



Analysis of stomach contents of freshwater stingrays (Elasmobranchii, Potamotrygonidae) from the middle Negro River, Amazonas, Brazil

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Abstract. Potamotrygonid stingrays are restricted to Neotropical rivers and information on their diet remains scarce. Thus, the prey composition of four freshwater stingray species from the middle Negro River was studied using stomach contents analysis: *Potamotrygon motoro* (n=40), *Potamotrygon orbignyi* (n=27), *Potamotrygon* sp. “cururu” (n=26), and *Paratrygon aiereba* (n=34). Prey items were identified up to the lowest taxonomic level possible and analyzed with the Index of Relative Importance (%IRI). Teleosteans, crustaceans and insect larvae were consumed by all species in distinct proportions. *Potamotrygon motoro* fed mainly on palaemonid shrimps (33.8%) and trichodactylid crabs (48.7%). Stomach contents of *Potamotrygon orbignyi* were composed principally by insects (96.4%), with predominance of gomphid dragonfly larvae. *Potamotrygon* sp. “cururu” fed on crustaceans (shrimps and crabs: 49.4%) and insect larvae (30.2%). *Paratrygon aiereba* consumed mainly teleosteans (94.8%), which were composed by a wide variety of species. These results suggest a food partitioning among the four freshwater stingray species, based on the use of different microhabitats and foraging substrates. Different mechanisms of prey detection and capture may also contribute to the differences of prey composition in the stomach contents of these stingray species.

Key words: feeding, potamotrygonids, aquatic communities, diet

Resumo. Análise dos conteúdos estomacais de espécies de raias de água doce (Elasmobranchii, Potamotrygonidae) do médio rio Negro, Amazonas, Brasil. As raias Potamotrygonidae são restritas aos rios neotropicais e pouco se conhece sobre a alimentação dessas espécies. Com base nisso, a composição de presas foi estudada para quatro espécies de raias do médio rio Negro: *Potamotrygon motoro* (n=40), *Potamotrygon orbignyi* (n=27), *Potamotrygon* sp. “cururu” (n=26) e *Paratrygon aiereba* (n=34), por meio de análise de conteúdos estomacais. Os itens alimentares foram identificados até o menor nível taxonômico possível, e os resultados foram analisados utilizando o Índice de Importância Relativa (%IRI). As quatro espécies alimentaram-se de peixes, crustáceos e insetos, porém em proporções diferenciadas. Os exemplares de *P. motoro* consumiram principalmente camarões Palaemonidae (33,8%) e caranguejos Trichodactylidae (48,7%). Os conteúdos estomacais de *Potamotrygon orbignyi* foram compostos majoritariamente por insetos (96,4%), com predomínio de larvas de libélula Gomphidae, enquanto *Potamotrygon* sp. “cururu” consumiu crustáceos (camarões e caranguejos: 49,4%) e larvas de insetos (30,2%). *Paratrygon aiereba* ingeriu predominantemente peixes (94,8%). Estes resultados indicam uma possível partilha de recursos alimentares entre essas quatro espécies de raias, baseada no uso de diversos microhabitats e substratos de forrageamento. Mecanismos diferenciados de detecção e captura de presas também podem contribuir para as diferenças observadas na alimentação dessas raias de água doce.

Palavras-chave: alimentação, potamotrygonídeos, comunidade aquática, dieta

Introduction

Elasmobranchs occur in all aquatic ecosystems and are dominant predators of communities where they live (Camhi *et al.* 1998, Wetherbee & Cortés 2004). This group presents complex sense organs and a variety of feeding mechanisms and mechanics that make them highly competitive with marine tetrapods and teleosteans (Compagno 1990, Maruska 2001, Motta 2004).

While increasing attention has been given to the diet and feeding behavior of sharks, batoids have generally been overlooked (Lowe *et al.* 1996, Heithaus 2001, Kyne & Bennett 2002, Ebert & Cowley 2003). Detailed information about the feeding habits of elasmobranchs will provide a better understanding of their natural history, their role in aquatic ecosystems, and the position of each species in the trophic levels within their particular environments (Cortés 1999, Wetherbee & Cortés 2004).

Only few species of skates and rays occupy the apex of the food chain in marine environments and most of them are predators of benthic communities (Kyne & Bennett 2002, Gilliam & Sullivan 1993), consuming primarily mollusks, crustaceans and teleosteans. There are exceptions to this generalization, however, due to the plasticity of their feeding habits, ontogenetic variations of their diets, and the geographic distribution or availability of different types of prey (Compagno 1990, Wetherbee & Cortés 2004).

