanal fisheries. The present study aimed to characterize the diet of this species captured off Pernambuco. We obtained 77 stomachs with contents: 52.27% female, 47.72% male. DW ranged from 15 to 37.80 cm mean of 26.53 ± 4.82 cm. Four higher taxonomic prey categories were identified: Arthropoda, Annelida, Chordata and Sipuncula. The most important item in the diet was Polychaeta (40.65% IRI), followed by *Leptochela serratorbita* (30.35% IRI), suborder Dendrobranchiata (10.18% IRI), order Isopoda (4.19% IRI), and *Meiosquilla schmitti* (3.56% IRI). *H. marianae* displays specialized predatory habit ($B_A = 0.06$) primarily based on shrimps (42.36% IRI) and polychaetes (40.65% IRI). There was no significant difference in the diet by sex or in sexual development stages. However, neonates fed on decapods crustaceans (70.68%) and polychaetes (17.09%). For immatures, decapod crustaceans (69.5%) and polychaetes (25.86%) made up most of their diet. The adults feed mainly on polychaetes (62.79%) and crustacean decapods (30.47%), with a slight increase of fish (5.46%). The trophic level (TL) estimated was based on stomach content analysis was 3.66 and estimated on the analysis of stable isotopes being 3.29, which classified the species as a mesopredator.

Keywords: *Dasyatis marianae*; elasmobranchs; endemic ray; trophic ecology; South America

INTRODUCTION

In tropical regions, the most numerous groups of Batoidea are the Myliobatiformes (Ebert & Compagno, 2007; Aschliman *et al.*, 2012). The rays of the Myliobatiformes order account for 43% of all batoids and for more than 22% of all known species of Chondrichthyes (Ebert & Compagno, 2007; Weigmann, 2016).

In Brazil, out of 26 species of the Myliobatiformes order (Rosa & Gadig, 2014), seven species of the Dasyatidae family occupy coastal and estuarine environments (Bigelow & Schroeder, 1953; McEachran & Carvalho, 2002; Santos & Carvalho, 2007). *Hypanus marianae* (Gomes, Rosa & Gadig, 2000) is endemic to coastal areas of northeastern Brazil (Bender *et al.*, 2013;

Costa *et al.*, 2017), occurring in artisanal fisheries (Yokota & Lessa, 2007) where it is locally named “big-eye-stingray” or “coã-stingray”.

Adult specimens of big-eye-stingray occupy coral reef areas, with seagrass beds as the preferred habitat of females, while immature stingrays concentrate on sandy bottoms, near the beach and on sandbanks (Gomes *et al.*, 2000; Costa *et al.*, 2015). Coastal sandbanks are likely the primary nursery areas for this species (Yokota & Lessa, 2006).

The feeding biology of elasmobranchs have been investigated in the last decade in order to describe the diet of the species (Wetherbee & Cortés, 2004; Navia *et al.*, 2007; Albo-Puigserver *et al.*, 2015; López *et al.*, 2016; Wilga *et al.*, 2016); to understand the role of given species in marine ecosystems (Wetherbee &

Cortés, 2004; Navia *et al.*, 2007; Albo-Puigserver *et al.*, 2015; Madigan *et al.*, 2015; López *et al.*, 2016); to estimate predation impacts on economically valuable or threatened species (Myers *et al.*, 2007) as well as to elucidate the biomechanic processes involved in predation strategies (Dean *et al.*, 2005; Wilga *et al.*, 2016). However, studies on the feeding biology of elasmobranchs are more frequently focused on shark species.

Quantitative studies on Dasyatidae stingrays' diet and trophic ecology being scarce (Aguiar & Valentin, 2010; Shibuya & Rosa, 2011) though, this researches may contribute to a better understanding of the mesopredators' role and can help in the development of ecosystem-based management approaches (Heithaus *et al.*, 2008; Rocchi *et al.*, 2017), as this information increases the understanding of the fishing impacts on mesopredator species (Grubbs *et al.*, 2016).

