

Diet of the freshwater stingray *Potamotrygon motoro* (Chondrichthyes: Potamotrygonidae) on Marajó Island (Pará, Brazil)

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Received July 25, 2008 – Accepted February 10, 2009 – Distributed February 28, 2010

(With 2 figures)

Abstract

The stomach contents of 137 examples of *Potamotrygon motoro* caught in 3 locations (Muaná, Afuá and Lake Arari) on Marajó Island were analysed. The values of the Index of Relative Importance (IRI) and its respective percentage (%IRI) were calculated. The level of repletion 1 (¼ full) was the most representative for both sexes, as well as for immature and mature specimens. Most of the food items found were well-digested. The food items identification indicated the presence of 15 orders, including insects, mollusks, crustaceans, annelids and fish. Differences in diet were observed among the locations studied when comparing %IRI, crustaceans being the most preferred in Afuá, fish in Lake Arari and mollusks in Muaná.

Keywords: fish feeding, Estuarine habitats, Chondrichthyes.

Dieta da raia de água doce *Potamotrygon motoro* (Chondrichthyes: Potamotrygonidae) na Ilha de Marajó (Pará, Brasil)

Resumo

O conteúdo estomacal de 137 exemplares de *Potamotrygon motoro* provenientes de 3 localidades (Muaná, Afuá e Lago Arari) na ilha de Marajó foi analisado. Os valores do Índice Relativo de Importância (IRI) e respectiva porcentagem (%IRI) foram calculados. O nível de repleção 1 (¼ cheio) foi o mais representativo para ambos os sexos, assim como para exemplares imaturos e maduros. A maioria dos itens alimentares analisados encontrava-se bastante digerido. A identificação dos itens alimentares indicou a presença de 15 ordens, incluindo insetos, moluscos, crustáceos, anelídeos e peixes. Diferenças na dieta entre os locais amostrados foram observadas ao se comparar as %IRI, sendo crustáceos o item preferencial em Afuá, peixes no Lago Arari e moluscos em Muaná.

Palavras-chave: alimentação de peixes, ambientes estuarinos, Chondrichthyes.

1. Introduction

The Amazon Estuary involves the Amazon and Tocantins Rivers discharge area, as well as an archipelago of river-ocean islands where Marajó Island is located. Marajó Island occupies an area of approximately 50 thousand km² and is the largest river-ocean island in the world (Cruz, 1987). This region shelters both marine and freshwater ichthyofauna, including freshwater stingray species (Barthem, 1985; Sanyo Techno Marine, 1998). It presents heterogeneous environments, forming a mosaic of differentiated habitats among its vegetation types. These habitats, such as lakes, lagoons, beaches,

streams and mangroves, are important for many fish species including elasmobranchs, since they have differentiated patterns of habitat use and preferences (Carrier et al., 2004).

The Potamotrygonidae family is considered unique among elasmobranchs since its members are completely adapted and restricted to freshwater environments (Thorson et al., 1983; Rosa, 1985). The feeding studies in continental stingrays have shown variations in composition and frequency of certain food items, as well as intra and inter-specific diet differences according to

habitat (Charvet-Almeida, 2001; 2006; Rincon, 2006). These studies indicate that potamotrygonids could be generally classified as carnivorous, preying on fish, crabs, shrimps, insects and mollusks.

Potamotrygon motoro (Natterer in Müller and Henle, 1841) (Ocellate River Stingray) is a widely distributed species, present from the Orinoco River (Venezuela) down to La Plata River, but is absent from the Atrato-Magdalena (Maracaibo) basin and Paranaíba River (Rosa, 1985). This species is popular in the ornamental fish trade (Araújo, 1998), is polychromatic (Rosa, 1985), is highly fecund in relation to other potamotrygonids (Charvet-Almeida et al., 2005) and poorly known, with sparse life history and population data available at the present moment (Drioli and Chiaramonte, 2005).

Research on fish feeding habits (diet) and feeding strategies has grown in importance in ichthyologic studies. Such studies provide information for better understanding of how ecosystems function, and consequently on how to manage them correctly (Zavala-Camin, 1996).