Few studies have been undertaken to examine the diets and feeding habits of potamotrygonid rays, as is also true with several other groups of marine rays. Achenbach & Achenbach (1976) were the first to determine the diet of stingrays from that family, and these authors reported ontogenetic changes in terms of the prey consumed.

Rosa *et al.* (1987) described the stomach contents of specimens of *Plesiotrygon iwamae* from the Solimões and Napo Rivers in Brazil, and reported finding teleosteans, insects and decapods. This analysis was based on only three individuals, which did not allow them to determine the importance of each prey category. Lasso *et al.* (1996) analyzed the diets of *Potamotrygon orbignyi* and *Paratrygon aiereba* from the Apure River, Venezuela, and identified insectivorous and piscivorous habits respectively.

Pantano-Neto (2001) examined the diet and anatomy of the orobranchial muscles of two *Potamotrygon* species from Brazil and observed that *P. motoro* fed mainly on insects while *P. henlei* fed especially on gastropods. This author also

demonstrated a strong relationship between the feeding habits and the oral muscles of these species, with *P. motoro* having a higher relative biomass of muscles responsible for suction (*e.g. depressor hyomandibulae*), while *P. henlei* had stronger adductor muscles (*e.g. adductor mandibulae*) that aid in breaking its prey apart.

Rincon-Filho (2006) found a high predominance of Ephemeroptera in the diet of *P. orbignyi* in the upper Tocantins River, Brazil. The same author also reported teleosteans, mollusks and crustaceans as the main prey items in the diets of three other potamotrygonid species (*Paratrygon aiereba*, *Potamotrygon henlei* and *P. sp* “n”) from the same sampling site, although these findings were based on very few individuals. Lonardoni *et al.* (2006) found that *P. falkneri* and *P. motoro* fed mainly on teleosteans and insects, respectively, in the upper Paraná River, Brazil. These authors observed seasonal fluctuations of the dominant prey items for the two species, and both consumed mollusks during the flooding season. Overall, studies of batoids have shown that marine and freshwater species both consume the same broad prey categories (teleosteans, crustaceans and mollusks). The greatest difference between them is the considerable importance of insect larvae in the diets of freshwater stingrays (as insects are essentially absent in marine environments).

Comparisons of the dietary compositions recorded in different studies have been complicated by the use of various different indices. Cortés (1997) proposed the use of an Index of Relative Importance (IRI) for interpreting dietary data in elasmobranchs. However, earlier diet analyses carried out by Cortés (1997) as well as some later studies by Pantano-Neto (2001) and Lonardoni *et al.* (2006), for example, use only direct counts, such as frequency of occurrence, volume and/or numbers. This lack of standardization has resulted in incomplete interpretations, however, as they are based solely on the frequency of occurrence of the prey items.

Feeding ecology involves food partitioning and different levels of competition among species, especially in environments with high biodiversity. Despite the similarities between the anatomical features of potamotrygonid species and their co-occurrence in the Negro River, each population has its own distinct microhabitats and feeding habits. According to Araújo (1998), juveniles of *Potamotrygon motoro* occur in tributaries on both margins of the Negro River, whereas juveniles of *Paratrygon aiereba* occur at the mouths of these tributaries as well as on nearby sand beaches; adults of both species occur in the main river channel.

Juveniles and adults of *P. orbignyi* have been associated with sandy beaches, and *Potamotrygon* sp. (locally known as “cururu”) is frequently observed in flooded forests (“igapós”). These findings suggest that sympatric potamotrygonid species may exploit different feeding grounds and prey types, which may lessen competitive interactions.

The present study analyzes the prey composition in the stomachs of the co-occurring stingrays *Potamotrygon motoro*, *P. orbignyi*, *Potamotrygon* sp. “cururu” and *Paratrygon aiereba* in the middle course of the Negro River during the dry season, using Cortés’ methodology

(Cortés 1997).

Material and Methods

The present study was undertaken from December 2001 to March 2007 by a team from the Project Freshwater Stingrays Monitoring Plan (FAPEAM, Amazonas Funding Agency). Sampling was carried out during the low (dry season) to rising (rainy season) hydrological periods, from September to March (Fig. 1). Stingray sampling is seasonal, with an inverse relationship being observed between capture vulnerability and the water level in the river (see Araújo 1998).

