

1 **Trophic plasticity of omnivorous fishes in natural and human-dominated**  
2 **landscapes**

3 *Mayara Pereira Neves*<sup>1,2,5,\*</sup>, *Rosilene Luciana Delariva*<sup>3</sup>, *Daniel M. Perkins*<sup>4</sup>, *Clarice*  
4 *Bernhardt Fialho*<sup>2</sup>, *Pavel Kratina*<sup>5,\*</sup>

5 **Affiliations:**

6 <sup>1</sup> *Department of Biosciences, Rice University, Houston, TX, United States.* [mayara-](mailto:mayara-nevesbio@hotmail.com)  
7 [nevesbio@hotmail.com](mailto:nevesbio@hotmail.com), ORCID: <https://orcid.org/0000-0003-2523-3874>

8 <sup>2</sup> *Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia, Instituto*  
9 *de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 90650-001,*  
10 *Brazil.* [clarice.fialho@ufrgs.br](mailto:clarice.fialho@ufrgs.br), ORCID: <https://orcid.org/0000-0001-7908-5534>

11 <sup>3</sup> *Programa de Pós-Graduação em Conservação e Manejo de Recursos Naturais, Centro de*  
12 *Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná, Cascavel, PR,*  
13 *85819-110, Brazil.* [rosilene.delariva@unioeste.br](mailto:rosilene.delariva@unioeste.br), ORCID: [https://orcid.org/0000-0002-](https://orcid.org/0000-0002-6489-2437)  
14 [6489-2437](https://orcid.org/0000-0002-6489-2437)

15 <sup>4</sup> *School of Life and Health Sciences, Whitelands College, University of Roehampton,*  
16 *London, SW15 4JD, United Kingdom.* [daniel.perkins@roehampton.ac.uk](mailto:daniel.perkins@roehampton.ac.uk), ORCID:  
17 <https://orcid.org/0000-0003-0866-4816>

18 <sup>5</sup> *School of Biological and Behavioural Sciences, Queen Mary University of London,*  
19 *London, E1 4NS, United Kingdom.* [p.kratina@qmul.ac.uk](mailto:p.kratina@qmul.ac.uk), ORCID: [https://orcid.org/0000-](https://orcid.org/0000-0002-9144-7937)  
20 [0002-9144-7937](https://orcid.org/0000-0002-9144-7937)

21

22 **\*Corresponding authors:**

23 Mayara P. Neves, [mayara-nevesbio@hotmail.com](mailto:mayara-nevesbio@hotmail.com)

24 Pavel **Kratina**, [p.kratina@qmul.ac.uk](mailto:p.kratina@qmul.ac.uk)

25

26 **Running head:** The role of trophic plasticity in Anthropocene

27 **Keywords:** Agriculture, land-use changes, tropical stream ecosystems, stable isotopes,  
28 trophic niches, urbanization.

29 **Author contribution statement**

30 MPN, RLD and CBF designed study; MPN and RLD conducted fieldwork; MPN identified  
31 the specimens, conducted laboratory and statistical analyses; MPN, PK and DMP  
32 interpreted the data; MPN and PK wrote the manuscript; all authors edited the manuscript.

33 **Data availability statement**

34 We will archive the data and code in the DRYAD repository once the article is accepted.

35 **Scientific significance statement**

36 Omnivory plays a critical role in species coexistence and stability of ecological  
37 communities and ecosystems. Yet, we know very little about how trophic plasticity of  
38 animals facilitates species coexistence in the context of intensive land use changes. We  
39 combined a suite of diet tracing methods and surveys of basal resources, invertebrate prey,  
40 and vertebrate top predators in natural, agricultural, and urban streams, to show how  
41 ecological communities are restructured through trophic plasticity of dominant fish species.

42 Our findings do not only advance evolutionary-ecology conceptually, but also have strong  
43 implications for the conservation of stream biodiversity facing intensive land-use changes.

