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Ontogenetic and seasonal variation in the trophic niche of a threatened Amazonian freshwater stingray revealed by diet and stable isotope analyses

Paulo Arthur Abreu Trindade
pauloaatrindade@gmail.com
Universidade Federal do Pará - UFPA
Friedrich Wolfgang Keppeler
Universidade Federal do Pará - UFPA
Marcelo Ândrade
Universidade Federal do Pará - UFPA
Pedro Tarcio Souza Rocha
Universidade Federal do Pará - UFPA
Lucio Brabo
Universidade Federal do Ceará
Carson Jeffres
University of California, Davis
Tommaso Giarrizzo
Universidade Federal do Ceará

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Ontogenetic and seasonal variation in the trophic niche of a threatened Amazonian freshwater stingray revealed by diet and stable isotope analyses

Paulo Arthur de Abreu Trindade (0000-0002-3723-9117)[1*], Friedrich Wolfgang Keppeler (0000-0002-5165-1298)[1,2], Marcelo Ândrade (0000-0002-3573-5774)[1], Pedro Tarcio Souza Rocha (0000-0002-1141-9566)[1]; Lucio Brabo (0000-0001-9005-4798)[2], Carson Jeffres (0000-0001-6532-6851)[3], Tommaso Giarrizzo (0000-0002-5116-5206)[1,2]

[1] Núcleo de Ecologia Aquática e Pesca da Amazônia – NEAP, Universidade Federal do Pará - UFPA, Belém, Pará, Brazil.

[2] Instituto de Ciências do Mar (LABOMAR), Universidade Federal do Ceará (UFC), Fortaleza, Brazil.

[3] Center for Watershed Sciences, University of California, Davis, California, USA.

*Corresponding Author: Adress - Avenida Perimetral da Ciência, km 01, s/n, sala 05, 1° andar, Belém, Guamá, Pará, 66075-750 – Brazil. Email - pauloaatrindade@gmail.com

Abstract

Ontogenetic changes and seasonal variation in river flow influence trophic interactions in freshwater ecosystems, but their relative effects on tropical freshwater elasmobranchs remain poorly understood. We investigated how these factors affect the trophic ecology of the threatened freshwater stingray *Potamotrygon leopoldi*, endemic to the Xingu River (Amazon Basin), in a regulated river section influenced by the Belo Monte Hydroelectric Complex. We combined stomach content and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses to examine variation in diet composition, trophic niche breadth, prey size, trophic position, and resource assimilation according to body size, sex, and river discharge. *Potamotrygon leopoldi* had a broad trophic niche and an opportunistic diet, consisting mainly of mollusks, crustaceans, fish, and aquatic insects. Ontogeny was the main driver of trophic variation. Larger individuals consumed larger prey, had broader isotopic niches, and assimilated resources from a wider range of basal carbon pathways than juveniles. River discharge had a secondary effect, influencing prey diversity and resource assimilation, whereas differences between sexes were negligible. Stomach contents and stable isotopes revealed complementary aspects of recent prey consumption and longer-term resource assimilation. Overall, our results indicate that resource use changes throughout the life cycle, mainly in association with body size, while hydrological variation further influences trophic patterns in *Potamotrygon leopoldi*.

Keywords Neotropical. Amazon. Elasmobranch. Feeding Ecology. Habitat.

Introduction

Effective conservation of threatened species depends on a solid understanding of their basic ecology, particularly feeding biology and trophic interactions. Diet influences energy acquisition, population dynamics, and the role species play within food webs (Elton 1927; Mittelbach 1981). In predators, trophic interactions are especially important because they regulate prey populations and contribute to the organization and functioning of ecological communities (Romanuk et al. 2011). Despite their ecological importance, many threatened species remain poorly studied, limiting our ability to understand their ecological roles and predict how they may respond to ongoing environmental change.

Freshwater stingrays of the subfamily Potamotrygoninae are a distinctive, yet still poorly known, component of Neotropical freshwater ecosystems. As the only elasmobranch lineage fully adapted to freshwater environments (Fontenelle et al. 2021), they occupy high trophic positions and may contribute to linking energy pathways across aquatic food webs. However, many species are increasingly affected by habitat degradation, harvesting for the ornamental fish trade, and hydrological changes associated with river impoundment (Charvet et al. 2018). Among them, the white-spotted freshwater stingray *Potamotrygon leopoldi* Castex & Castello, 1970, is endemic to the Xingu River basin and is currently classified as Vulnerable by the IUCN Red List (Charvet et al. 2022). Its restricted distribution, relatively slow life history, and occurrence in a river system undergoing major hydrological alteration make this species of particular conservation concern and highlight the need for a better understanding of its ecological requirements.

The Xingu River is one of the largest clearwater tributaries of the Amazon and supports a highly endemic fish fauna shaped by marked seasonal fluctuations in river discharge (Zuluaga-Gómez et al. 2016). In recent years, part of the basin has experienced substantial changes in its natural flow regime associated with the operation of the Belo Monte Hydroelectric Complex (Ferreira et al. 2025). Because river flow influences habitat availability, prey distribution, and the exchange of energy across aquatic food webs, seasonal changes in discharge are expected to affect the feeding ecology of aquatic predators. Evaluating how *P. leopoldi* responds to these hydrological conditions is therefore important not only for understanding its trophic ecology but also for interpreting the ecological consequences of flow regulation in large tropical rivers.

Hydrological conditions are not the only source of trophic variation within predator populations (Vazquez and Lucifora 2026). Resource use also changes throughout individual development as body size increases and morphological, behavioral, and energetic constraints are progressively relaxed (Werner and Gilliam 1984). These ontogenetic changes often result in alterations in prey size, diet composition, trophic position, and niche breadth (Klecka and Boukal 2013). In contrast, trophic differences between males and females are generally less pronounced and, when present, are often associated with sexual dimorphism, reproductive investment, or habitat use. While these mechanisms are well documented for many freshwater predators, little is known about how they interact with seasonal hydrological variation to shape the feeding ecology of freshwater stingrays (Nakazawa et al. 2013).

Evaluating trophic ecology often requires combining complementary approaches because each provides information at different temporal scales. Stomach content analysis identifies recently consumed prey with high taxonomic resolution but reflects only a short period of feeding activity and may be affected by differential digestion rates (Araújo et al. 2007; Jung et al. 2015). Stable isotope analysis complements this information by integrating assimilated resources over longer time scales, with $\delta^{15}N$ providing an estimate of trophic position and $\delta^{13}C$ indicating the origin of basal carbon sources and habitat use (Post 2002; Fry 2006). Together, these approaches provide a more comprehensive view of feeding ecology and have become increasingly valuable for investigating trophic relationships in aquatic ecosystems, although they have rarely been applied jointly to freshwater stingrays (Layman et al. 2012; Márquez-Velásquez et al. 2019).