Elasmobranchs perform an important role in energy transfer among the higher trophic levels (Wetherbee and Cortés, 2004). Precise descriptions of diets in this group are hampered by the plasticity of their feeding habits that often result in ontogenetic and spatiotemporal shifts (Achenbach and Achenbach, 1976; Wetherbee and Cortés, 2004).

Some aspects of the feeding habits and diet of *Potamotrygon motoro* were previously studied by other authors using varying methodologies (Pântano-Neto and Souza, 2002; Lonardoni et al., 2006; Melo et al., 2007; Shibuya et al., 2007; Silva and Uieda, 2007). Results from these researchers demonstrated that this species has feeding plasticity and diet variability. Nevertheless, few of them were carried out in the Amazon region and none in the Amazon River mouth region. Taking into account that *P. motoro* is caught as an ornamental and food resource in this region, the understanding of its biology is essential for adequate management and conservation purposes.

The main objective of this study was to characterise the diet of *Potamotrygon motoro* taken from three sampling points on Marajó Island, evaluating possible differences between localities, sexes and sexual maturity stages (immature or juvenile and mature or adult).

2. Material and Methods

Seventeen sampling periods of approximately seven days each were carried out and distributed over the dry, rainy and transitional seasons, between 2005 and 2007. The specimens were caught with bottom longlines and other fishing gears (seine nets, gillnets, spears, and handlines) mentioned in the literature as being efficient methods for catching freshwater stingrays (Barthem,

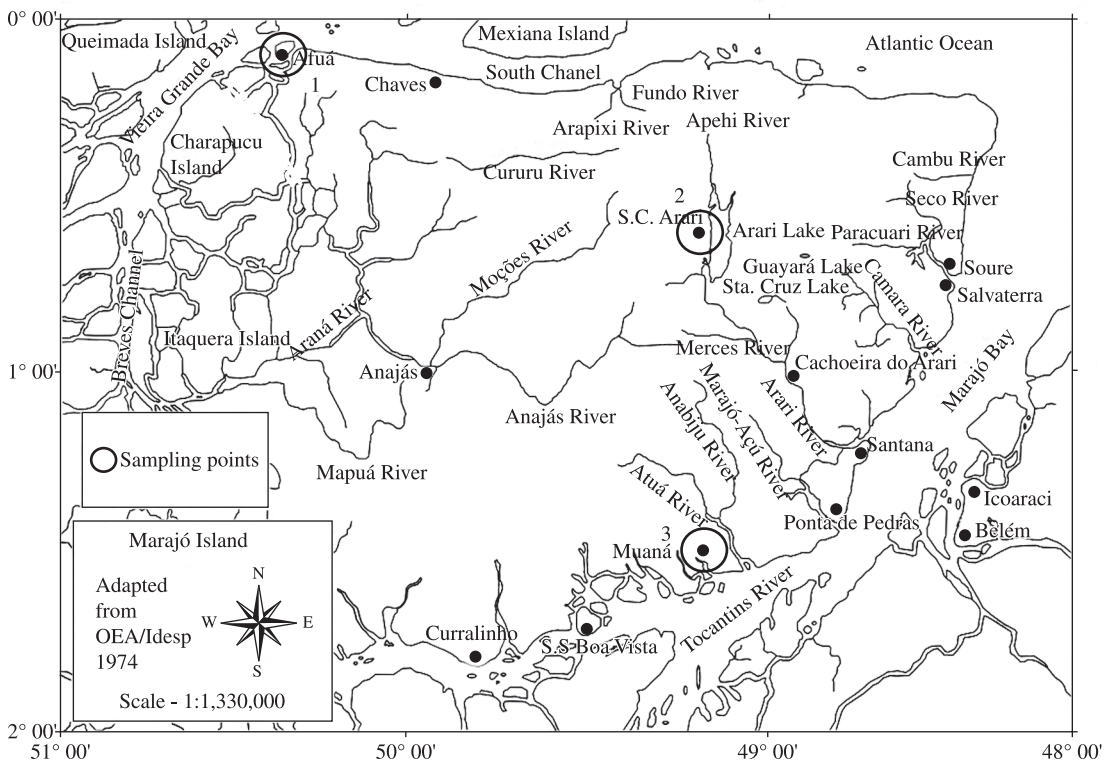


Figure 1. Marajó Island map depicting the sampling points: (1) Afuá, (2) Arari Lake and (3) Muaná.