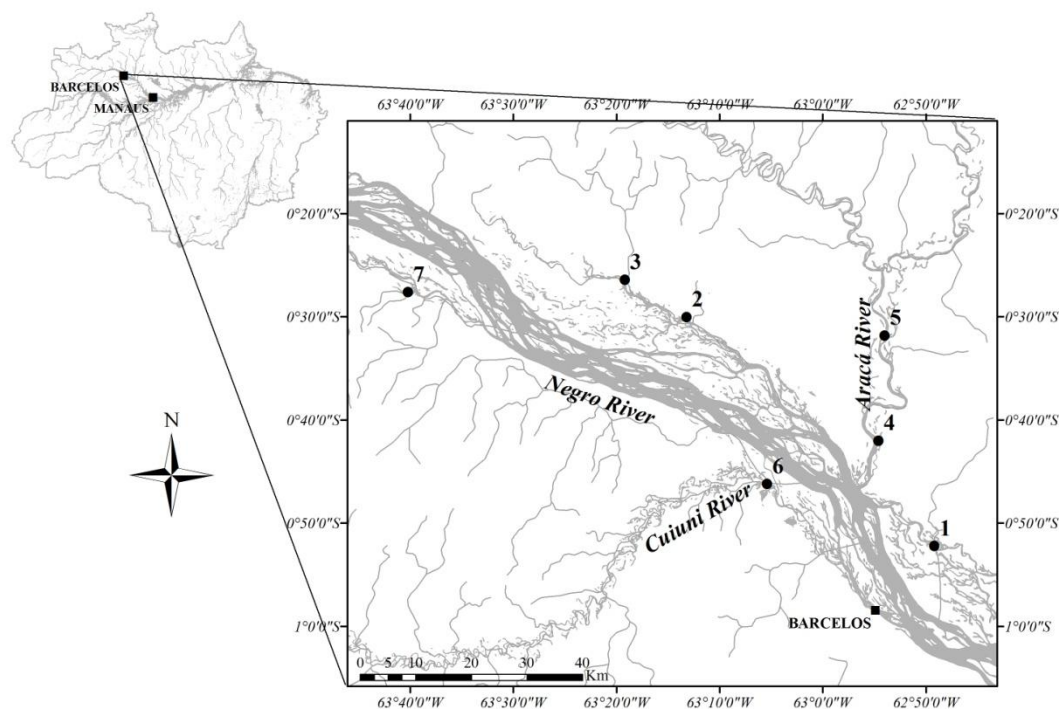


Figure 1. Sampling sites of *Potamotrygon motoro*, *P. orbignyi*, *Potamotrygon* sp. “cururu” and *Paratrygon aiereba*, in the middle Negro River, Barcelos, Amazonas State, Brazil. 1. Zamula Stream; 2 – Daraquá Stream; 3 – Itu River; 4 – Maqui Lake; 5 – Demeni River; 6 – Cuiuni River; 7 – Arirahá River.

Neonates and juveniles of *P. motoro*, *P. orbignyi* and *Paratrygon aiereba* as well as juveniles and adults of *Potamotrygon* sp. “cururu” were collected using a dip net. Adults of *P. motoro* and *Paratrygon aiereba* were collected using bottom longlines, while *P. orbignyi* adults were collected by gillnets. All specimens were collected by local fishermen during the night and in different habitats (sandy and muddy beaches, river channels, lakes and streams).

Stingrays were euthanized with clove oil (Eugenol), and their stomachs were immediately removed and injected with a 10% formalin solution

to halt digestion. After a few days of immersion in formalin, the stomachs were gently washed and conserved in 70% ethanol. All stingray specimens were identified according to Rosa (1985) and maintained in 10% formalin. Total length (TL), disc width (DW), total weight (TW) and sex were recorded for each individual.

Stomach contents were analyzed with the aid of a stereoscopic microscope. Prey items were sorted, weighted (g), identified to the lowest taxonomic level possible and subsequently counted and maintained in 70% ethanol. Prey items were identified using specific identification keys (e.g.

Géry 1977, Burgess 1989, Keith *et al.* 2000, Melo 2003) and were also compared with specimens from the Zoological Collections of the National Institute for Amazonian Research – INPA, with the assistance of specialists for each taxonomic category.