44

45 **Abstract:** The persistence of diverse communities and functioning ecosystems under  
46 increasing anthropogenic pressure relies on food web rewiring and the ability of animals to  
47 expand or change their diet in disturbed ecosystems. We combined a suite of diet tracing  
48 techniques to study trophic plasticity in omnivorous fishes, eco-morphologically similar  
49 species with high competition potential, across different human land-uses in subtropical  
50 streams. We found that the proportion of native forest cover, associated with intensive land-  
51 use, altered the isotopic composition of fishes, which were more enriched in  $^{13}\text{C}$ , without  
52 affecting the carbon isotope ratios of their prey and basal resources. There was also  
53 evidence for a non-linear effect of native forest cover on the  $\delta^{15}\text{N}$  values of basal resources,  
54 macroinvertebrates, and omnivorous fishes, indicating that nutrient pollution from  
55 agriculture propagated through stream food webs. The most widely distributed fish species  
56 shifted their diet from autochthonous resources to terrestrial invertebrates and sedimentary  
57 organic matter in disturbed streams. Moreover, the isotopic niche of this fish species was  
58 broader in streams with higher fish species richness, indicating combined impacts of  
59 environmental change and competition on species coexistence. Therefore, our findings  
60 showed that the dominance and trophic niche breadth of dominant omnivores depend not  
61 only on the availability of resources but also on the interactions with their putative  
62 competitors.

63

## 64 **Introduction**

65 Pervasive land use change is among the primary causes of biodiversity loss and food web  
66 simplification, altering ecosystem functioning worldwide (Etard et al. 2022; Kemp et al.  
67 2023; Moi et al. 2023). The maintenance of healthy functioning ecosystems and high  
68 species diversity under increasing human pressure relies on food web rewiring and the  
69 ability of animals to shift their diet in disturbed ecosystems with scarce resources (Kemp et  
70 al. 2023; Lu et al. 2016). In this context, omnivores play a functional role in enhancing food  
71 web stability as adaptive foraging allows them to adjust their diet (Kratina et al. 2012),  
72 facilitating their persistence in the face of human-induced disturbances (Carvalho et al.  
73 2019). However, the ecological mechanisms underlying this trophic plasticity are not fully  
74 understood.

75 Omnivores have a broad diet and the ability to feed on resources from different  
76 trophic positions (Ingram et al. 2012; Gutgesell et al. 2022). Tropical environments contain  
77 a high diversity and abundance of omnivorous species (Fricke et al. 2023), where  
78 generalism and opportunism are common strategies (Garcia et al. 2017; Costa-Pereira et al.  
79 2017; Neves et al. 2021). For instance, common omnivorous tetra fishes exploit different  
80 aquatic microhabitats and consume many types of animal and plant resources (Delariva and  
81 Neves 2020), allowing them to respond to seasonal variability in resource availability  
82 (Costa-Pereira et al. 2017; Neves et al. 2021; Gutgesell et al. 2022). Due to their trophic  
83 plasticity, omnivores are generally resilient to anthropogenic stressors and many  
84 omnivorous species even proliferate in highly degraded environments (Larentis et al. 2022).

85 Changes in environmental characteristics modify the diversity and availability of  
86 food resources for fishes. Human disturbance, such as deforestation, can reduce

87 allochthonous inputs, causing habitat homogenization, and increased consumer reliance on  
88 autochthonous carbon sources (Doi 2009). Such habitat homogenization can, in turn, reduce  
89 trophic niche breadth of generalist animals as predicted by MacArthur's habitat  
90 heterogeneity hypothesis (MacArthur 1972). However, there is currently no consensus on  
91 how human disturbances affect the trophic niches of omnivores, even across studies that  
92 use a consistent analytical approach. There is some empirical evidence that the trophic  
93 niches of mammals and birds exhibit a variable response to modified landscapes (Magioli  
94 et al. 2019). Similarly, the trophic niches of some neotropical birds became narrower in  
95 human-modified landscapes, but not for all trophic guilds (Navarro et al. 2021). In New  
96 Zealand, streams with high sedimentation and human disturbances were associated with  
97 compression of fish trophic niches (Burdon et al. 2019). By contrast, nitrogen pollution  
98 appears to expand the trophic niches of Brazilian fishes (Carvalho et al. 2019). Finally,  
99 trophic niche breadths of macroinvertebrates in Croatian streams (Price et al. 2019) or fish  
100 meso-predators in Sabah, Malaysian Borneo (Wilkinson et al. 2021) were not influenced by  
101 different types of land-use.