The combination of traits such as endemism, small body and feeding of invertebrates, may drive *H. marianae* to a high-risk probability of extinction (Bender *et al.*, 2013) since its preferred habitats are highly sensitive to environmental changes. In IUCN assessments, *H. marianae* is classified as DD (Data Deficient) due to the lack of information necessary to adequately assess its extinction risk (Rosa & Furtado, 2016). Thus, the objective of the current study is to analyze the diet and to verify the occurrence, or not, of sexual and ontogenetic changes in *H. marianae*, landed along the Pernambuco coast. Furthermore, the trophic level of the species will be inferred through the stomach contents and stable isotope analysis.

MATERIALS AND METHODS

Sample collection

The specimens of *Hypanus marianae* were obtained from artisanal fisheries off Pernambuco coast, Brazil (7°37'33.6"S, 34°48'52.4"W to 8°53'19.0"S, 35°08'59.8"W). The sample sites were Ponta de Pedras (PP), Itamaracá (IT), Sirinhaém (SI) and São José da Coroa Grande (SJ), where the individuals are captured as bycatch in fish-weirs, in beach seine, shrimp trawl and longline, respectively (Fig. 1).

After sampling, the specimens were sexed, photographed and weighed (in g). The disc width (DW) was measured (in cm), and the sexual development stages were determined by macroscopic analysis of the reproductive organs, according to the criteria established by ICES (2012).

The stomachs were removed and then stored in 4% formalin solution. After 48 h, the stomachs were excised, washed with tap water and the food contents were

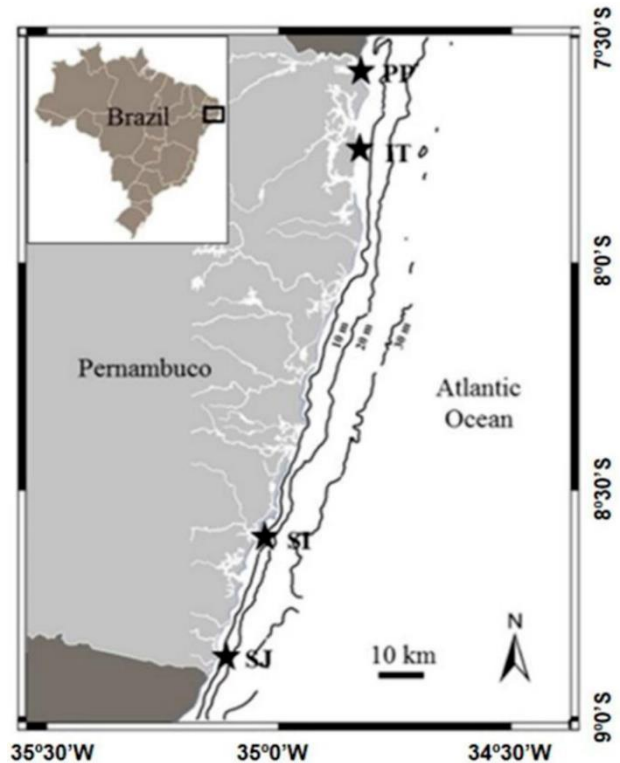


Figure 1. Locations of landings of *Hypanus marianae* in Pernambuco, Brazil, in Ponta de Pedras (PP), Itamaracá (IT), Sirinhaém (SI) and São José da Coroa Grande (SJ) from July 2013 to October 2016.

retained in a sieve mesh of 0.1 cm and preserved in 70°GL ethanol. The material was analyzed under a stereoscopic microscope, and food components were identified to the lowest possible taxonomic level. After, the items were separated, counted, and individually weighed to the nearest 0.0001 g (Cortés, 1997). The excess ethanol was removed with a paper towel (Magnusson *et al.*, 2003; Aguiar, 2010).

Algae and sediment were considered accidental ingestions and thus were excluded from the analysis (as well as all unrecognized materials). Stomachs showing only unidentifiable material were treated as empty stomachs and were excluded from the analysis.