The percentages of frequency of occurrence (%F.O.), numbers (%N), and weight (%W) were calculated for each prey category according to the following equations (Hyslop 1980):

$\%F.O. = 100 \times Fi/n$, where: **Fi** is number of stomachs containing a particular prey taxon; **n** is total number of stomachs with any prey.

$\%N = 100 \times Ni/n$, where: **Ni** is total number of prey of a particular taxon; **n** is the total number of all prey identified.

$\%W = 100 \times Wi/n$, where: **Wi** is the total weight of prey of a particular taxon; **n** is the total weight of all prey in the stomachs.

The Index of Relative Importance (IRI) was calculated combining %F.O., %N, and %W (Pinkas *et al.* 1971), replacing volumetric percentage for weight percentage: $IRI = (\%P + \%N) \times \%F.O.$ The IRI was transformed into a percentage (%IRI; *cf.* Cortés 1997), for comparisons among the studies. Empty stomachs and those presenting only amorphous substances (unidentified digested contents) were not considered in the IRI calculation. The results of %W, %N and %F.O. were used to prepare three-dimensional graphics to aid in interpreting the contributions of each prey item categories to the stingrays' diets (Cortés 1997).

Results

Forty specimens of *Potamotrygon motoro*, with disc widths (DW) ranging from 17.0 to 56.0 cm, 27 specimens of *P. orbignyi* (DW: 17.0 – 48.2 cm), 26 *Potamotrygon* sp. “cururu” (DW: 14.9 – 31.0 cm), and 34 *Paratrygon aiereba* (DW: 18.4 – 67.0 cm) were analyzed. The numbers of empty stomachs, those with only amorphous substances, and those presenting at least one prey item for each stingray species are summarized in Table I.

Prey items that were found in all four species included insect larvae, mollusks, crustaceans and teleosteans. Palaemonid shrimps and dragonfly larvae (Odonata: Gomphidae) were present in distinct proportions in the stomachs of all of the species analyzed; mollusks were observed in the stomachs of only one specimen of *P. motoro*. A total of 18 taxonomic groups were found in all species. The results of percentages of number, weight and frequency of occurrence and the Index of Relative Importance (IRI) of prey items are shown in Table I.

The relative importance of prey categories (%IRI) indicated differences among the stingray species. *Potamotrygon motoro* had decapods as its

dominant prey (70.0%), followed by teleosteans (26.5%). Insects and mollusks had minor importance for this species, with %IRI values of 3.4% and 0.1% respectively. *Potamotrygon orbignyi* fed mainly on insects (96.4%), with a low participation of teleosteans (2.7%) and crustaceans (0.9%). *Potamotrygon* sp. “cururu” had the largest %IRI for crustaceans (49.4%), followed by insects (30.2%) and teleosteans (20.4%). *Paratrygon aiereba* consumed primarily teleosteans (94.8%), while crustaceans and aquatic insects (both with 2.6%) had lower participations in its diet.

Palaemonid shrimps had high importance for *P. motoro* (33.8%), in contrast for the other three species. Dragonfly larvae were the dominant item in the diet of *P. orbignyi*, having a 73.9% importance level, while this prey item presented only 23.7% IRI for *P. sp.* “cururu”. Crustaceans represented almost 50% IRI for *P. sp.* “cururu”; they were not the dominant prey item in this species' diet because the percentages of frequency of occurrence of insect larvae and crustaceans were similar but the low contribution of the weight of crustaceans made them the second most important item for *P. sp.* “cururu”.

The three-dimensional graphic representations of the %F.O., %N and %W values illustrate the relative importance of the different prey categories to each stingray species (Fig. 2). *Potamotrygon motoro* (Fig. 2a), *P. orbignyi* (Fig. 2b) and *Paratrygon aiereba* (Fig. 2c) had up to two dominant prey items in their diets, while *P. sp.* “cururu” (Fig. 2d) showed a more homogeneous participation of various prey taxa. Crustaceans were dominant in the diet of *Potamotrygon motoro*, although teleosteans were also significant; mollusks were a rare item in the diet of this species. Insects were the dominant prey of *P. orbignyi*. Insects were also consumed by *Potamotrygon* sp. “cururu”, but in similar proportions to both crustaceans and teleosteans. *Paratrygon aiereba* had teleosteans as its dominant prey, while invertebrates were rare in its diet.