Despite the ecological and conservation importance of *P. leopoldi*, little is known about how individual development and seasonal variation in river flow influence its feeding ecology. In this study, we investigated how ontogeny and hydrological conditions shape trophic niche dynamics in this threatened freshwater stingray inhabiting a regulated stretch of the Xingu River. By combining stomach content and stable isotope analyses, we evaluated variation in diet composition, prey size, trophic position, niche breadth, and assimilated resources in relation to body size, sex, and river discharge. We expected larger individuals to consume larger prey and exhibit broader trophic niches than juveniles, reflecting ontogenetic changes in resource use. We also predicted that seasonal changes in river discharge would influence prey diversity and the contribution of assimilated resources by altering habitat availability and prey assemblages. Finally, because sexual dimorphism in *P. leopoldi* is modest relative to ontogenetic differences, we expected trophic differences between males and females to be limited.

Material and Methods

Study Area

The Xingu River is a tributary to the Amazon River and is approximately 2,300 km long (Camargo and Júnior 2008). Due to its underlying geology, it is described as a clear water river with low suspended material, high transparency and low autochthonous productivity (Lucas et al. 2021). The climate is typical of an equatorial rainforest and considered tropical, hot and humid, with an average annual temperature ranging from 17.5 to 24.5 °C, and average annual precipitation ranging from 2,066 mm to 2,379 mm (Camargo et al. 2015). The study was conducted in the Volta Grande do Xingu (**Fig. 1**), located in the Middle Xingu River, known for its natural beauty and diversity of flora and fauna, which was affected by the construction and operation of the Belo Monte Hydroelectric Complex (Pimental Dam upstream of the Volta Grande and Belo Monte Dam downstream) (Freitas et al. 2022). The main impacts include the homogenization and reduction of river flow in an approximately 100 km stretch of the river that encompasses the Volta Grande rapids (Keppeler et al. 2022), which is now known as the Reduced Flow Section (RFS). The ecological effects of this environmental perturbation are still not fully understood.

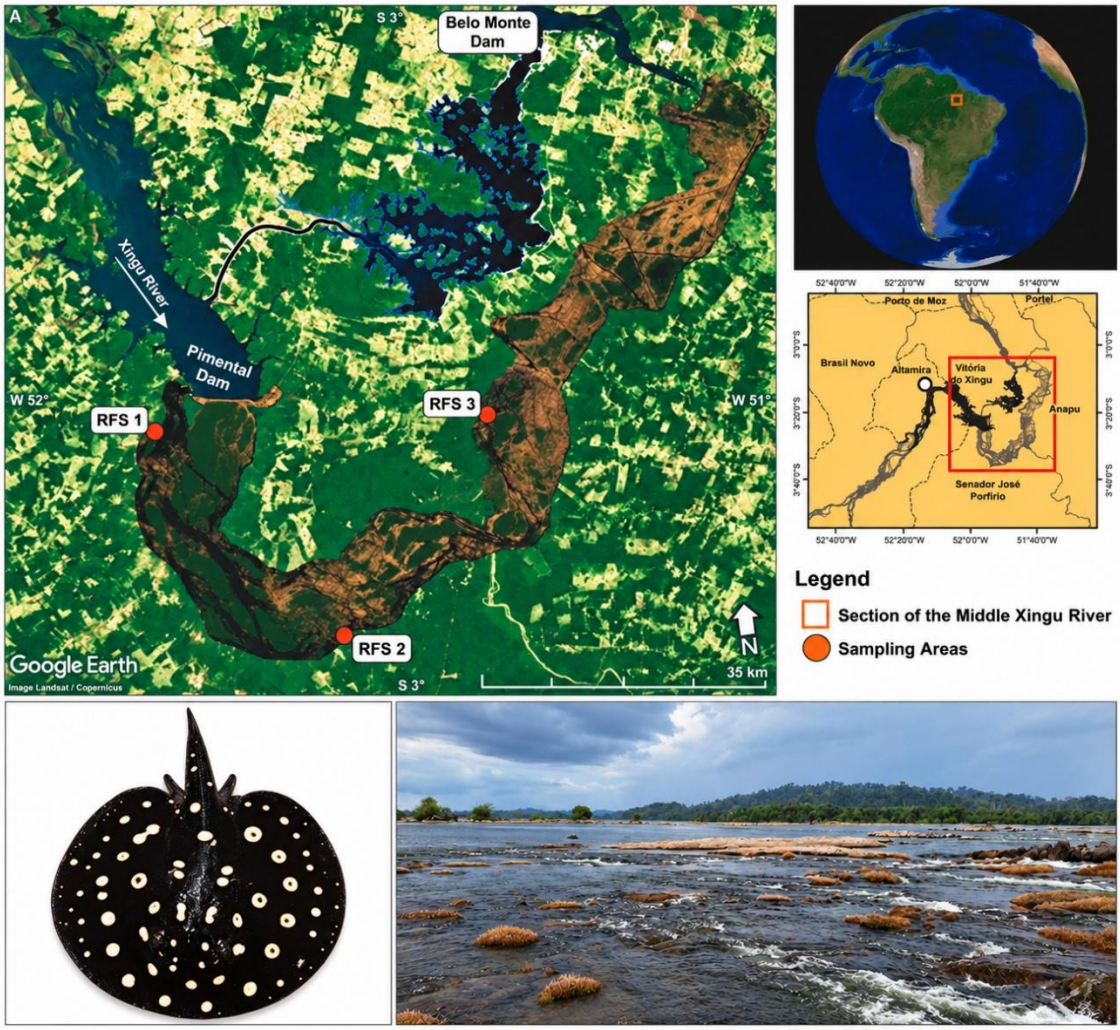


Fig. 1 Location of the Reduced Flow Section (RFS), Volta Grande do Xingu, (highlighted in orange) of the Middle Xingu River, Pará, Brazil, resulting from the construction of the Belo Monte dam (including the Pimental Dam). Orange circles indicate sampling areas where *Potamotrygon leopoldi* were collected. The images below are of the species *P. leopoldi* and its habitat of rapids

Fish Sampling

Individuals of *P. leopoldi* were captured in three sampling areas within the Reduced Flow Stretch (RFS 1, RFS 2, and RFS 3) during the flood (April) and dry (October) seasons, using longlines and cast nets between April 2020 and October 2022 (**Fig. 1**). All individuals were fixed in 10% formaldehyde and sent to the laboratory at the Federal University of Pará, where disc width (DW) and total weight (TW) were measured and sex was determined based on the presence or absence of claspers. Individuals were classified as juveniles or adults according to Charvet et al. (2018).