Quantitative analysis of diet

A prey accumulation curve (Cortés, 1997) was built using EstimateS[®] version 7.5.1 to verify whether the number of analyzed stomachs was sufficient to represent the species diet, based on the reduction of standard deviations. Thus, the percentage frequency of occurrence (O%), the numerical occurrence or number percentage (N%) and weight (W%) were calculated. The index of relative importance (IRI) was determined according to Pinkas *et al.* (1971), and also calculated as

a percentage (%IRI) (Cortés, 1997): %IRI = (IRI / \sum IRI) \times 100.

The diet niche breadth was estimated using Levin's index B_i (Krebs, 1999): $B_i = 1 / \sum P_j^2$, where P_j is the fraction by IRI of each food j in the diet. The values were standardized for B_A (Krebs, 1999): $B_A = (B_i - 1) / (N - 1)$, where N is the number of classes, and B_A ranges from 0 to 1. Low values of B_A indicate diets dominated by few prey items (specialist predators), while higher values indicate generalist diets.

The trophic level (TL) of the species, based on stomach contents, was also calculated (Cortés, 1999): $TL_k = 1 + (\sum_{j=1}^n P_j \times TL_j)$, where TL_k is the trophic level of the species (k), P_j is the proportion of the prey category (j) in predator diet (k); n is the total number of prey categories, and TL_j is the trophic level of the prey group (j). We used the trophic level values of the prey categories suggested by Ebert & Bizzarro (2007) for trophic level analysis in batoids.

The weight data of the food items were grouped into five categories of prey, suggested by Ebert & Bizzarro (2007): decapods (crustacean decapods); fishes (fish teleost); amphipods (amphipods and isopods); polychaetes (polychaetes worms) and other crustaceans (crustaceans not included in the decapod order).

Based on the weight of each prey category, the data of food items were transformed in square-root, and a Bray-Curtis similarity coefficient was used to construct a similarity matrix (Jacobsen & Bennet, 2012).

The one-way similarity analysis (ANOSIM) using the similarity matrix, was conducted considering individuals as samples and sex and sexual development stages as factors. The R -value resulted by the ANOSIM analysis (the strength of the factors on the samples) and the significance level (P) was evaluated using a random permutation test with 999 permutations (Clarke & Gorley, 2006). When $R = 0$, there are no differences among the groups (sex and sexual development stages) or the samples (stomach) in each group. When $R = 1$, the samples of the groups are similar, but the groups are different. Statistical significance was set at $P < 0.05$. Diet similarity was also analyzed by non-metric multidimensional scaling analysis (nMDS).

Finally, the percentage of similarity (SIMPER) was used to estimate the contribution of each prey category to the diet of each factor (sex or sexual development stages). The analyses were performed using the software Primer v.6 (Clarke & Gorley, 2006).

Muscle tissue collection

Muscle samples of *H. marianae* were obtained from eight individuals, taken from the lower portion of the pectoral fin and stored frozen. As stipulated by Post

(2002), muscle tissue samples from an herbivorous species were obtained ($n = 2$) to be used as a baseline. The species chosen were the teleost fish *Sparisoma axillare* (Steindachner, 1878), an herbivore (Rosa & Moura, 1997) that occurs in the same coral reef environment as *H. marianae*, which reflects the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ value of the base of the food chain (Post, 2002).

Stable isotope analysis

The collected material was cut in 1 cm² cubes and placed in test tubes, following the methodology suggested by Kim & Koch (2012) for extraction of lipids from the tissue by the addition of 10 mL of petroleum ether. The test tubes were then shaken in Ultrasonic Washer (Ultracleaner 700 - Unique) for 15 min. The petroleum ether solution was discarded, and the procedure repeated. After, 10 mL of deionized water was added to extract the urea accumulated in the tissue; the test tube was stirred for 15 min, and then the deionized water solution was discarded and the process repeated twice.