Discussion

Similar general food categories were consumed by *Potamotrygon motoro*, *P. orbignyi*, *P. sp.* “cururu” and *Paratrygon aiereba*; however, each species had different predominant prey items. In the current study, crustaceans (especially trichodactylid crabs) were the main prey item for *Potamotrygon motoro*. Pantano-Neto (2001) reported that *P. motoro* from the Cristalino River, a tributary of the Araguaia River, in Mato Grosso State, Brazil, consumed large amounts of insect larvae (99%), although this author used a different method for calculating the feeding index (Kawakami & Vazzoler 1980). Rincon-Filho (2006),

however, noted that the specimens examined by Pantano-Neto (2001) were actually chromatic variants of *P. orbignyi*, which would explain the observed differences in their diets. Individuals of *P. motoro* from the upper Paraná River had insect larvae as their dominant prey item, as well as some mollusks and teleosteans (Lonardon et

al. 2006, Silva & Uieda 2007). The present study found a similar percentage of teleosteans and crustaceans, although teleosteans numbers and weight percentages indicated a lower contribution to the diet of *P. motoro* as compared to crustaceans (%N, %W and %F.O. values higher than 50%).

Table I. Prey items found in the stomachs of *Potamotrygon motoro*, *P. orbignyi*, *P. sp.* “cururu” and *Paratrygon aiereba*, from Negro River basin, expressed in percentages of number (%N), weight (%W) and frequency of occurrence (%F.O.), Index of Relative Importance (%IRI). Number of stomachs with contents (C), stomachs with amorphous substance (A) and empty stomachs (E) are presented for each potamotrygonid species.

| Items | <i>P. motoro</i> (C=32; A=5; E=3) | | | | <i>P. orbignyi</i> (C=12; A=5; E=10) | | | | <i>Potamotrygon sp.</i> “cururu” (C=17; A=1; E=8) | | | | <i>Paratrygon aiereba</i> (C=15; A=6; E=13) | | | |
|-------------------------|--------------------------------------|-------------|-------------|-------------|---|-------------|-------------|-------------|--|-------------|-------------|-------------|--|-------------|-------------|-------------|
| | %N | %W | %F.O. | %IRI | %N | %W | %F.O. | %IRI | %N | %W | %F.O. | %IRI | %N | %W | %F.O. | %IRI |
| FISHES | 29.8 | 33.2 | 53.2 | 26.5 | 3.6 | 32.7 | 9.1 | 2.7 | 12.7 | 55.0 | 29.4 | 20.4 | 77.8 | 99.8 | 50.0 | 94.8 |
| Characiformes | | | | | | | | | | | | | | | | |