1985; Lasso et al., 1996; Charvet-Almeida, 2001; 2006; Rincon, 2006). Longlines were the main fishery gear used. They were usually set at dusk and recovered at dawn, remaining in the water for a period varying from two to twelve hours. Fishing during daytime was impossible due to the presence of bait competitors (Characidae, mainly *Serrasalmus* spp.). Nets were used only as complementary gears and in a non-standardised way.

The potamotrygonids were caught off three sampling points on Marajó Island, namely: Afuá (0° 09' S and 50° 23' W), Muaná (01° 31' S and 49° 13' W) and Lake Arari (0° 39' S and 49° 10' W) (Figure 1).

Afuá is located on the northwestern portion of Marajó Island. There are many river channels (depths ranging mostly from 0.5 to 6 m) and islets in this area, which is highly influenced by the Amazon River discharge and by tidal variations. Muaná is on the southeastern part of the island, north of the Marajó Bay. There are several river channels (similar depth ranges as in Afuá) and flooded areas, influenced mainly by the Tocantins River discharge. Tidal change variations are intense. Arari is the largest lake on Marajó Island, located on the central eastern part of the island. Around this lake there are flooded grass fields and many streams that drain towards it. Water level in this environment seasonally varies with rainfall but limited tidal influence was noted in this area. Muddy substrates were predominant in all sampling points.

The freshwater stingrays were anaesthetised, killed and eviscerated through a ventral semi-circular incision performed below the scapulocoracoid cartilage. Stomachs had their extremities tied to avoid content loss, were removed, labeled, fixed in formaldehyde buffered (sodium tetraborate) solution and conserved in 70% ethanol.

During laboratory analysis, stomach contents were emptied onto a Petri dish and examined under a stereoscopic magnifier (Nikon® SMZ-10A). The food items were weighed on a precision scale (0.001 g - Sartorius LC621S) but excess liquid was removed with soft paper towels previously to weighing.

The stomach content analysis was carried out using the most specific taxonomic level. Afterwards, the previously identified food items were sent to specialists in each of the groups found to confirm their identification.

The values for each food item were calculated as follows:

- a) Percentage frequency of occurrence (% F.O. = $100 \times F_i / n$, where: F_i = number of times the food item occurred; and n = total number of stomachs with food items).
- b) Percentage by number (% N = $100 \times N_i / n$, where: N_i = number of items of each taxon; and n = total number of items of all taxa).
- c) Percentage by weight (% W = $100 \times W_i / n$, where: W_i = total weight of one item (taxon); and n = sum of all the weights of all stomach contents).

The Index of Relative Importance (IRI) (Pinkas et al., 1971) was calculated in a modified way (Hacunda,

1981), where the percentage by volume originally used was substituted for a percentage by weight. This modification was needed owing to the difficulty in calculating the percentage by volume for very small food items. The IRI modified method was based on the following equation: $IRI = \% F.O. \times (\% P + \% N)$.

The IRI was also transformed into a percentage (% IRI) for a better interpretation of the data in accordance with the recommendations in the literature (Cortés, 1997). A three-dimensional graphic representation of the stomach content data was prepared with Statistica 7.0 (Statsoft, Inc.).

The stages of sexual maturation were obtained from another study in progress on the reproductive characteristics of this species in the same region (personal observation of M. P. Almeida). In the present study, the sexual maturity stages were divided into immature (juvenile) and mature (adult).

The levels of repletion were observed and the following values were attributed: 0 = empty; 1 = ¼ full; 2 = up to half full; 3 = ¾ full and 4 = full. The degree of digestion of the items followed the scale suggested by Zavalla-Camin (1996), who used the following values: 1 = undigested food; 2 = external parts partially digested; 3 = external parts and muscular mass partially digested; 4 = only the axial skeleton and part of the muscular mass present; and 5 = only fragments are found.

Direct comparisons were made with the calculated %IRI values regarding the diet between sampling points, sexes, and immature and mature specimens. The degrees of repletion between immature and mature specimens and between sexes were compared by using the Chi-Square Test (χ^2 , degree of freedom DF = 1; level of significance $P < 0.05$) (Zar, 1999; Ayres et al., 2003).

Empty stomachs or those containing only amorphous substance were not considered for analysis. The spiral valves were also not taken into account for analysis since they presented contents at a very advanced stage of digestion.