After the extraction of lipids and urea, *H. marianae* muscle tissue samples were lyophilized. Finally, the material was macerated and subjected to stable isotope analysis on a mass spectrometer.

The values of δ were calculated from the reference standards of carbon (Pee Dee Belemnite) and nitrogen (atmospheric air), according to equation (Peterson & Fry, 1987):

$$\delta_{\text{sample}} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \times 1,000 \right],$$

where R represents the ratio of the heaviest to the lightest isotope ($^{15}\text{N}:^{14}\text{N}$; $^{13}\text{C}:^{12}\text{C}$).

Trophic level estimate

The trophic level of the species was calculated using the formula described by Post (2002):

$$TL_{\delta^{15}\text{N}} = TL_{\text{baseline}} + \frac{(\delta^{15}\text{N}_{\text{predator}} - \delta^{15}\text{N}_{\text{baseline}})}{1.5\text{‰}},$$

where $TL_{\delta^{15}\text{N}}$ is the trophic position of the predator, TL_{baseline} is the trophic position of the baseline ($TL_{\text{baseline}} = 2$), $\delta^{15}\text{N}_{\text{predator}}$ is the $\delta^{15}\text{N}$ value of the consumer, $\delta^{15}\text{N}_{\text{baseline}}$ is the $\delta^{15}\text{N}$ value of the baseline and 1.5‰ is the expected enrichment in $\delta^{15}\text{N}$ per trophic level.

The enrichment constant of $\delta^{15}\text{N}$ used was based on a study by Galván *et al.* (2016), which estimated the trophic discrimination factors (TDF) and turnover rates in blood and muscle of the small nose fanskate *Sympterygia bonapartii* (Müller & Henle, 1841) through controlled feeding. The results found by Galván *et al.* (2016) led to an estimated TDF value for *S. bonapartii* of 1.5‰. Since the enrichment constant

may vary according to the species studied (Post, 2002; Hussey *et al.*, 2014), the estimated value for *S. bonapartii* was used because it lives in a benthic habitat similar that of *H. marianae*.

RESULTS

Out of 89 of *Hypanus marianae* specimens, 77 had a disc width (DW) of 15 to 37.80 cm (mean 26.53 ± 4.82 cm) and had identifiable stomach contents. Females represented 52.27% (15 to 37.80 cm DW) and males 47.72% (15.10 to 30.7 cm DW) of the total sample. The prey accumulation curve tends to an asymptote in the last five points of the curve (Fig. 2), meaning that the sample size was sufficient to represent the diet of the species.

The four most frequent prey categories identified in this study were Arthropoda (Amphipoda, Decapoda, Isopoda and Stomatopoda); Annelida (Polychaeta); Chordata (Teleostei) and Sipuncula (Table 1).

The most important item in *H. marianae* diet was the class Polychaeta (40.65% IRI), followed by *Leptochela serratorbita* (Spence Bate, 1888) (30.35% IRI), suborder Dendrobranchiata (10.18% IRI), order Isopoda (4.19% IRI), and *Meiosquilla schmitti* (Schmitt, 1940) (3.56% IRI) (Table 1).

The similarity analysis (ANOSIM) indicated no significant differences in feeding between the sexes (Global R = 0.04; $P = 0.09$) or in relation to sexual development stages (Global R = 0.02; $P = 0.23$). Results were also corroborated using non-metric multidimensional scaling analysis (nMDS), which revealed overlap in diet between the sexes and among the sexual development stages (Fig. 3).

H. marianae showed low value in diet breadth based on Levin's index ($B_A = 0.06$). Data showed a specialist feeding strategy, with a diet primarily based on shrimps (suborder Dendrobranchiata, family Sergestoidea, *Rimapenaeus constrictus* (Stimpson, 1871), *Leptochela serratorbita* (Spence Bate, 1888), *Sicyonia parri* (Burkenroad, 1934) and *Pleoticus muelleri* (Spence Bate, 1888)) with 42.36% of IRI and polychaetes (40.65% IRI) (Table 1). Results corroborate with the similarity percentage analysis (SIMPER), which showed prey categories that most contributed to the diet of all groups (Table 2).