| Characidae | | | | | 3.6 | 32.7 | 9.1 | 5.1 | 1.8 | 38.9 | 5.9 | 7.8 | 2.8 | <0.1 | 5.6 | 0.8 |
| Curimatidae | 0.7 | 0.4 | 3.1 | <0.1 | | | | | 3.6 | 13.1 | 5.9 | 3.2 | | | | |
| Clupeiformes | | | | | | | | | | | | | | | | |
| Engraulididae | | | | | | | | | | | | | 30.6 | 1.1 | 5.6 | 9.6 |
| Gymnotiformes | | | | | | | | | | | | | | | | |
| Unidentified | | | | | | | | | | | | | 2.8 | 0.1 | 5.6 | 0.9 |
| Perciformes | | | | | | | | | | | | | | | | |
| Cichlidae | 20.4 | 20.2 | 9.4 | 6.9 | | | | | | | | | 2.8 | 42.3 | 5.6 | 13.6 |
| Siluriformes | | | | | | | | | | | | | | | | |
| Callichthyidae | | | | | | | | | | | | | 2.8 | 0.4 | 5.6 | 0.9 |
| Cetopsidae | | | | | | | | | | | | | 2.8 | 54.4 | 5.6 | 17.3 |
| Doradidae | 1.8 | 2.3 | 9.4 | 0.7 | | | | | 1.8 | 0.5 | 5.9 | 0.4 | 8.3 | 1.0 | 11.1 | 5.5 |
| Loricariinae | 0.7 | 1.0 | 6.3 | 0.2 | | | | | | | | | 2.8 | 0.3 | 5.6 | 0.9 |
| Synbranchiformes | | | | | | | | | | | | | | | | |
| Synbranchidae | 2.2 | 4.1 | 9.4 | 1.1 | | | | | | | | | | | | |
| Unidentified | 4.0 | 5.2 | 34.4 | 5.7 | | | | | 5.5 | 2.5 | 17.6 | 4.6 | 22.1 | 0.2 | 27.8 | 33.6 |
| MOLLUSKS | 1.1 | 0.8 | 3.1 | 0.1 | | | | | | | | | | | | |
| Caenogastropoda | | | | | | | | | | | | | | | | |
| <i>Pomacea</i> sp. | 1.1 | 0.8 | 3.1 | 0.1 | | | | | | | | | | | | |
| CRUSTACEANS | 54.9 | 62.9 | 75.0 | 70.0 | 3.6 | 1.6 | 18.2 | 0.9 | 47.3 | 34.7 | 58.8 | 49.4 | 11.1 | 0.1 | 22.2 | 2.6 |
| Decapoda | | | | | | | | | | | | | | | | |
| Euryrhynchidae | | | | | | | | | 34.5 | 7.9 | 17.6 | 24.2 | 2.8 | 0.1 | 5.6 | 0.8 |
| Palaemonidae | 42.5 | 3.5 | 40.6 | 33.8 | 3.6 | 1.6 | 18.2 | 1.4 | 5.5 | 0.8 | 17.6 | 3.6 | 8.3 | <0.1 | 16.7 | 7.6 |
| Trichodactylidae | 12.4 | 59.4 | 37.5 | 48.7 | | | | | 7.3 | 26.0 | 23.5 | 25.4 | | | | |
| INSECTS | 14.2 | 3.1 | 25.0 | 3.4 | 92.8 | 65.7 | 72.7 | 96.4 | 40.0 | 10.3 | 58.8 | 30.2 | 11.1 | 0.1 | 22.2 | 2.6 |
| Odonata | | | | | | | | | | | | | | | | |
| Cordulidae | 5.8 | 2.4 | 9.4 | 1.4 | | | | | | | | | | | | |
| Gomphidae | 3.3 | 0.5 | 9.4 | 0.6 | 25.5 | 62.9 | 54.5 | 73.9 | 14.5 | 6.2 | 35.3 | 23.7 | 2.8 | <0.1 | 5.6 | 0.8 |
| Libellulidae | 2.2 | 0.2 | 9.4 | 0.4 | | | | | 14.5 | 3.2 | 5.9 | 3.4 | | | | |
| Ephemeroptera | | | | | | | | | | | | | | | | |
| Leptophlebiidae | 0.7 | <0.1 | 3.1 | 0.1 | 67.3 | 2.8 | 18.2 | 19.6 | 7.3 | 0.7 | 11.8 | 3.0 | | | | |
| Unidentified | 2.2 | <0.1 | 6.2 | 0.2 | | | | | 3.7 | 0.2 | 5.9 | 0.7 | 8.3 | <0.1 | 16.7 | 7.5 |