In this study, plant fragments were not considered a food item, since this material is probably incidentally ingested by suction and is unlikely part of the diet of freshwater stingrays (Bragança, 2002; Charvet-Almeida, 2001; 2006).

3. Results

A total of 137 stomachs was analysed (81 males / 56 females), from Afuá ($n = 8$), Lake Arari ($n = 121$) and Muaná ($n = 8$). Of the total number of stomachs analysed, 90% ($n = 123$) contained food items, the rest contained only amorphous substance.

Detailed analysis of the diet verified that about 15 major prey orders were identified among this species' stomach contents. Decapoda was the most representative food item order considering FO, N, W and IRI percentages. The second most important order in terms of %IRI

was Siluriformes (Osteichthyes). The remaining orders represented less than 5% (Table 1).

The families that could be identified in the samples were: Hyriidae, Corbiculidae and Ampullaridae (Gastropoda); Grapsidae, Trichodactylidae and Palaemonidae (Malacostraca); Formicidae, Gomphidae, Libelulidae, Gryllotalpidae, Polymitarciidae and Scarabeidae (Insecta); Sternopygidae, Acestorhynchidae, Erythrinidae, Anostomidae, Cynodontidae, Characidae, Pimelodidae, Doradidae, Auchenipteridae, Callichthyidae, Loricaridae, Cichlidae and Engraulidae (Osteichthyes).

It was also possible to identify some items to a taxonomic level of genus or species, such as: *Diplodon* sp., *Corbicula* sp. and *Pomaceae* sp. (gastropods); *Dilocarcinus pagei* Stimpson, 1861, *Macrobrachium* sp. (arthropods) and *Eigenmannia* sp., *Acestorhynchus* sp., *Hoplias* sp., *Leporinus* sp., *Pygocentrus nattereri* Kner, 1858, *Serrasalmus altispinnis* (Cuvier and Valenciennes, 1850), *Pimelodus* sp. and *Trachydoras* sp. (Osteichthyes). Among these, the ones representing the greatest %IRI were: *Pomaceae* sp. (gastropods); *Dilocarcinus pagei* (Malacostraca); Polymitarciidae (Insecta) and Callichthyidae (Osteichthyes). In Oligochaeta a more specific identification other than class was impossible to be achieved.

According to the three-dimensional graphic representation of the %IRI, the dominant food items in the

diet of *P. motoro* at Marajó Island were fish and crustaceans (Figure 2).

The IRI ranked five groups of food items considering the location and sex (Table 2). The dominant items were different for the different sampling points, crustaceans were predominant in Afuá (IRI = 97%), fish in Arari Lake (64%) and mollusks in Muaná (58%). The main food item was the same for both sexes within each sampling point. Nevertheless, when considering the sampling points altogether, IRI values for fish and crustaceans were dominant in the diet of *Potamotrygon motoro*.

In Afuá and Lake Arari there were no diet variations when comparing immature and mature specimens. However, in Muaná the dominant food item for immature specimens was crustaceans, whilst the sexually mature potamotrygonids preferred mollusks. On Marajó Island (locations altogether) the dominant food item for immature stingrays was crustaceans and fish for mature specimens (Table 3).

Repletion level 1 was the main result obtained for females (69%), males (64%), immature (46%) and mature specimens (69%). The Chi-Square Test (χ^2 , degree of freedom DF = 1; level of significance P < 0.05) indicated significant differences in repletion levels 0.1 and 2 when comparing immature and mature classes (Table 4).

Most of the food items were well-digested, pertaining to category 4 (31%) and 5 (37%). This characteristic posed a problem to precisely identify the food items. A digestion

Table 1. Results of the stomach content analysis for *P. motoro* (n = 137) indicating the percentage frequency of occurrence (%FO), percentage by number (%N), percentage by weight (%W), Index of Relative Importance (IRI) and its respective percentage (%IRI) for each taxonomic order identified.