102         Herein, we used a landscape disturbance gradient and combined stomach content  
103 and stable isotope analyses to quantify the diet and trophic niche of omnivorous fishes in a  
104 biodiversity hotspot in Southern Brazil. Despite its ecological relevance, the Atlantic  
105 Forest, a biome composed by the coastal forest or Atlantic rain forest and the tropical  
106 seasonal forest or semi-deciduous Atlantic Forest, has been dramatically reduced over the  
107 decades and replaced by agricultural and urban land use (Mohebalian et al. 2022). We  
108 tested the overarching hypothesis that, in response to the negative impacts of agriculture

109 and urbanization expansion, and associated reduction of native riparian forest, the trophic  
110 ecology of a dominant omnivorous species should change. Specifically, we predicted that:

111 1) Human disturbances could promote changes in resource use by omnivorous  
112 fishes and, consequently, alter their stable isotope composition. However, human  
113 disturbances can also affect the isotopic composition of basal resources, which may  
114 reverberate to the isotopic composition of macroinvertebrate prey and their fish  
115 consumers. Lower native riparian forest cover can expose the streams to high solar  
116 incidence, and consequently increase algal productivity. Therefore, we expected that  
117 omnivorous fishes would have higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in streams with lower  
118 coverage of native forest and high inputs of nitrogen from agricultural practices.

119 2) As the proportion of native forest cover decreases, stream ecosystems are more  
120 strongly affected by leaching and silting events due to the greater exposure to  
121 torrential summer storms. Consequently, omnivorous fishes would be forced to  
122 switch their consumption and assimilation from abundant, high-quality  
123 autochthonous resources in forested areas to a low nutritional resource (e.g.,  
124 detritus) and allochthonous (terrestrial invertebrates) resources in areas with low  
125 coverage of native forest. Alternatively, omnivorous fishes in more open canopy  
126 streams would rely more on autochthonous resources, if these reaches have higher  
127 algal primary productivity.

128 3) The trophic niche of common omnivorous fish would become narrower in  
129 disturbed streams due to habitat homogeneity and low diversity of resources, but  
130 these effects could also be modulated by interactions with potential interspecific  
131 competitors. We addressed this hypothesis by applying niche theory, which predicts

132 that in the absence of competitors, species can contract their trophic niches and  
133 consume their preferred food items. However, to reduce competitive pressure from  
134 potential competitors, species would expand their trophic niches through consuming  
135 alternative food items.

136

## 137 **Methods**

### 138 *Study area and field sampling*

139 Iguaçú and Piquiri rivers and their tributaries belong to the second largest basin in South  
140 America, the Paraná River (Abell et al. 2008). There is less than 4% of Atlantic Forest  
141 currently remaining in Paraná State and the native forest has been gradually replaced by  
142 monocultures, livestock farmland and other agroindustrial activities.

143 In the region between the Iguaçú and Piquiri river basins, we surveyed seven headwater  
144 streams (2<sup>nd</sup> and 3<sup>rd</sup> orders) across a gradient of native forest cover (5-75%, Table S1; Fig.  
145 S1) in the December of 2017 (wet season). We estimated human land uses in the areas  
146 encompassing each stream, by demarcating the catchment above each sampling site and  
147 inputting geographical coordinates of the sampling sites into Quantum GIS software (QGIS  
148 version 2.18.10). Using the GRASS plugin in QGIS, we opened the MDE raster, and with  
149 the ‘r.watershed’ and ‘r.water.outlet’ tools, we delimited the catchment area for each  
150 sampling site (range: 6.6 to 18.2 km<sup>2</sup>; Table S1). We used the 2017 land use cover data  
151 from the MapBiomias website (MapBiomias Project 2021) as a basis to calculate the  
152 different land use covers inside the polygon of each delimited catchment site. We  
153 calculated the area (km<sup>2</sup>) for the following land uses: urbanized area - paved area,  
154 residential and industrial area; agricultural area - pastures, plantations of annual and