Neonates (young of the year) of *H. marianae* displayed a diet based on decapod crustaceans, polychaetes, and amphipods, whereas immature individuals displayed a diet mostly of decapod crustaceans and polychaetes. Similarly, the adults fed mainly on polychaetes, decapod crustaceans and fish (Table 2). Teleostei fish were found in seven examined stomachs,

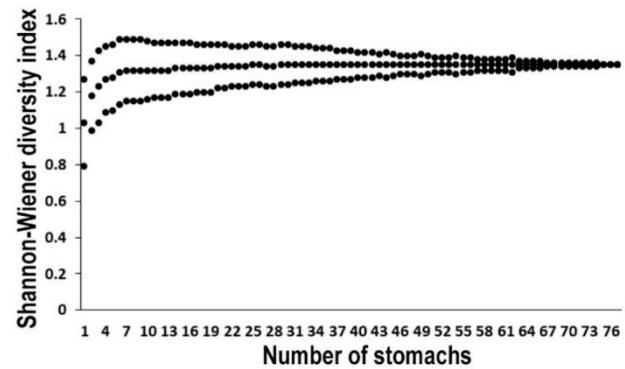


Figure 2. Cumulative average and standard deviation of the Shannon-Wiener diversity index of food items in the stomachs of *Hypanus marianae* off Pernambuco coast, Brazil from July 2013 to October 2016.

two adult males and five adult females; one was pregnant.

The trophic level (TL) estimated for *H. marianae* based on stomach content analysis was 3.66. The mean $\delta^{15}\text{N}$ value was 11.68 ± 0.92 , and the estimated TL was 3.29 ± 0.61 (Table 3).

DISCUSSION

The cumulative prey curve for *Hypanus marianae* tended to an asymptote in 72 stomachs, more than twice the value found by Costa *et al.* (2015), in samples from the state of Rio Grande do Norte, whose asymptote was reached at 31 stomachs, probably because in this work almost twice as many food items were identified, compared to Costa *et al.* (2015). The early stabilization of the curve is justified by the small niche breadth found for *H. marianae* ($B_A = 0.06$), indicating a reduced diversity of food items in the diet, which is typical of specialized diets, as is the case for *Rhinobatos percellens* from southern Brazil (Levin's index $B_A = 0.06$) (Carmo *et al.*, 2015).

H. marianae is associated with coral reefs (Gomes *et al.*, 2000; Costa *et al.*, 2015, 2017), reflecting a feeding strategy based on the predation of benthic reef organisms, such as small crustaceans and polychaetes. Similarly, in Australia and the Caribbean, the diets of *Pastinachus ater*, *Himantura uarnak*, *Neotrygon kuhlii*, *Taeniura lymma*, *Urogymnus asperrimus* (O'Shea *et al.*, 2013) and *Hypanus americanus* are predominantly made up of polychaetes and shrimp (Tilley *et al.*, 2013).

Although polychaetes have been a significant item in the diet of *H. marianae*, accounting for 40.65% of the IRI, shrimp (suborder Dendrobranchiata, family Sergestoidea, *Rimapenaeus constrictus*, *Leptochela serratorbita*, *Sicyonia parri* and *Pleoticus muelleri*) corresponded to 42.36% of IRI. Some other batoid from

Table 1. Occurrence (O), frequency of occurrence (FO%), number (n), numerical frequency (N%), weight in grams (W), weight frequency (W%) relative importance index (IRI), relative importance index in percentage (IRI%) and order of importance (OI-IRI) of the food items in diet of *Hypanus marianae* off Pernambuco coast, from July 2013 to October 2016.