Class	Order	%FO	%N	%W	IRI	% IRI
Oligochaeta	Anelida	0.81	3.83	0	3.10	0.05
Malacostraca	Decapoda	50.81	39.88	43.28	4238.57	67.62
Osteichthyes	Characiformes	10.48	3.26	24.10	286.73	4.57
	Clupeiformes	0.81	0.19	0.09	0.23	0
	Gymnotiformes	0.81	0.57	0.10	0.54	0.01
	Perciformes	14.52	5.36	5.05	151.15	2.41
	Siluriformes	34.68	10.92	18.34	1014.74	16.19
	Unidentified fish	24.19	8.24	2.89	269.23	4.30
Insecta	Coleoptera	1.61	0.38	0.19	0.92	0.01
	Ephemeroptera	12.10	15.13	0.12	184.53	2.94
	Hymenoptera	0.81	0.19	0.00	0.15	0
	Odonata	7.26	1.72	0.07	13.00	0.21
	Orthoptera	2.42	0.57	0.07	1.55	0.02
	Unidentified insects	1.61	0.57	0	0.92	0.01
Gastropoda	Veneroida	0.81	0.19	0.01	0.16	0
	Caenogastropoda	7.26	8.43	5.56	101.57	1.62
	Unionoida	1.61	0.38	0.09	0.76	0.01
	Unidentified molluscs	0.81	0.19	0.04	0.19	0
	Total	173.41	100	100	6268.03	100

The numbers in bold correspond to the highest values.

Table 2. Percentage Index of Relative Importance (%IRI) calculated for the food item groups of *P. motoro* by location (1, 2 and 3 and overall total) and sex (M and F).

Food item / sex	Afuá (1)			Arari Lake (2)		
	M (n = 4)	F (n = 2)	Total (n = 6)	M (n = 42)	F (n = 68)	Total (n = 110)
Crustaceans	95.04	92.48	97.15	24.29	33.00	30.84
Insects	0.00	6.42	0.63	1.61	7.20	4.93
Mollusks	0.81	1.10	0.91	-	0.01	0.00
Fish	4.15	-	1.31	74.10	59.79	64.23
Annelids	-	-	-	-	-	-
Total	100	100	100	100	100	100

Food item / sex	Muaná (3)			Total - Marajó Island (1+2+3)		
	M (n = 2)	F (n = 6)	Total (n = 8)	M (n = 76)	F (n = 48)	Total (n = 124)
Crustaceans	36.35	42.18	39.22	43.39	31.79	41.92
Insects	-	3.15	1.86	4.85	0.71	4.42
Mollusks	63.65	52.49	57.65	1.47	0.16	1.19
Fish	-	-	-	50.24	67.34	52.44
Annelids	-	2.18	1.27	0.05	-	0.03
Total	100	100	100	100	100	100

The numbers in bold correspond to the highest values.

Table 3. Percentage Index of Relative Importance (%IRI) calculated for the food item groups of *Potamotrygon motoro* by location (1, 2 and 3 and overall total) and stage of sexual maturity.

Food item / sexual maturity stage	Afuá (1)		Arari Lake (2)		Muaná (3)		Total - Marajó Island (1+2+3)	
	Immature (n = 4)	Mature (n = 2)	Immature (n = 3)	Mature (n = 107)	Immature (n = 4)	Mature (n = 4)	Immature (n = 11)	Mature (n = 113)
Crustaceans	95.1	95.6	22.1	31.1	79.5	17.6	78.5	33.9
Insects	0.0	3.3	1.7	5.0	8.1	0.3	2.3	4.8
Mollusks	0.8	1.1	x	0.0	8.2	82.1	3.4	0.7
Fish	4.1	0.0	76.2	63.9	x	x	14.3	60.6
Annelids	x	0.0	x	0.0	4.2	x	1.5	x
Total	100	100	100	100	100	100	100	100

The numbers in bold correspond to the highest values.

Table 4. Chi-Square Test (χ^2) comparing stomach repletion levels to sex and sexual maturity.

Repletion level	Sex (n)		χ^2	p	Sexual maturity (n)		χ^2	p
	Female	Male			Immature	Mature		
0	4	7	0.818	0.547	1	10	7.364	0.016
1	56	36	4.348	0.048	6	86	69.565	0.000
2	13	9	0.727	0.522	4	18	8.909	0.006
3	8	3	2.273	0.228	2	9	4.455	0.070
4	0	1	1.00	1.000	0	1	1.00	1.000
Total	81	56	-	-	13	124	-	-

The numbers in bold correspond to statistically significant values.

degree 5 was more frequent when females (34%) and males (46%) were considered separately. The most frequent results were degree 4 (40%) for the immature specimens and degree 5 for the sexually mature specimens (39%).