155 perennial crops and silviculture; and forested area - included areas of riparian forest and  
156 remnants of native forest. To estimate the proportion of native forest cover associated with  
157 each stream site, we calculated the proportions of the urbanized, agricultural, and forested  
158 areas from the total area (km<sup>2</sup>) of each stream watershed. The native forest cover (%) was  
159 then used as a predictor in the subsequent analyses.

### 160 ***Physical and chemical habitat characteristics***

161 We measured the key environmental variables that can be influenced by native forest cover  
162 (Table S1), as well as the mean stream width (m), depth (m) and flow velocity (m<sup>3</sup> s<sup>-1</sup>) at  
163 five stream cross-sections. We used a Horiba U-50 multiparameter water quality probe for  
164 *in situ* measurements of water temperature (°C), pH, dissolved oxygen concentration (mg  
165 L<sup>-1</sup>) and saturation (%), oxidation reduction potential (ORPmv), specific electric  
166 conductivity (μS cm<sup>-1</sup>), turbidity (NTU) and total solids (μS cm<sup>-1</sup>). We replicated all these  
167 measurements three times across the stream reach in each stream. From each stream, we  
168 collected a 1-L water sample for laboratory analysis of chlorophyll- $\alpha$  (μg L<sup>-1</sup>), total  
169 phosphorus (μg L<sup>-1</sup>), total nitrogen (mg L<sup>-1</sup>), ammonium NH<sub>4</sub> (mg L<sup>-1</sup>), soluble reactive  
170 phosphorus, SRP (μg L<sup>-1</sup>), and nitrate NO<sub>3</sub> (mg L<sup>-1</sup>) concentrations, following the approach  
171 described by Mackereth et al. (1978) and Apha (2005).

### 172 ***Fishes, their invertebrate prey, and basal resources***

173 In each stream, we sampled the fish assemblages in a 50 m reach using electrofishing,  
174 making three passes of 40 min. The abundance of Characidae species comprised 50.9% to  
175 75.5% of total fish abundance in the sampled streams. Within this family, we focused our  
176 analysis on all omnivorous fish species (six species) of genus *Astyanax*, *Bryconamericus*



177 and *Psalidodon*. These included *Psalidodon* aff. *paranae* (Eigenmann 1914) in sites S3 and  
178 S5, *Astyanax lacustris* (Lütken 1875) in S3 and S7, *Psalidodon* aff. *gymnodontus*  
179 (Eigenmann 1911) in S2, *Astyanax* aff. *fasciatus* Cuvier, 1819 in S7, and *Bryconamericus*  
180 *ikaa* (Casciotta, Almirón and Azpelicueta 2004) in S1. *Psalidodon bifasciatus* (Garavello  
181 and Sampaio 2010) was a dominant species and was collected from all sampled streams.  
182 Thus, the analysis of *P. bifasciatus* allowed us to quantify changes in the trophic ecology of  
183 a dominant species across a broad land use gradient and the potential effects of competition  
184 with species of close phylogenetic proximity and high morphological similarity. In  
185 addition, *P. bifasciatus* was less abundant in streams with co-occurrence of other characid  
186 species (S1, S2, S3, S5 and S7; mean numerical abundance: 33.5%) than in streams without  
187 the presence of another characid species (S4 and S5, mean numerical abundance: 71.4%).