Potamotrygon orbignyi had insectivorous feeding habits, mainly consuming dragonfly larvae. Similar results were reported by Lasso et al. (1996)

and Rincon-Filho (2006) for individuals from the Apure and Tocantins river basins respectively, and both studies encountered mainly Ephemeroptera (the

second most important group of insects for *P. orbignyi* from Negro River basin). The predominance of insects in the diet of *P. orbignyi* is possibly related to its small mouth, which makes capturing large prey such as crabs and teleosteans more difficult. Additionally, this stingray species

lives near sandy beaches where insect larvae are abundant. Bragança *et al.* (2004) investigated the feeding biology of *P. orbignyi*, *P. scobina* and *Plesiotrygon iwamae* and found considerable quantities of crustaceans and insects (Diptera) in their stomachs.

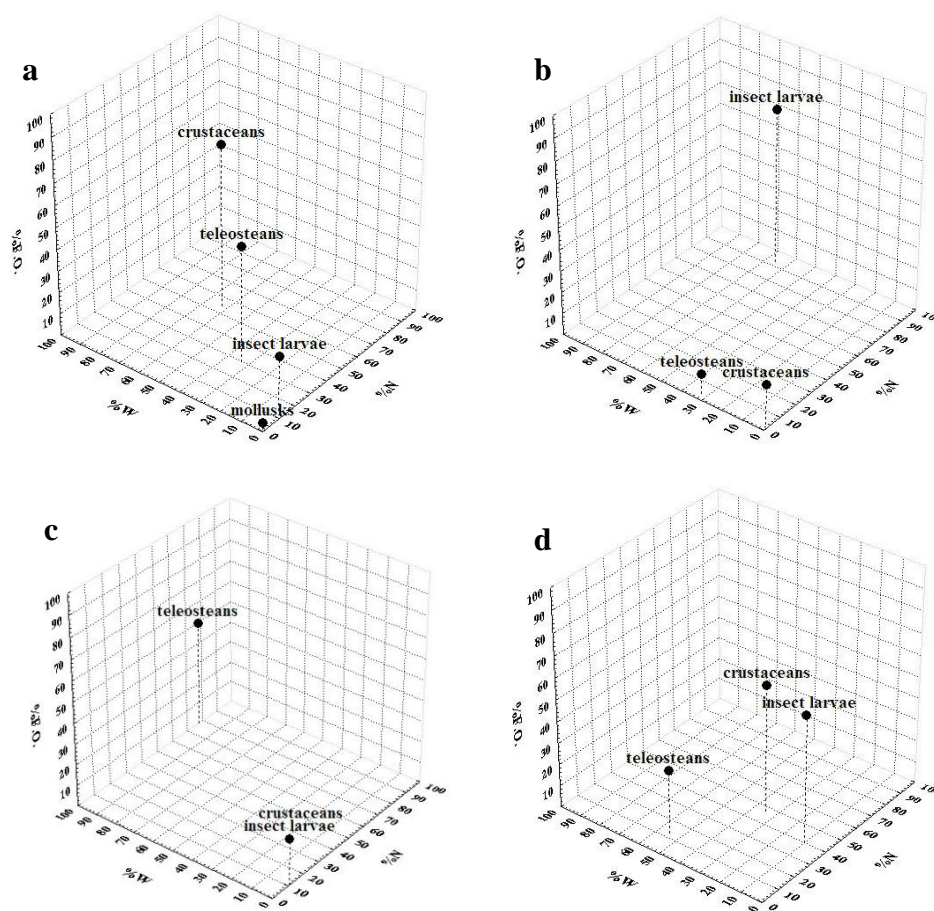


Figure 2. Graphic representation of the diet of *Potamotrygon motoro* (a), *P. orbignyi* (b), *Paratrygon aiereba* (c), and *Potamotrygon* sp. “cururu” (d), using percentages of weight (%W), number (%N) and frequency of occurrence (%F.O.).

The diet of *Potamotrygon* sp. “cururu”, which had never been previously examined, indicated generalist feeding habits based on the consumption of trichodactylid crabs, euryrhynchid shrimps and gomphid dragonflies. Teleosteans remains were encountered, but this prey item apparently contributed very little to the diet of this stingray species. According to Araújo (1998), “cururu” stingrays occur in flooded forests (“igapós”) where its main prey are common and abundant (Melo 2003, Fidelis *et al.* 2008). There is no information available about this species during the dry season when there are no flooded forest sites; these stingrays may change their diets or simply reduce their feeding activities during this period, which remains to be investigated.

Paratrygon aiereba demonstrated strongly piscivorous habits, consuming a wide variety of teleosteans commonly found in the river channel (*e.g.* Cetopsidae and Doradidae) and near sandy beaches (*e.g.* Engraulididae and Loricariinae), where adults and juveniles of *P. aiereba* are common, respectively. Lasso *et al.* (1996) and Rincon-Filho (2006) reported similar results for stingray specimens from the Apure River (Venezuela) and Tocantins River (Brazil), although they did not identify the prey fish to species level.

The varied consumption patterns of a given stingray species at different locations may be a consequence of particular habitat features at each site, as observed for *P. motoro* (Lonardoni *et al.* 2006) and *P. orbignyi* (Bragança *et al.* 2004,

Rincon-Filho 2006), and indicates that at least some stingrays are able to adapt to different environments and different prey availabilities. This same situation was not observed for *Paratrygon aiereba*, however, as it consistently exhibited a strongly piscivorous diet, differing only in terms of the fish taxa consumed.

More than 50% of the specimens of *P. orbignyi* and *Paratrygon aiereba* had empty stomachs or stomachs containing only amorphous substances (see Table I). Simpfendorfer (1998) and Barry (2002) considered empty stomachs or those containing only amorphous substances as indicating regurgitation due to excessively distended stomach walls. The empty stomachs observed with *P. orbignyi* and *Paratrygon aiereba*, however, may also have been caused by capture stress (*q.v.* Wetherbee & Cortés 2004), as being hooked for long periods of time may well cause regurgitation of their stomach contents. The high occurrence of empty stomachs and prey in advanced degrees of digestion may also suggest that the specimens analyzed were not feeding frequently, for prey items in different stages of digestion would otherwise have been expected (Wetherbee & Cortés 2004).