4. Discussion

Feeding habits and diet studies have been through various revisions and proposals of new methodologies to analyse data in different animal groups in the recent

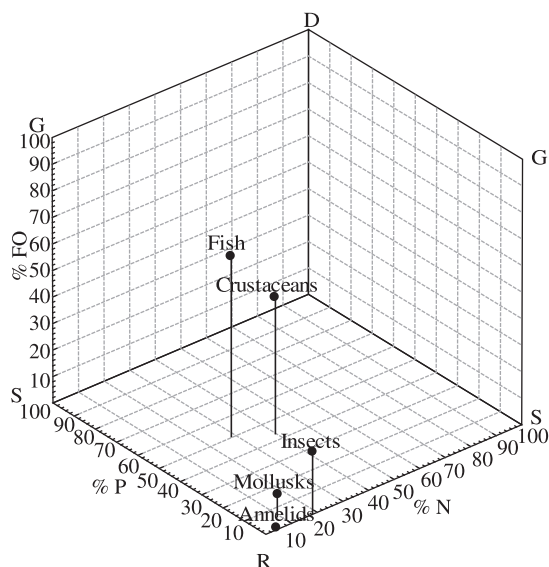


Figure 2. Three-dimensional graphic representation of the Percentage of the Index of Relative Importance (%IRI) obtained for the food items of *P. motoro* on Marajó Island, where: D = dominant food category and R = rare food category (referring to the prey), and G = generalised diet and S = specialised diet (referring to the predator).

past. Cortés (1997) presented the first critical revision on the study methods used in fish feeding based on a stomach content analysis with a specific approach for elasmobranchs. It recommended the use of the Index of Relative Importance (IRI) (Pinkas et al., 1971), its percentage and also indicated the use of a three-dimensional graphic representation, as being the most appropriate (Cortés, 1997).

When considering the relative environment similarity and the wide distribution range of *P. motoro* in the Neotropical region (Rosa, 1985), little or no diet variation was expected. The greatest variety in food items found for *P. motoro* on Marajó Island occurred with the bony fish and insect categories, both of them with at least 5 different orders. Despite this large variety, insects always showed low %IRI values, and never represented more than 3% of the samples taken.

Until now *P. motoro* seems to be the freshwater stingray species that displays the most varied feeding habits. This affirmation is based on the results available from this and other studies. Pântano-Neto and Souza (2002) in the Cristalino River (Goiás) region identified a diet based on insects, mainly Chironomidae (Diptera); Melo et al. (2007) on the Juruá River Basin (Acre) indicated this specie to be insectivorous; Shibuya et al. (2007) showed that the preferential food item were crustaceans in the Negro River Basin (Amazonas); Lonardon et al. (2006) showed that *P. motoro* preferred mollusks in the flood season and insects in the dry season in the Paraná River (Paraná) and Silva and Uieda (2007) demonstrated that this species fed on both insects and fish in the Paraná

River (São Paulo and Mato Grosso). Results from these authors demonstrate that this species has feeding plasticity and diet variability.

Certainly the feeding plasticity of *P. motoro* has contributed to its wide geographical distribution and favours its expansion into new areas/habitats. *P. motoro* is considered an invading species in the Paraná River (Agostinho et al., 1997; Lonardon et al., 2006; Garrone-Neto et al., 2007) and probably it is capable of adapting to the food items available in its new distribution range.

Few other species of freshwater stingrays have had their feeding habits studied in such detail. In comparison to the diet variability of *P. motoro*, some other species usually exhibit a far more stable feeding preference. This stability was seen in *Paratrygon aiereba* (Müller and Henle, 1841) that seems to be an exclusively fish eating species (Lasso et al., 1996; Charvet-Almeida, 2006; Shibuya et al., 2007); *Potamotrygon orbignyi* (Castelnau, 1855) is considered an insectivorous species (Lasso et al., 1996; Bragança, 2002; Silva et al., 2006; Rincon, 2006; Melo et al., 2007; Shibuya et al., 2007) and *Potamotrygon leopoldi* Castex and Castello, 1970 is a species that feeds predominantly on gastropods (Charvet-Almeida, 2006).