Prey's item	O	%FO	n	%N	W	%W	IRI	%IRI	OI-IRI
Phylum Sipuncula	8	10.39	13	1.41	3.79	3.32	49.19	0.87	11
Phylum Annelida									
Class Polychaeta	49	63.64	85	9.25	30.69	26.88	2299.30	40.65	1
Phylum Arthropoda									
Class Crustacea									
Order Decapoda									
Suborder Pleocyemata									
Family Palinuridae	2	2.60	2	0.22	0.06	0.05	0.69	0.01	19
Family Scillaridae									
<i>Scyllarides</i> sp.	1	1.30	3	0.33	0.27	0.24	0.73	0.01	19
Suborder Dendrobranchiata	29	37.66	113	12.30	3.43	3.00	576.09	10.18	3
Family Penaeidae									
<i>Rimapenaeus constrictus</i>	7	9.09	23	2.50	3.73	3.27	52.48	0.93	10
<i>Sicyonia parri</i>	2	2.60	4	0.44	0.12	0.11	1.41	0.02	18
<i>Pleoticus muelleri</i>	3	3.90	4	0.44	1.78	1.56	7.75	0.14	15
Family Caridae									
<i>Leptocheila serratorbita</i>	30	38.96	340	37.00	8.08	7.07	1717.00	30.35	2
Family Sergestoidae	3	3.90	95	10.34	0.54	0.47	42.12	0.74	13
<i>Acetes americanus</i>	1	1.30	1	0.11	0.01	0.01	0.16	<0.01	20
Suborder Brachyura	8	10.39	10	1.09	5.79	5.07	63.97	1.13	9
Family Portunidae									
<i>Callinectes</i> sp.	14	18.18	20	2.18	9.45	8.27	190.01	3.36	6
<i>Callinectes exasperatus</i>	2	2.60	5	0.54	2.96	2.60	8.16	0.14	15
<i>Microphrys bicornutus</i>	1	1.30	1	0.11	1.47	1.29	1.82	0.03	17
Family Upogebiidae									
<i>Upogebia</i> sp.	6	7.79	7	0.76	0.98	0.86	12.63	0.22	14
Order Amphipoda	3	3.90	10	1.09	0.10	0.09	4.58	0.08	16
Order Isopoda	16	20.78	96	10.45	1.09	0.95	236.82	4.19	4
Class Cephalocarida	1	1.30	1	0.11	0.01	0.01	0.15	<0.01	20
Order Stomatopoda	11	14.29	24	2.61	2.45	2.15	67.98	1.20	8
Family Squillidae									
<i>Meiosquilla schmitti</i>	12	15.58	50	5.44	8.53	7.47	201.19	3.56	5
Phylum Chordata									
Class Actinopterygii									
Teleost	7	9.09	7	0.76	8.48	7.42	74.40	1.32	7
Family Holocentridae									
<i>Holocentrus adscensionis</i>	2	2.60	5	0.54	20.38	17.85	47.77	0.84	12
Total	77	100	919	100	114.18	100	5656.40	100	

the Brazilian coast inhabiting the marine substrate also feeds mainly on polychaetes and crustaceans. The species *Hypanus americanus* (Aguilar, 2010), *Hypanus guttatus* (Silva *et al.*, 2001; Carvalho-Neta & de Almeida, 2002; Gianeti, 2011), *Urotrygon microphthalmum* (Santander-Neto, 2015), *Rioraja agassizii*, *Zapteryx brevirostris* and *Rhinoptera bonasus* (Bornatowski *et al.*, 2014) have shrimp or polychaetes as main groups of prey of the diet, similar to *H. marianae*.