Bethea *et al.* (2007) reported that plants were frequently found in the stomach contents of bonnethead shark *Sphyrna tiburo* and were considered a prey item for this species - even though its presence may only be the result of their feeding behavior, as undigested plants were rarely found in the spiral valve of sharks. Some authors (*e.g.* Bragança *et al.* 2004, Lonardoni *et al.* 2006) consider plants as making up at least part of the diets of potamotrygonid species. In the current study, plants were rare among the stomach contents of the potamotrygonid species analyzed, and were not considered a prey category. These discrepancies in interpreting the role of plant material in the diet of freshwater stingrays clearly indicate the need for more detailed studies on its nutritional contribution.

The IRI interpretation proposed by Cortés (1997) has been employed in many studies of the diet and feeding habits of elasmobranchs (*e.g.* Ebert & Cowley 2003, Rincon-Filho 2006, Collins *et al.* 2007). There had been a lack of standardization of feeding analyses before Cortés's study due to the use of various methods proposed by a number of different authors - making comparative studies more difficult (see Hyslop 1980, Kawakami & Vazzoler 1980 and Costello 1990).

Some studies carried out after Cortés' review (1997) (such as Pantano-Neto 2001 and Lonardoni *et al.* 2006) did not adopt %IRI, but rather used percentage of frequency of occurrence

(%F.O.). Their findings showed a high participation of insect larvae (more than 90%F.O.) in the diet of *P. motoro*, whereas the current study observed 71.9% teleosteans, 78.1% crustaceans, and a low occurrence of insect larvae (37.5%) for the same stingray species.

Mollusks (*Pomacea* sp.) were found in the stomach contents of only one specimen of *P. motoro*. Low conductivity, an acid pH, and low levels of calcium and phosphorus in the waters of the Negro River are supposed to limit the occurrence of mollusks (Sioli 1953, Goulding *et al.* 1988, Volkmer-Ribeiro *et al.* 1998), which may explain the rarity of this prey item in the diet of freshwater stingrays. However, more than 50% of the diet of potamotrygonid species (*e.g.* *Potamotrygon henlei*) from clear waters rivers (Cristalino and Paraná Rivers) has been reported to be composed of gastropods and bivalves (Pantano-Neto 2001, Lonardoni *et al.* 2006).

Dragonfly larvae were present in the diets of all of the stingrays species analyzed, and with particularly high participation in the diets of *Potamotrygon* sp. "cururu" (23.7%) and *P. orbignyi* (73.9%). The high %IRI of this prey type for *P. orbignyi* reflects its habitat of sandy beach areas along the Negro River where insect larvae are commonly found buried in the sand (Fidelis *et al.* 2008). The consumption of large amounts of gomphid dragonfly larvae also provides us with indirect information about the foraging tactics of this stingray species, which digs up prey buried in the sand.

A temporal investigation of the diets of *P. motoro* and *P. falkneri* from Upper Paraná River was undertaken by Lonardoni *et al.* (2006) and demonstrated differences in their diets during the dry and flooding seasons. The largespot stingray (*P. falkneri*) had piscivorous feeding habits in the dry season but malacophagous habits in the flooding season. The ocellate river stingray (*P. motoro*) fed mainly on aquatic insects in both seasons, but also consumed mollusks during the flooding season. The present study have shown that the four stingray species that co-occur in the Negro River consumed similar prey items, although with distinct individual %IRI values that presumably reflect differences between the habitats where each species is found. Seasonal, ontogenetic and sex variations on their diet may also exist and should be considered in future studies.

The present results corroborated observations from previous studies that the diets of the same species of Potamotrygonidae from distinct locations cannot be generalized. The abundance and

availability of prey items in the environment where these stingrays live strongly influence their diets, which would at least partially explain the differences encountered in the published data on the subject. These differences may be particularly important in short term studies, and the results presented here must be considered carefully in light of its restriction to the dry and rising seasons. Information concerning changes in prey consumption linked to seasonal variations is essential for a better understanding of the role of locally available prey items and the specific habitats used by potamotrygonid species during the annual hydrological cycles of Amazonian rivers.

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