Differences in the diet between sexes were expected, as observed in *Potamotrygon leopoldi* in the Xingu River region (Charvet-Almeida, 2006). In this group of stingrays the reproductive strategy (matrotrophic viviparity with trophodermata - Charvet-Almeida et al., 2005) depends on lipid reserves accumulated in the female liver (Baldrige, 1970; Craik, 1978) and therefore is directly related to the feeding process. However, the dominant item in the diet of *P. motoro* was the same for both sexes on Marajó Island.

Several elasmobranch species have diet changes associated to ontogeny and sexual maturity (Lowe et al., 1996; Muto et al., 2001). Some diet differences were noted between immature and mature specimens. These were observed in Muaná and in the overall results. According to the overall data, immature stingrays seemed to prey mainly on crustaceans, while mature specimens on fish. However, this predominance varied among sampling points and is possibly associated to prey availability in each area. In the Paraná River (Tocantins River drainage) an ontogenetic diet shift was registered for *Potamotrygon orbignyi* (Rincon, 2006). Rincon (2006) pointed out that a higher diversity of food items (mainly insects) was consumed by larger stingrays and that this shift was not associated to sexual maturity.

The presence of plant fragments in potamotrygonid species stomach content analysis (Charvet-Almeida, 2001; 2006; Rincon, 2006; Lonardon et al., 2006) contribute to the idea that these stingrays present speculative habits, as indicated by Zuanon (1999). The presence of small quantities of these fragments in the stomachs also suggests that these particles are not actually part of these stingray's diet, but are probably ingested accidentally by suction during the feeding process (Bragança, 2002).

Despite being highly regarded, the IRI (Pinkas et al., 1971) holds the premise that the analysis should be performed with all items at the same identification level in order to avoid results distortions. Since food items found in elasmobranchs are usually highly digested, the results almost always require higher taxa groupings (e.g. insects, crustaceans, etc.).

In the present study, it was observed that the food items examined were well-digested, and therefore it was difficult to identify the samples at more specific taxonomic levels. Given the condition of items, at higher taxa grouping, *P. motoro* ingested predominantly fish and crustaceans. However, when the food item analysis was performed at an order level, the crustacean group became the most representative, followed by fish, producing, therefore, an inversion in importance of the main food items observed. Crustaceans were represented by a single order (Decapoda), while fish comprised of at least five orders. If fish were grouped under a single taxonomic category (e.g. teleosts), the %FO and %P and consequently IRI values would be higher than the ones calculated for crustaceans. Cortés (1997) considered IRI efficient to describe species diets; however, he also recognised that that IRI values depend a lot on the taxonomic resolution levels attained in the analysis.

The occurrence of high percentages of empty stomachs, or stomachs with low levels of repletion, was evident in the sample studied and is a common characteristic observed among marine stingrays (Wetherbee et al., 1990), as well as in other freshwater stingray species (Charvet-Almeida, 2001; Bragança, 2002; Bragança et al., 2004; Rincon, 2006; Charvet-Almeida, 2006). These results suggest that potamotrygonids possibly feed on small quantities, perhaps several times a day. An hourly sampling standardisation throughout a 24-hour period to check precisely the feeding time could not be done since daytime fisheries were impracticable due to bait competitors. This way, results obtained in the present study corresponded mainly to nighttime sampling. The same problems with daytime fisheries and similar methodologies were adopted by other freshwater stingray diet researchers in the Amazon region (Bragança, 2002; Bragança et al., 2004; Charvet-Almeida, 2006; Rincon, 2006).

Results demonstrated that in the different environments studied *P. motoro* varied its feeding habits and always displayed differentiated diets with main food items %IRI values higher than 50%. This variability in feeding can be related to the abundance/availability of specific food items in each sampling point or to a specific feeding behaviour expressed in different locations.

Acknowledgements — The authors are grateful to the specialists consulted to help identify the food items in their respective specialisation: Dr. Luiz Ricardo Simone, (MZUSP - mollusks); Dr. Dr. Jansen S. Zuanon (INPA - fish); Dr. Célio Magalhães (INPA - crustaceans) and Dr. Bento Mascarenhas (MPEG - insects). The leading author is thankful to the National Counsel of Technological and Scientific Development for the doctoral grant.

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