Specimens were collected under permit No. 02001.011114/2020-52, issued by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), as part of an ichthyofauna monitoring program associated with the Belo Monte Hydroelectric Plant. Considering the conservation

121 status of this endemic species, the sampling strategy aimed to minimize capture pressure on wild
122 populations, and the number of individuals collected for this study was deliberately limited.
123
124 *Tissue sampling and baseline sources*
125 Prior to fixation of the whole body in formaldehyde, stomachs and muscle tissue (~5 g) from the
126 ventral region of the body were taken from each specimen, labeled, stored in plastic bags and preserved on
127 ice for transport to the laboratory, where they were frozen at -20°C until processing. Additionally, we
128 sampled primary carbon sources (C3 plants, C4 plants, periphyton, phytoplankton) and potential prey
129 (freshwater sponges, crustaceans, fish, mollusk, zooplankton, terrestrial and aquatic insects) for stable
130 isotope analysis. Samples of riparian vegetation and aquatic macrophytes were collected manually, and
131 periphyton samples were scraped from rocks and submerged wood with tweezers. Phytoplankton and
132 zooplankton were collected by filtering the water through a 20 μm and 64 μm conical trawl net, respectively.
133 Mollusk, aquatic insects, crustaceans and freshwater sponges were sampled with a dip net and/or collected
134 by hand in shallow waters. Terrestrial insects were collected manually in the riparian forest. Small fish (e.g.
135 Loricariidae and Characidae) that can be eaten by stingrays were collected with gill nets, cast nets and dip
136 nets. Primary sources and potential prey samples were individually bagged and preserved on ice, returned
137 to the lab and froze at -20°C until processing.

138 *Stomach content analysis*
139 The stomachs were carefully removed, and their contents were placed in Petri dishes for analysis
140 under a stereomicroscope (Nikon SMZ800N) at magnification from 8× to 32×. Prey were identified to the
141 lowest possible taxonomic level using the specialized literature of Bicudo and Bicudo (1970) for algae,
142 Mugnai et al. (2010) for invertebrates and Camargo and Estupiñan (2011) for fish. After taxonomic
143 identification, the diet items were counted and weighed on an analytical balance (0.001 g). The contribution
144 of each prey to the diet of *P. leopoldi* was estimated to be using three main indices: prey specific abundance
145 (*Pi*), frequency of occurrence (*Fi*) and index of relative importance (*IRI*), *Pi* and *Fi* were calculated as
146 follows:

147 $$Pi = \frac{\sum Si}{\sum St}$$

148 $$Fi = \frac{Ni}{N}$$

149 Where *Si* is the weight of the stomach contents composed of prey *i*, *St* is the weight of the total
150 stomach contents of those predators that ingested prey *i*, *Ni* is the number of predators with prey *i* in their
151 stomach and *N* is the total number of predators analyzed.

152 The Index of Relative Importance (*IRI*) was modified and replaced by the Prey-Specific Index of
153 Relative Importance (*PSIRI*) to incorporate numerical (*%PNi*) and weight (*%PWi*) percentage contributions
154 as follows (see Brown et al. 2012):

155 $$\%PSIRI = \frac{\%FO_i \times (\%PN_i + \%PW_i)}{2}$$

156 For analysis of composition and feeding strategy, ingested items were classified into 7 general
157 categories: mollusk, detritus (items that could not be categorized were included as debris.), crustaceans,
158 plant material, fish, aquatic insects and terrestrial insects.

159 *Stable isotope analysis*

160 The muscle tissue samples and basal sources were washed in distilled water and dried in an oven
161 at 60ºC for 24 hours (Burgess and Bennett 2017). Subsequently, with the aid of a porcelain mortar and
162 pestle, the samples were macerated until they turned into a fine powder and placed in tin capsules and sent
163 for analysis. The encapsulated samples were analyzed at the UC Davis Stable Isotope Facility – University
164 of California, Davis to obtain Carbon and Nitrogen isotopic signatures. The natural isotopic ratios found in
165 the tissues of the individuals ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were expressed by the delta (δ) notation ($\delta^{13}C$ and $\delta^{15}N$,
166 respectively), in per mil (‰) of the international standards: PBD (Peedee Belemnite Limestone) for carbon
167 and atmospheric air for nitrogen. $\delta^{15}N$ is positively correlated with trophic level due to its natural
168 enrichment of 2-3‰ during assimilation of ingested material into consumer tissue (Peterson and Fry 1987;
169 Post 2002). $\delta^{13}C$ varies among primary producers and detrital pathways at the base of food chains and
170 largely reflects differences in photosynthetic pathways (C3, C4, CAM) as well as inorganic carbon sources
171 assimilated by plants (Peterson and Fry 1987). Consequently, variation in $\delta^{13}C$ and $\delta^{15}N$ of animals has
172 been proposed as an indicator of trophic niche differences (Layman et al. 2012).

173 The carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic signature values of the species and sources were
174 calculated using the equation: $\delta X = [R_{sample}/R_{standard} - 1] \times 1000‰$. Where X is ^{13}C and ^{15}N and R is the
175 isotopic ratio $^{13}C/^{12}C$ and $^{15}N/^{14}N$ (Peterson and Fry 1987). Before the analysis, in the case of samples with
176 lipid concentrations greater than 5% (C:N > 3.5 for aquatic animals), a lipid correction was performed for
177 the data set using the mathematical expression proposed by Post et al. (2007).

178 *River flow data*

179 River flow data for each sampling date was obtained from the ONS website
180 (https://www.ons.org.br/). The flow corresponds to the amount of water released by the Pimental dam
181 (powerhouse outflow + spilled flow + flow from other structures), which regulates the flow of the Xingu
182 River in the RFS.

183 *Rarefaction and extrapolation analysis*

184 To determine whether the number of stomachs analyzed accurately described the diet of *P.*
185 *leopoldi*, rarefaction and extrapolation (R/E) sampling curves were created, based on sample size for species
186 richness that can be rarefied to sample sizes smaller or extrapolated to a larger sample size, guided by an
187 estimated asymptotic species richness (Colwell et al. 2012). Incidence data was used, with the main
188 measures of species diversity: species richness (q = 0), Shannon diversity (q = 1) and Simpson diversity (q
189 = 2) (Hsieh and Chao 2016). Prey accumulation curves were created considering hydrological periods
190 (flood and drought), ontogenetic groups (juvenile and adult), and sex (female and male).