According to Costa *et al.* (2015), shrimp of the suborder Dendrobranchiata (29.0% of IRI) and the class of Polychaeta (12.2% of IRI) were the second and the fourth most important items in the diet of *H. marianae*. However, Shibuya & Rosa (2011) indicated shrimp as the main item, with 70.57% of IRI, whereas polychaetes were only 0.76% of IRI. Overall, the decapod crustaceans tended to be one of the most important prey items for the stingrays (Bornatowski *et al.*, 2010, 2014; Pardo *et al.*, 2015) because shrimp and

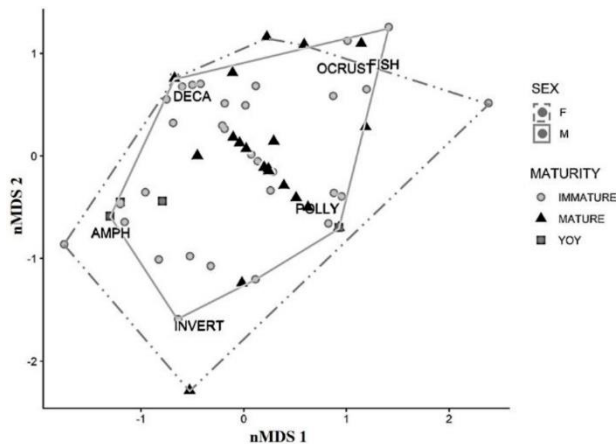


Figure 3. Non-metric multidimensional scaling analysis (nMDS) of *Hypanus marianae* based on the weight of the diet items: females distribution polygon (dashed line), males distribution polygon (solid line), young of the year (square), immature (circle) mature (triangle). AMPH: Amphipoda; DECA: Decapoda; OCRUS: Other Crustacea; POLLY: Polychaete; INVERT: Invertebrates; FISH: fishes; YOY: young of the year.

polychaetes are abundant prey for coralline stingrays (O'Shea *et al.*, 2013).

The specialized diet of *H. marianae* based on shrimp and polychaetes can be due to the small mouth aperture, which limits prey size (Tilley *et al.*, 2013). This consideration also fits for *Zapteryx brevirostris*, which feed on the same prey (Bornatowski *et al.*, 2014).

It is known that some batoids capture and process their prey through movements of the oral cavity, allowing strict internal orientation of the water flow (Dean *et al.*, 2005). As observed by Shibuya & Rosa (2011), stomach contents of *H. marianae* suggest similar foraging behavior due to the presence of many preys from the same group, presenting a similar degree of digestion as well as the occurrence of sediments.

Furthermore, ANOSIM revealed no significant differences in diet between the sexes (Global R = 0.04; $P = 0.09$), similar to *Hypanus longus* (López-García *et al.*, 2012); *Urotrygon rogersi* (Navia *et al.*, 2011); *Dasyatis chrysonota* (Ebert & Cowley, 2003); *Bathyraja brachyrops* (Belleggia *et al.*, 2008) and *Bathyraja magellanica* (Barbini *et al.*, 2010). Nonetheless, nMDS showed an overlap of prey categories used by males and females (Fig. 3). The different contributions of the same food category for male and female diets (Polychaeta and Decapod Crustacea) (Table 2) can reflect sexual segregation for this species (Yokota & Lessa, 2006). Thus, teleostean fish in the adult female diet of *H. marianae* may be related to the use of different habitats. Thus, corroborating the observation by Yokota & Lessa

(2007) and Costa *et al.* (2015) about ontogenetic and sexual segregation for *H. marianae*, which is also reinforced by Motta *et al.* (2009), who reported fishes from Scaridae family in the contents of a pregnant female captured in northern Brazil (Maranhão State).

Teleost fish are bigger and faster than shrimp, which explains the presence of this item almost exclusively in the adult diet - something that is related to their higher predation capacity (Sisneros & Tricas, 2002a) and their larger mouth width enabling the catch of larger prey. Size-related shifts in the diet for *N. kuhlii* (Pardo *et al.*, 2015) were reported with decreasing importance of polychaetes and the increased importance of carid shrimp and teleost fishes. Although there are no significant differences in the diet among the sexual development stages for *H. marianae* (Global R = 0.02; $P = 0.23$; Fig. 3), the SIMPER showed an ontogenetic change in the contribution of each item in the diet of the stingray (Table 2). Also, a diet shift in batoids during ontogeny is widely known (Sisneros & Tricas, 2002b; Kyne *et al.*, 2008; Jacobsen & Bennett, 2011; Vaudo & Heithaus, 2011; Yick *et al.*, 2011; López-García *et al.*, 2012; Ruocco & Lucifora, 2016).