188 For stomach content analysis, all specimens were anesthetized in eugenol, then  
189 fixed in 10% formaldehyde and later preserved in 70% alcohol. Subsequently, the  
190 specimens were identified, measured, and weighed in the laboratory. For the stable isotope  
191 analysis, during the sampling, adult specimens of each species were immediately  
192 euthanized via spinal section, identified, measured, and weighed *in situ*. The specimens  
193 were stored on ice for further processing in the laboratory. The fish samples were collected  
194 with authorization from the Instituto Chico Mendes de Conservação da Biodiversidade  
195 (license numbers 30182, 25039-1) and approved by the Ethics Committee on Animal Use  
196 of the Universidade Federal do Rio Grande do Sul (CEUA – 32734), following the  
197 protocols in their ethical and methodological aspects for the use of fish. Voucher specimens  
198 were deposited in the ichthyology collection of the Departamento de Zoologia at the  
199 Universidade Federal do Rio Grande do Sul (Table S2).

200 We sampled larval aquatic insects (Ephemeroptera, Trichoptera, Plecoptera, Hemiptera,  
201 Coleoptera, Megaloptera, Odonata, Chironomidae, Simuliidae) and other aquatic  
202 invertebrates (*Aegla* and Atyidae) using a kick net (0.04 m<sup>2</sup> and 200 µm mesh size) across  
203 the entire same stream reach. We sampled terrestrial invertebrates (Araneae, Coleoptera  
204 adult, Odonata adult, Auchenorrhyncha adult, Hemiptera adult, Hymenoptera, Orthoptera,  
205 Lepidoptera adult, Diptera adult) using an entomological net from riparian vegetation  
206 around each stream. We collected Oligochaeta manually in the soil around the streams.

207 Basal resources we sampled included riparian vegetation (terrestrial leaves of  
208 pteridophytes, monocotyledons, and seeds of dicotyledons), aquatic biofilm, particulate  
209 organic matter (POM) and sedimentary organic matter (SOM). We also sampled biofilm by  
210 scraping and washing rocks with distilled water. For POM, we collected a 1-L water sample  
211 per stream and filtered the water sample using a filtration apparatus attached to a vacuum  
212 pump using fiber filters (Macherey-Nagel® filters). We randomly sampled SOM from three  
213 sections of each stream. We froze all fish samples, prey invertebrates and basal resources at  
214 -20 °C freezer until processing in the laboratory (Table 3).

### 215 ***Laboratory procedures***

216 We analyzed the stomach contents of 309 fish specimens (Table S5). To reduce the  
217 variance caused by ontogenetic diet shifts, we inspected the gonads of all sampled fish and  
218 selected only adult specimens for further analyses. We removed fish stomachs and used  
219 optical and stereoscopic microscopes (Opton TIM-2B WF10X) to identify their contents to  
220 the lowest taxonomic resolution possible (usually family or genus). We quantified the diet  
221 items using the volumetric method, i.e., the total volume of a food item taken by the fish

222 population is given as a percentage of the total volume of all stomach contents (Hyslop  
223 1980), using graduated test tubes and a glass counting plate (Hellawell and Abel 1971).

224 We analyzed a total of 266 samples of basal resources, prey, and fishes for nitrogen  
225 ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) stable isotope ratios (Table S3). First, we washed all basal  
226 resources and prey with distilled water. For smaller taxa (e.g., Chironomidae and  
227 Simuliidae), we pooled at least ten individuals into a single sample. For fish samples, we  
228 dissected dorsal muscle tissue and washed it with distilled water (Neves et al. 2021). Then,  
229 we lyophilized all samples, homogenized them using mortar and pestle and stored them in 2  
230 mL Eppendorf tubes. Subsequently, we weighed  $1.6 \pm 0.2$  mg for dry animal tissue, or  $3.6$   
231  $\pm 4.2$  mg for basal resources, into tin capsules and analyzed the samples at the Center for  
232 Nuclear Energy in Agriculture (CENA) at the University of São Paulo, Brazil. To  
233 determine the stable isotope ratios, we used a mass spectrometer system in the continuous-  
234 flow (CF-IRMS) mode with a Carlo Erba elemental analyzer (CHN 1110), coupled to a  
235 Delta Plus mass spectrometer (Thermo Scientific). We present all values in delta notations,  
236 which represent the deviation of stable isotope ratios ( $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$ ) from universal  
237 standards: PDB limestone for carbon and atmospheric nitrogen for nitrogen. We did not  
238 correct the fish muscle  $\delta^{13}\text{C}$  values for lipids because all C:N ratios were below 3.5,  
239 indicating negligible lipid content in the samples (Hoffman et al. 2015).