191 *Diet composition and feeding strategy*

192 To explore variation in the composition of prey ingested by *P. leopoldi*, a Bray-Curtis Distance-
193 based Redundancy Analysis (db-RDA) was conducted. As predictor variables, we used river flow, body
194 size, and sex. The significance of the terms was calculated using an ANOVA-like permutation test (n=999)
195 (Legendre and Legendre 2012). We describe the importance of prey items in the stomach content using the
196 graphical method proposed by Costello (1990) and modified by Amundsen et al. (1996) (Fig. S2,
197 Supplementary Material). This analysis uses prey specific abundance (*Pi*) and frequency of occurrence (*Fi*)
198 indices to describe diet patterns, particularly regarding specialization and generalism (Costello, 1990;
199 Amundsen et al. 1996).

200 *Diversity and size of ingested prey*

201 Linear regression was used to investigate how the diversity of prey ingested by *P. leopoldi* varies
202 according to body size, river flow, and sex. In this case, prey diversity was calculated using the Simpson
203 index, which gives less weight to rare species than the Shannon index and richness itself. Then, a mixed
204 model was used to investigate variation in prey body size. The response variable was log-transformed (prey
205 weight), while the predictors were *P. leopoldi* body size, sex, and river flow. We consider the identity of

206 the individual as a random effect in this model because we consider that prey within the same stomach
207 cannot be considered as independent sampling units.

208 *Isotopic niche*

209 To explore how the isotopic niche defined by $\delta^{13}C$ and $\delta^{15}N$ varies between juveniles and adults,
210 we used standardized ellipse areas (SEA) using Bayesian inference that was proposed by Jackson et al.
211 (2011). The Bayesian approach generates a distribution of values (posteriori) that reflects the uncertainty
212 associated with the sampling process and that can affect the size, location and shape of the ellipse. This
213 uncertainty is greater for smaller sample sets. The difference between the SEAs of the two ontogenetic
214 groups was calculated statistically using the posterior distribution that was generated (Jackson et al. 2011).
215 The correction suggested by Jackson et al. (2011) standard ellipse area estimates (SEAc) were applied to
216 eliminate any potential bias caused by different sample sizes. Linear regressions were also conducted to
217 explore how body size, river flow, and sex (predictors) influence $\delta^{13}C$ and $\delta^{15}N$ signatures of *P. leopoldi*.

218 *Trophic links probabilities*

219 Diet shifts and resource assimilation were assessed using the Bayesian hierarchical EcoDiet
220 framework (Hernvann et al. 2022), which integrates stomach content and stable isotope data with prior
221 information. The model represented the food web exploited by *P. leopoldi*, including major prey groups
222 (sponges, mollusks, crustaceans, fish, aquatic and terrestrial insects) and basal resources (zooplankton,
223 periphyton, and detritus). Given ontogenetic dietary differences, separate models were fitted for adults and
224 juveniles.

225 Priors consisted of a binary matrix describing trophic links among taxa and a diet proportion
226 matrix, informed by literature on the system and related species and by expert knowledge (Almeida et al.
227 2008; Zuluaga-Gómez et al. 2016; Shibuya et al. 2016; Márquez-Velásquez et al. 2019; Pagliarini et al.
228 2020; Shibuya 2022). Model inputs included $\delta^{15}N$ and $\delta^{13}C$ values from muscle tissue and stomach content
229 data collected in the Xingu River. As stomach content data were available only for *P. leopoldi*, trophic links
230 for other taxa relied primarily on isotope data and literature information.

231 Trophic discrimination factors were set to 3.4‰ for $\delta^{15}N$ and 0.8‰ for $\delta^{13}C$ (Vander Zanden and
232 Rasmussen 2001). Models were fitted using Markov chain Monte Carlo simulations (100,000 iterations;
233 50,000 burn-in), and convergence was confirmed using the Gelman–Rubin statistic (< 1.1; Gelman and
234 Rubin 1992).

Software and packages

All analysis was conducted in R (R Core Team 2026). Rarefaction analyses were carried out in the iNEXT package (Hsieh and Chao 2016), db-RDA and permutation analysis in the vegan package (Oksanen et al. 2022), the Hierarchical Bayesian Models in the EcoDiet package (Hernvann et al. 2022), standardized ellipses areas in the SIBER package (Jackson and Parnell 2023), and mixed models in the lme4 package (Bates et al. 2015).

Results

Overall, 37 specimens of *P. leopoldi* were captured, the disc width ranged from 32.0 to 67.0 cm ($\bar{x} = 49.1 \pm 10.1$ cm), and the total weight ranged from 1,800 to 15,000 g ($\bar{x} = 7{,}386 \pm 4{,}039$ g). Of these, 26 individuals were females (DW = 32.0 – 67.0 cm; $\bar{x} = 51.5 \pm 10.8$) and 11 were males (DW = 34.0 – 49.6 cm; $\bar{x} = 43.3 \pm 5.1$ cm). Stomach contents were analyzed for all individuals, while stable isotope analysis was conducted on 34 of them.

Rarefaction and extrapolation curve

Although richness estimators did not reach asymptotic values, the accumulation curves demonstrated that during the dry period there was greater diversity of food items (**Fig. 2a**). Among the ontogenetic groups, juveniles had slightly higher prey item diversity (**Fig. 2b**), while between females and males there was an overlap in accumulation curves (**Fig. 2c**). For richness and Shannon diversity indices, asymptotes were not reached, indicating that additional stomach samples would likely reveal a greater number of prey items.