Some factors related to diet shift are habitat change, an increase in the size of prey relative to an increase in the size of the predator, differences in movement patterns and an improvement in the ability to capture prey (Wetherbee & Cortés, 2004; Aguiar & Valentim, 2010; Grubbs, 2010).

Costa *et al.* (2015) suggest that the use of diverse habitats may result in different prey availability in each area, and this may explain the SIMPER results. In this study, neonates are found on the beaches, whereas larger individuals are found at greater depths, similar to reports on the species in other areas (Yokota & Lessa, 2007).

The trophic level estimated for *H. marianae* based on stomach content analysis (3.66) and $\delta^{15}\text{N}$ value (3.29) places the species at an intermediate level of the food chain, as a mesopredator (Cortés, 1997; Ebert & Bizarro, 2007). Both the trophic levels (obtained through SCA and SIA) presented values expected for the Dasyatidae family, ranging between 3.16 and 4.08 (Jacobsen & Bennett, 2013).

Hypanus marianae in Brazil occupies a trophic level (TL = 3.6) similar to *H. americanus* (TL = 3.52) in the Caribbean (Tilley *et al.*, 2013), *Urotrygon aspidura* (TL = 3.7) and *U. rogersi* (TL = 3.5) in Colombia (Navia *et al.*, 2016), *N. kuhlii* (TL = 3.58), *Neotrygon annotata* (TL = 3.57), and *Neotrygon picta* (TL = 3.55) in Australia (Jacobsen & Bennett, 2012), *Rhinoptera bonasus* (TL = 3.4) in Brazil (Bornatowski *et al.*, 2014) and *Myliobatis goodei* (TL = 3.2) in Patagonia (Molina & Cazorla, 2015), because their diet is based mainly on invertebrates.

Table 2. Items contributing to the diet differentiation between sexes and among the sexual development stages of *Hypanus marianae* according to Similarity Percentage analysis (SIMPER). YOY: young of the year.

Sample number	Sex		Sexual development stages		
	Female (%)	Male (%)	YOY (%)	Immature (%)	Mature (%)
	39	38	7	42	28
Decapods	73.01	37.98	70.68	69.5	30.47
Polychaetes	21.36	57.01	17.09	25.86	62.79
Fishes	3.93	0.02	-	0.01	5.46
Amphipods	0.62	0.83	12.23	0.95	-
Other crustaceans	0.59	3.53	-	2.66	0.99
Sipuncula	0.49	0.62	-	1.02	0.29

Table 3. Mean values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N} \pm \text{SD}$ (standard deviation) and estimated trophic level through stomach content analysis (TL SCA) and stable isotope analysis (TL $\delta^{15}\text{N}$) for *Hypanus marianae*. *Rosa & Moura (1997).

	Sample number	Mean $\delta^{13}\text{C}$	SD	Mean $\delta^{15}\text{N}$	SD	TL SCA	TL $\delta^{15}\text{N}$
<i>Sparisoma axillari</i>	2	-17.11	1.80	9.75	0.16	2.0*	-
<i>Hypanus marianae</i>	8	-14.06	0.87	11.68	0.92	3.66	3.29

Similarly, to other Myliobatiformes mesopredators (O'Gorman & Emmerson, 2009), *H. marianae* can play an important ecological role in the environment, controlling the populations of reef invertebrates used as prey, and being food source of other species (Vaudo & Heithaus, 2011) such as reef sharks (Costa *et al.*, 2015) and other stingrays such as *H. americanus* (Branco-Nunes *et al.*, 2016). Linking top predators to lower trophic levels, this species can promote the energy flow of the ecosystems it inhabits.

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