## 240 **Statistical analyses**

241 *Effects of native forest cover on isotopic composition of omnivorous fishes,*  
242 *macroinvertebrate prey, and basal resources*

243 To test our first hypothesis, we estimated the effects of native forest cover (%) on  $\delta^{15}\text{N}$  and  
244  $\delta^{13}\text{C}$  values of basal resources, aquatic macroinvertebrates, and omnivorous fishes, using  
245 generalized additive models (GAMs) in the *mgcv* package (Wood 2017). We tested whether  
246 linear or polynomial relationships provided a better fit to the data comparing models  
247 (Akaike's information criterion, AIC), RMSE values and adjusted  $R^2$ . The regression lines  
248 and 95% confidence intervals predicted from GAMs were obtained using the *stat\_smooth*  
249 function (Wickham, 2016). To test the effects of the native forest cover (%) on diet  
250 consumption and assimilation, trophic niche, trophic position, and fish condition, we  
251 focused on *P. bifasciatus* due to its wide distribution (present in all streams: S1 to S7). For  
252 this model species, we explored the main causes of the expansion or contraction of isotopic  
253 niches and its dominance in streams under different human disturbances. Thus, in the  
254 subsequent analyses we focused on stomach content and stable isotopes data of *P.*  
255 *bifasciatus* (derived from 10 to 16 individuals within each stream; Table S2).

256 *Relationships between environmental variables, diet consumption, isotopic composition,*  
257 *and resource assimilation of a widely distributed omnivorous fish*

258 To test our second hypothesis, we investigated changes in diet composition across a  
259 gradient of native forest cover. We also tested for the potential underlying environmental  
260 variables using the distance-based redundancy analysis (dbRDA) based on Bray-Curtis  
261 distance (Legendre and Anderson 1999) in the *vegan* package (Oksanen et al. 2020). We  
262 log-transformed (natural log) all the environmental variables due to the different  
263 measurement scales of each variable (i.e., oxygen concentration -  $\text{mg L}^{-1}$ ; specific electric  
264 conductivity -  $\mu\text{S cm}^{-1}$ ). The water temperature ( $^{\circ}\text{C}$ ) was converted to Kelvin, prior to the  
265 log-transformation. For the subsequent dbRDA analyses, we used native forest cover (%),

266 water temperature (K), ammonium  $\text{NH}_4$  ( $\text{mg L}^{-1}$ ), SRP ( $\mu\text{g NO}_3\text{-N/L}$ ) and nitrate  $\text{NO}_3$  ( $\text{mg}$   
267  $\text{L}^{-1}$ ) as most relevant water characteristics (see SI for more details on variable selection).  
268 We performed a permutation test for dbRDA, using the *anova.cca* function, to assess the  
269 statistical significance (Oksanen et al. 2020). Similarly, to test the influence of  
270 environmental variables on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *P. bifasciatus*, we also applied a dbRDA  
271 based on Euclidean distance (Legendre and Anderson 1999) with the same environmental  
272 variables as used for diet composition.