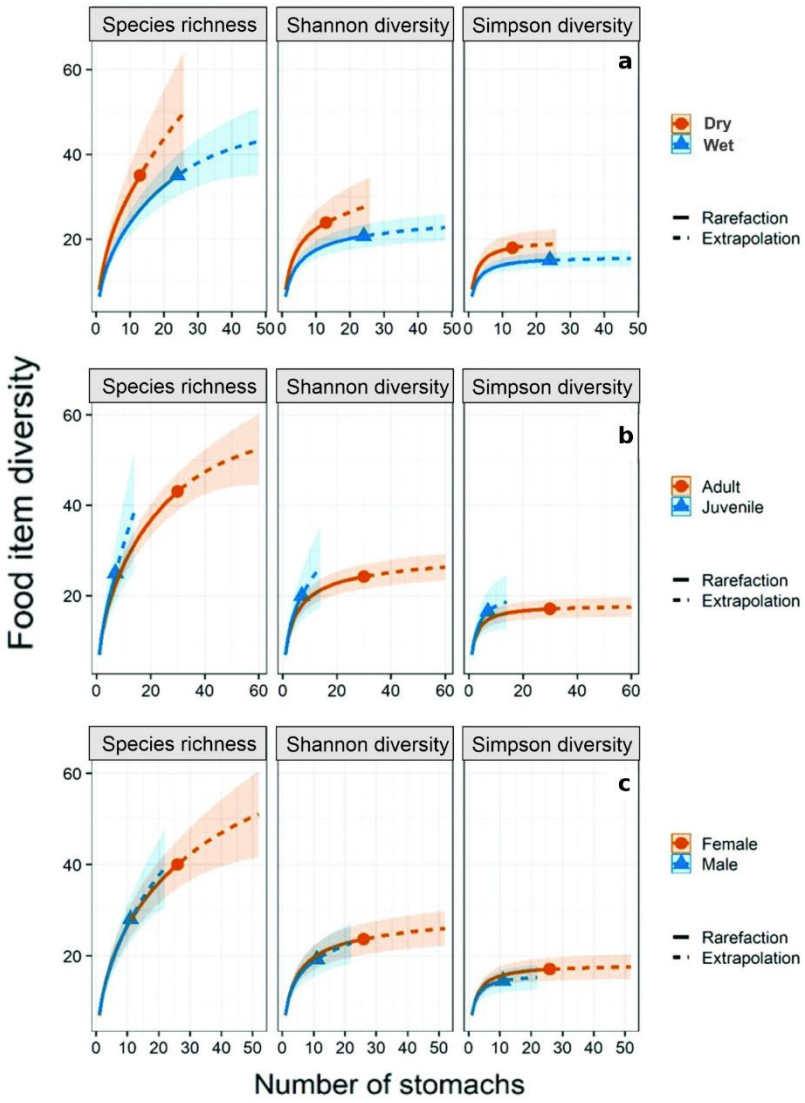


Fig. 2 Accumulation curves of the diversity of food items in relation to the number of stomachs collected from *Potamotrygon leopoldi* between: (**a**) hydrological periods (flood and drought), (**b**) ontogenetic phases (juvenile and adult) and (**c**) sex (female and male). Solid lines indicate rarefaction data, while dotted lines indicate extrapolation

Diet composition

A total of 7,853 items were recorded in the stomach contents of *P. leopoldi*. The group that represented the greatest relative importance was mollusk (*PSIRI* = 35.59%), especially, *Doryssa millepunctata* (PSIRI = 11.73%) and Gastropoda remains (PSIRI = 22.29%); followed by detritus (*PSIRI* = 16.94%), crustaceans (*PSIRI* = 9.29%), plant material (*PSIRI* = 7.80%), fish (*PSIRI* = 6.08%), aquatic insects (*PSIRI* = 3.78%) and terrestrial insects (*PSIRI* = 1.40%) (**Fig. 3; Table 1 – see Supplementary Material.pdf**).

The species *P. leopoldi* demonstrated a high dominance of mollusk in its diet, as well as a high frequency of occurrence and abundance of fish, detritus and crustaceans. Aquatic plants and insects had a frequency of occurrence above 50%, while terrestrial insects were the least abundant and frequent in food (**Fig. 3 and 4** – **see Supplementary Material.pdf**). db-RDA explained 21% of the variation in food items consumed. The water flow rate ($F_{(1,33)}$ = 3.36, P = 0.003) and body size ($F_{(1,33)}$ = 3.45, P = 0.003) of *P. leopoldi* had a significant effect on the distribution of ingested food prey. The water flow was positively associated with detritus and negatively associated with other prey (**Fig. 5**). On the other hand, the body size of *P. leopoldi* was negatively associated with fish consumption. There was no significant effect of sex on prey ingested ($F_{(1,33)}$ = 0.051, P = 0.101).

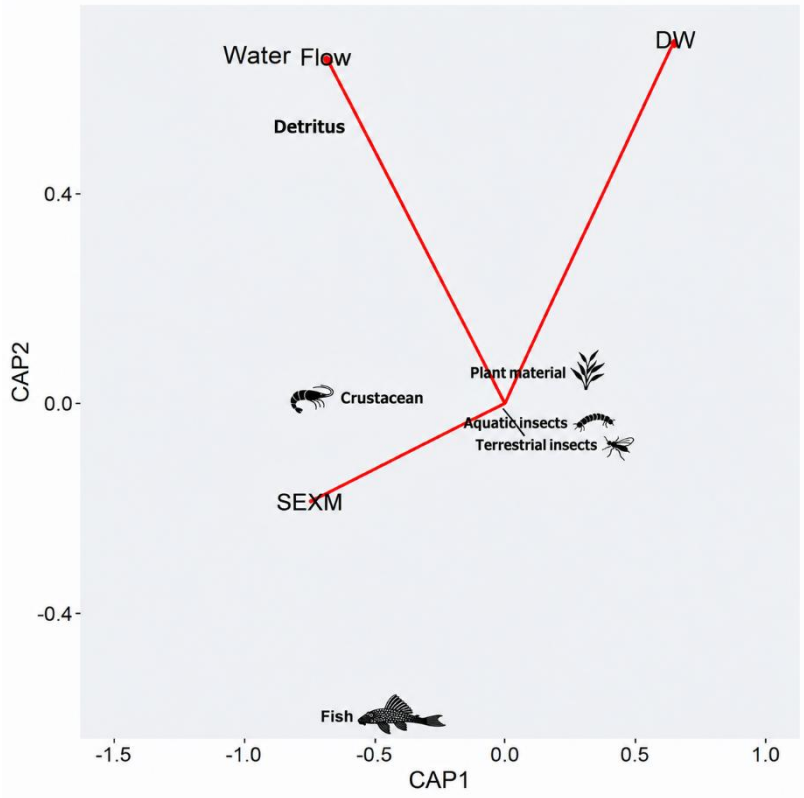


Fig. 5 Distance-based redundancy analysis (db-RDA) demonstrates the factors that are influencing the distribution of the main food groups of *P. leopoldi*. DW (Disc Width), SEXM (male rays)

Diversity and size of ingested prey

The model associated with prey diversity explained around 20% of the data. Disc width positively affected prey diversity (t = 1.949; p = 0.049) (**Fig. 6a**). On the other hand, water flow was negatively associated with prey diversity (t = -2.132; p = 0.040; **Fig. 6b**). There was no significant variation with sex (t = 0.717; p = 0.479).

The model associated with prey size explained only 5% of the data. The body size of *P. leopoldi* was positively associated with the size of the food item (t = 3.354; p < 0.001; **Fig. 6c**). No significant effect was observed associated with water flow (t = 0.267; p = 0.789) and sex (t = 0.385, p = 0.789).

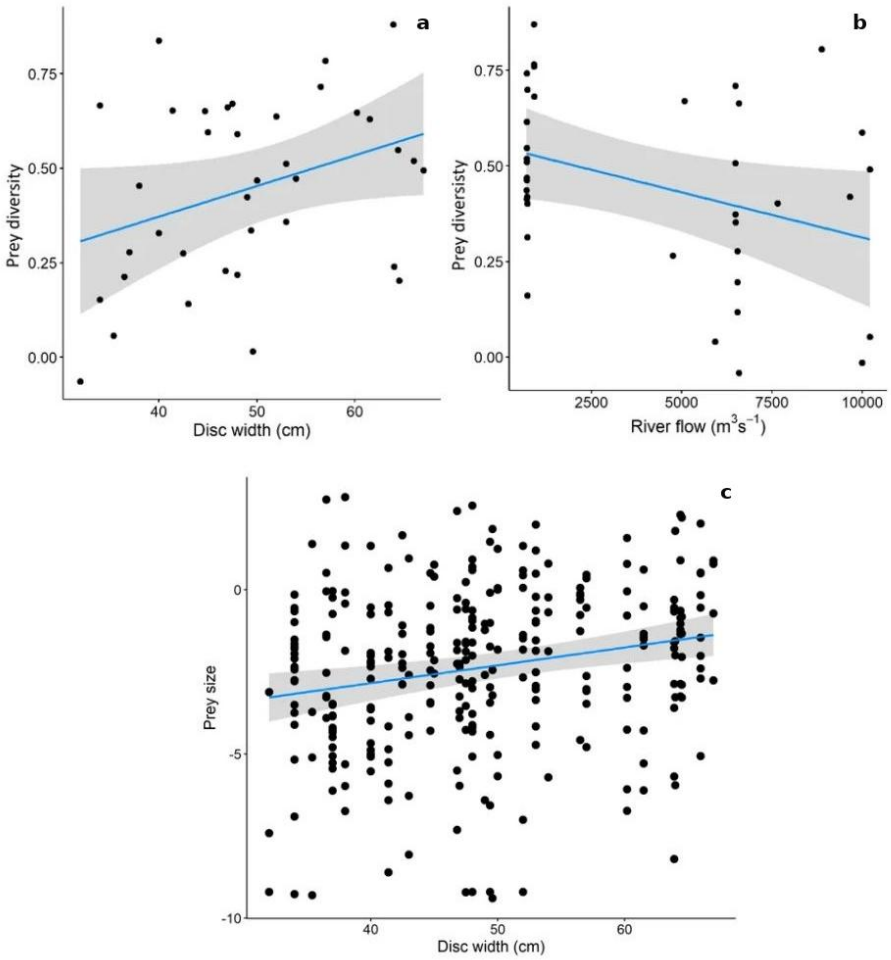
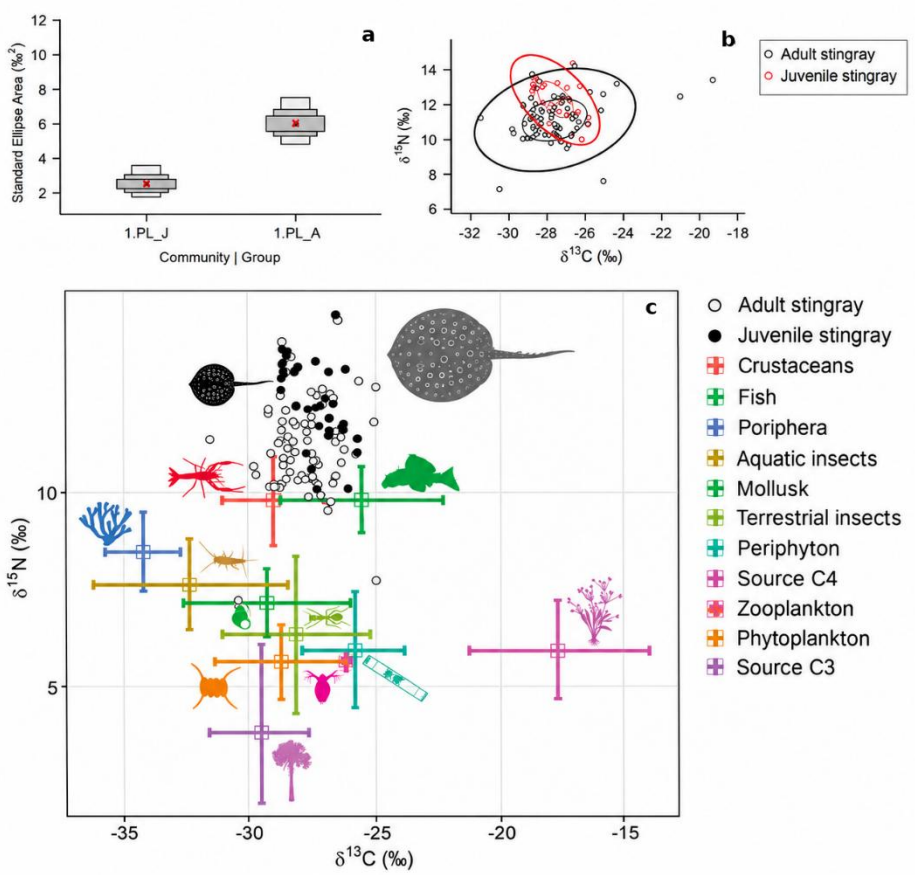


Fig. 6 Association of prey diversity of *P. leopoldi* with body size (Disc Width) (**a**) and River flow (**b**). Association of prey size in relation to Disc Width (cm) (**c**)

Isotopic niche

The isotopic niche breadth was smaller for juvenile than adult *P. leopoldi* (**Fig. 7a and 7b**). Overall, *P. leopoldi* had higher $\delta^{15}N$ than basal sources, invertebrates, and other smaller fishes. *P. leopoldi*, as well as all the other consumers analyzed, had isotope signatures more similar to phytoplankton and riparian vegetation (**Fig. 7c**).



297
298 **Fig. 7a and 7b** *P. leopoldi* (PL) isotopic niche space between ontogenetic phases (PL – Juvenile = *P.*
299 *leopoldi* Juvenile and PL - Adult = *P. leopoldi* Adult) presented as Bayesian ellipses. **7c**. Biplot showing δ
300 ^{13}C and δ ^{15}N isotopic signatures of basal resources and primary consumers in relation to *P. leopoldi*. The
301 mean source signatures are represented by the central squares with their respective standard deviations
302 (solid line)

303

304 Linear regressions (**Fig. 8**) explained about 42% of the variation in $\delta^{13}C$ and 46% of the variation
305 in $\delta^{15}N$. Lower $\delta^{13}C$ values were observed during the period of lower river flow ($t = 3.129$; $p = 0.002$).
306 Body size was positively associated with $\delta^{13}C$, but the effect was only marginally significant ($t = 1.771$; p
307 $= 0.0826$). While we did not observe a significant association between sex and $\delta^{13}C$ ($t = 1.409$; $p = 0.164$).
308 $\delta^{15}N$ was negatively associated with body size ($t = -4.970$; $p < 0.001$). River flow ($t = -1.506$; $p = 0.138$)
309 and sex ($t = 0.859$; $p = 0.394$) were not significant associated with $\delta^{15}N$ values.

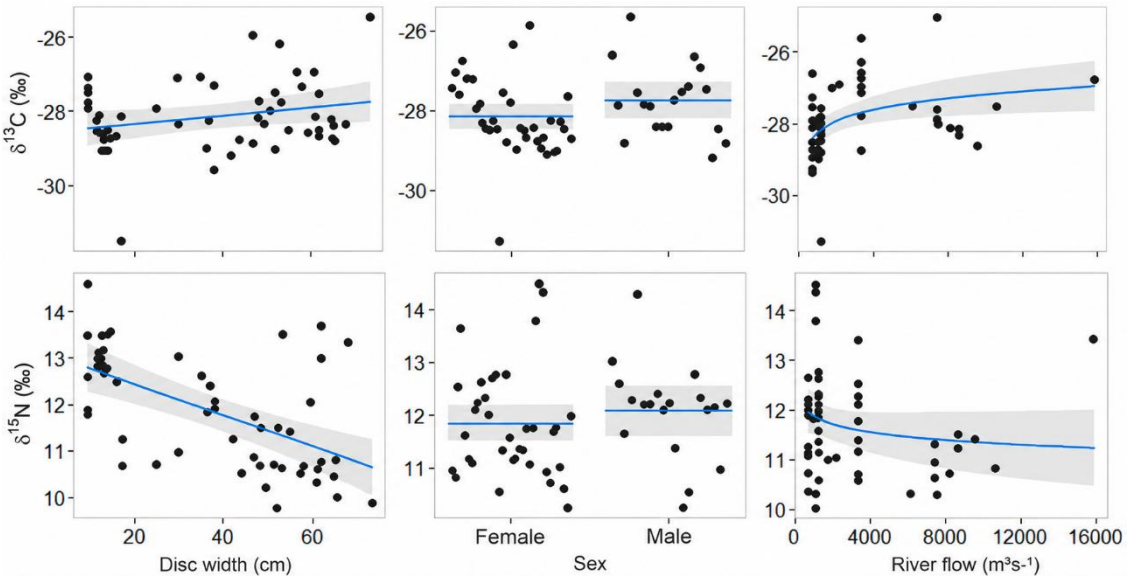


Fig. 8 Variation of isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of *Potamotrygon leopoldi* in relation to Disc Width (DW), River flow and sex, in the Reduced Flow Section (RFS) of the Middle Xingu River, Pará, Brazil

Trophic links probabilities

The EcoDiet model indicated that mollusks were the most assimilated prey, followed by crustaceans, aquatic insects, fish, terrestrial insects and zooplankton (**Fig. 9**). When analyzed by ontogenetic group, it was found that juvenile stingrays have fish, mollusks, aquatic and terrestrial insects, crustaceans and zooplankton as their main food sources, while adults have mollusks as their main food resource (**Fig. 9**).

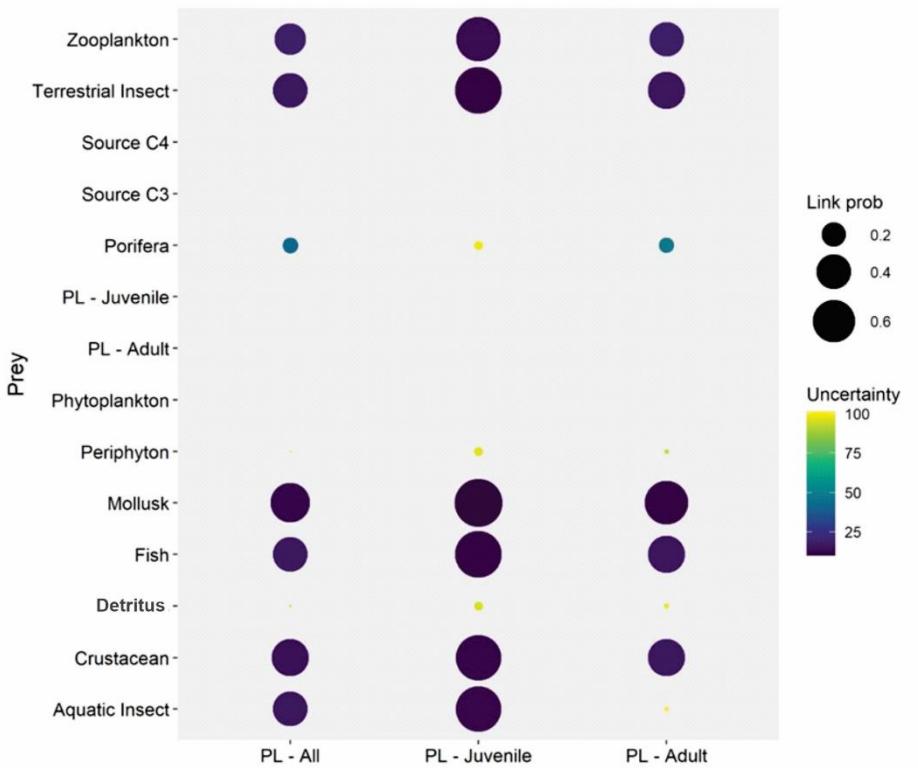


Fig. 9 Trophic links probabilities of *Potamotrygon leopoldi* (PL) according to the EcoDiet model. Results are presented for each ontogenetic phase (PL - Juvenile and PL - Adult) and for both phases combined (PL - All)

Discussion

Trophic niche breadth and diet composition

The combined stomach content and stable isotope analyses indicate that trophic variation in *P. leopoldi* is driven primarily by ontogenetic changes, whereas seasonal differences in river discharge represent a secondary source of variation. As individuals grow, they exploit a broader spectrum of prey and assimilate resources derived from multiple basal pathways, resulting in progressive expansion of their trophic niche. In contrast, hydrological conditions mainly influenced prey diversity and the relative contribution of assimilated resources, indicating that seasonal changes in river flow modify feeding opportunities without overriding ontogenetic patterns.

Broader trophic niches in adult stingrays likely reflect the relaxation of mechanical constraints associated with growth. As jaw strength and tooth mineralization increase, individuals become capable of handling a wider range of hard-bodied prey, particularly mollusks and crustaceans, thereby expanding their dietary breadth (Kolmann et al. 2016). Such flexibility is likely advantageous in dynamic river systems, where prey availability fluctuates seasonally. In the Xingu River, this capacity to exploit multiple food

resources may help buffer seasonal changes in habitat availability, especially within the regulated flow environment of the Reduced Flow Stretch.

The relatively small sample size (n = 37) should be considered when interpreting dietary richness and diversity, as it may have constrained the detection of less frequently consumed prey items. Although *P. leopoldi* was the most frequently captured stingray species during our sampling, the total number of individuals obtained over the two-year sampling period remained relatively low. Furthermore, the number of individuals collected for this study was deliberately limited to minimize capture pressure on wild populations, given the species' Vulnerable conservation status. This context is particularly relevant because the accumulation curves did not reach asymptotic values, suggesting that additional sampling could reveal further dietary items. Nevertheless, the integration of stomach content and stable isotope analyses provided complementary insights into the trophic ecology of *P. leopoldi*.

Effect of body size and ontogeny on diet

Ontogenetic changes in feeding ecology emerged as the primary source of trophic variation in *P. leopoldi*, indicating that individual development progressively reshapes resource use throughout the life cycle. As body size increased, juveniles shifted from a diet containing proportionally more fish to one dominated by mollusks and crustaceans. This transition is consistent with the progressive relaxation of morphofunctional constraints during growth (Summers et al. 2004). Juveniles possess less mineralized dentition and reduced mandibular strength, limiting their ability to process hard-bodied prey such as mollusks (Rutledge et al., 2019). As these structures develop, individuals become capable of exploiting mechanically resistant prey, a pattern widely documented among durophagous chondrichthyans (Edmonds et al. 2001; Kolmann and Huber 2009; Mara et al. 2010).

Stable isotope data provide an independent line of evidence supporting this ontogenetic transition. Adults occupied broader isotopic niches, suggesting the assimilation of resources derived from multiple basal carbon pathways, whereas juveniles showed narrower isotopic niches and relatively higher trophic positions. The negative relationship between disc width and $\delta^{15}N$ further indicates that smaller individuals rely proportionally more on fish and crustaceans, while larger stingrays increasingly exploit mollusks. Together, stomach content and isotope data reveal a coherent ontogenetic shift in both prey consumption and energy assimilation.

Ontogenetic niche shifts have been widely recognized as mechanisms that promote intraspecific trophic diversification and influence energy flow within aquatic food webs (Rosende-Pereiro et al. 2020). By altering prey selection, trophic position, and resource assimilation throughout development, individuals interact with different components of the food web as they grow. Our results extend this general ecological pattern to an endemic Amazonian freshwater stingray, reinforcing the importance of intraspecific variation as a mechanism structuring trophic interactions in tropical river ecosystems.

Effect of sex on diet

Sex had little influence on the feeding ecology of *P. leopoldi*. Stomach content and stable isotope analyses consistently showed extensive overlap in diet composition, trophic niche, and assimilated resources between males and females, indicating that both sexes exploit similar prey and habitats. These results suggest that trophic variation within the species is driven primarily by ontogenetic development rather than by sexual differentiation.

The absence of trophic segregation is consistent with the relatively modest sexual dimorphism observed in *P. leopoldi*, in which differences between males and females are associated mainly with reproductive structures rather than feeding morphology. Under these conditions, body size appears to exert a stronger influence on prey selection than sex itself. Similar patterns have been reported for other freshwater stingrays, where males and females occupy comparable trophic positions and exhibit broadly similar diets (Márquez-Velásquez et al. 2019). Together, these findings suggest that trophic partitioning is unlikely to represent an important mechanism for reducing intraspecific competition between sexes in this species.

Effect of river discharge and the flood pulse on diet

Seasonal variation in river discharge influenced feeding patterns in *P. leopoldi*, although its effects were secondary to those associated with ontogenetic development. Both stomach content and stable isotope analyses indicated greater dietary diversity during the dry season, when river discharge is lower and rocky substrates become more exposed. Because *P. leopoldi* is closely associated with rapids and rocky habitats, seasonal fluctuations in water level likely modify access to preferred foraging areas rather than directly affecting feeding behaviors.

One plausible explanation is that low-water conditions increase the availability of benthic habitats supporting mollusks, crustaceans, and aquatic insects, which constitute the principal prey of *P.*

leopoldi. During periods of higher discharge, these habitats become partially submerged or less accessible, potentially reducing prey availability and leading to a narrower spectrum of exploited resources (Goulding 1980; Gerking 1994). Rather than indicating reduced feeding activity, this pattern suggests that seasonal hydrology modifies the spatial distribution and accessibility of prey resources.

Stable isotope data provide complementary evidence for this interpretation. Seasonal shifts in $\delta^{13}C$ values indicate changes in the relative contribution of basal carbon sources, whereas variation in $\delta^{15}N$ suggests corresponding changes in energy pathways supporting the diet. Together, these results indicate that river discharge influences not only the prey consumed but also the basal resources sustaining food webs available to this species. Similar responses have been reported for other Potamotrygoninae, in which seasonal changes in prey availability associated with the Amazonian flood pulse strongly influence feeding ecology (Shibuya 2022).

Final considerations and ecological implications

Our findings indicate that trophic variation in *P. leopoldi* is structured primarily by ontogenetic development, whereas seasonal variation in river discharge acts mainly by modulating resource availability. As individuals grow, changes in morphology expand the range of prey they can exploit, leading to shifts in diet composition, trophic position, and the assimilation of energy from different basal pathways. Seasonal changes in river flow, in turn, influence access to preferred habitats and alter the diversity of resources available without overriding these ontogenetic patterns.

Together, stomach content and stable isotope analyses provide complementary evidence that individual development and hydrological conditions operate at different ecological scales. Ontogeny determines how resources are exploited throughout the life cycle, whereas seasonal variation modifies the environmental context in which these feeding strategies are expressed. This hierarchical relationship helps explain how feeding ecology is maintained in a highly dynamic tropical river while remaining responsive to seasonal fluctuations in habitat availability.

Although our study focuses on an endemic Amazonian freshwater stingray, the ecological processes identified here are likely relevant to other benthic predators inhabiting flood-pulse rivers. By demonstrating that trophic organization emerges from the interaction between life-history traits and seasonal hydrology, our results contribute to a broader understanding of how intraspecific variation shapes food-web dynamics in tropical freshwater ecosystems.

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Author contributions

Conceptualization: Tommaso Giarrizzo and Paulo Arthur de Abreu Trindade; Methodology: Paulo Arthur de Abreu Trindade and Lucio Brabo; Formal analysis and investigation: Friedrich Wolfgang Keppeler and Paulo Arthur de Abreu Trindade; Writing - original draft preparation: Paulo Arthur de Abreu Trindade; Writing - review and editing: Marcelo Ândrade, Pedro Tarcio Souza Rocha, Lucio Brabo and Carson Jeffres; Supervision and Project administration: Tommaso Giarrizzo.

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Conflict of Interest

The authors declare that they have no competing interests.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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