273 As part of the second hypothesis, to investigate how native forest cover (%) is related to  
274 the assimilation of resources by *P. bifasciatus*, we applied Bayesian stable isotope mixing  
275 models in the *MixSIAR* package (Stock and Semmens 2016). Mixing models provide  
276 quantitative estimates of diet contributions when the number of diet sources is low (Nielsen  
277 et al. 2018; Phillips et al. 2014). We pooled potential diet sources into four isotopically  
278 homogeneous categories: i) aquatic invertebrates, ii) terrestrial invertebrates, iii) aquatic  
279 biofilm and POM, and iv) SOM (See SI for more information about MixSIAR models and  
280 assumptions, Figs. S8-S9; Tables S9-S10). Fractionation is an important consideration  
281 when estimating a consumer's trophic position and resource assimilation (MixSIAR, Post  
282 2002). For *P. bifasciatus*, we used trophic discrimination factors (TDF) of  $1.3 \pm 0.3\%$  for  
283 C, and  $2.9 \pm 0.32\%$  for N (McCutchan et al. 2003). This enrichment is appropriate for  
284 muscle tissues of omnivorous fishes that consume mixtures of plant and animal diet  
285 (McCutchan et al. 2003). We also considered specific TDF values for plant resources  
286 (Bastos et al. 2017). Because this fish could assimilate bacteria and other microbes  
287 inhabiting basal plant resources, we followed the method of Neres-Lima et al. (2016) and

288 doubled the mean discrimination factor and the variability estimate (SD) by propagation of  
289 error ( $\sqrt{2 \cdot SD^2}$ ), yielding the values  $2.6 \pm 0.42\text{‰}$  for C, and  $5.8 \pm 0.45\text{‰}$  for N.

290 *Effects of native forest cover on trophic niches of a widely distributed omnivorous fish*

291 To test our third hypothesis, to understand the effects of native forest cover (%) on trophic  
292 niches of dominant omnivores, we calculated the trophic niche based on both stomach  
293 content and stable isotope analyses. We estimated the stomach contents niche breadth using  
294 the permutational analysis of multivariate dispersions (PERMDISP; Anderson 2006) in the  
295 *vegan* package (Oksanen et al. 2020, for specific details see SI – Table S6). PERMDISP  
296 tests whether group means, or group dispersions differ, considering the site as a driver. To  
297 estimate isotopic variance (isotopic niche) of omnivorous fishes, we calculated  
298 multivariate, ellipse-based metric ( $SEA_c$ , ‰) for each fish species (*P. bifasciatus* and other  
299 co-occurring Characidae species) in each stream using the *SIBER* package (Jackson et al.  
300 2011).

301 *Relationships among native forest cover, fish diversity and trophic niche of a dominant*  
302 *omnivore fish*

303 As part of the third hypothesis, to investigate the effects of both native forest cover (%) and  
304 co-occurring fish species on the trophic niche of the *P. bifasciatus*, we fitted generalized  
305 linear models (GLM) with the following structure:  $\log(SEA_c) \sim \text{native forest cover} * \text{fish}$   
306  $\text{richness}$ . We used  $SEA_c$  because stable isotopes provide a longer, time-integrated, measure  
307 of resource use than stomach contents. We used a Gaussian family distribution in the *glm*  
308 function, assuring that the model assumptions were met. We then ran a simplified version  
309 ( $\log(SEA_c) \sim \text{native forest cover} + \text{fish richness}$ ) of the first model and compared both

310 models using *anova* function. Because there was no significant interaction between the  
311 factors ( $F_{[1,3]} = 0.68$ ,  $p = 0.85$ ) or differences between the models with or without  
312 interaction ( $F_{[1,4]} = 0.04$ ,  $p = 0.86$ ), we fit the second simplified model.

313 Finally, to determine how both native forest cover (%) and co-occurring species  
314 were related to the dominance index (DI) of the *P. bifasciatus*, we also performed GLM  
315 with the following structure:  $DI \sim \text{native forest cover} * \text{fish richness}$ . A dominance index  
316 was calculated as the proportional abundance of *P. bifasciatus* within the fish assemblage in  
317 each stream (Perkins et al. 2018). We also tested the effects of native forest cover (%) on  
318 fish richness ( $\text{fish richness} \sim \text{native forest cover}$ ) using GAM models due to the indication  
319 of non-linear responses. All statistical analyses were performed in the language  
320 environment R, version 4.2.1 (R Core Team 2022).

321

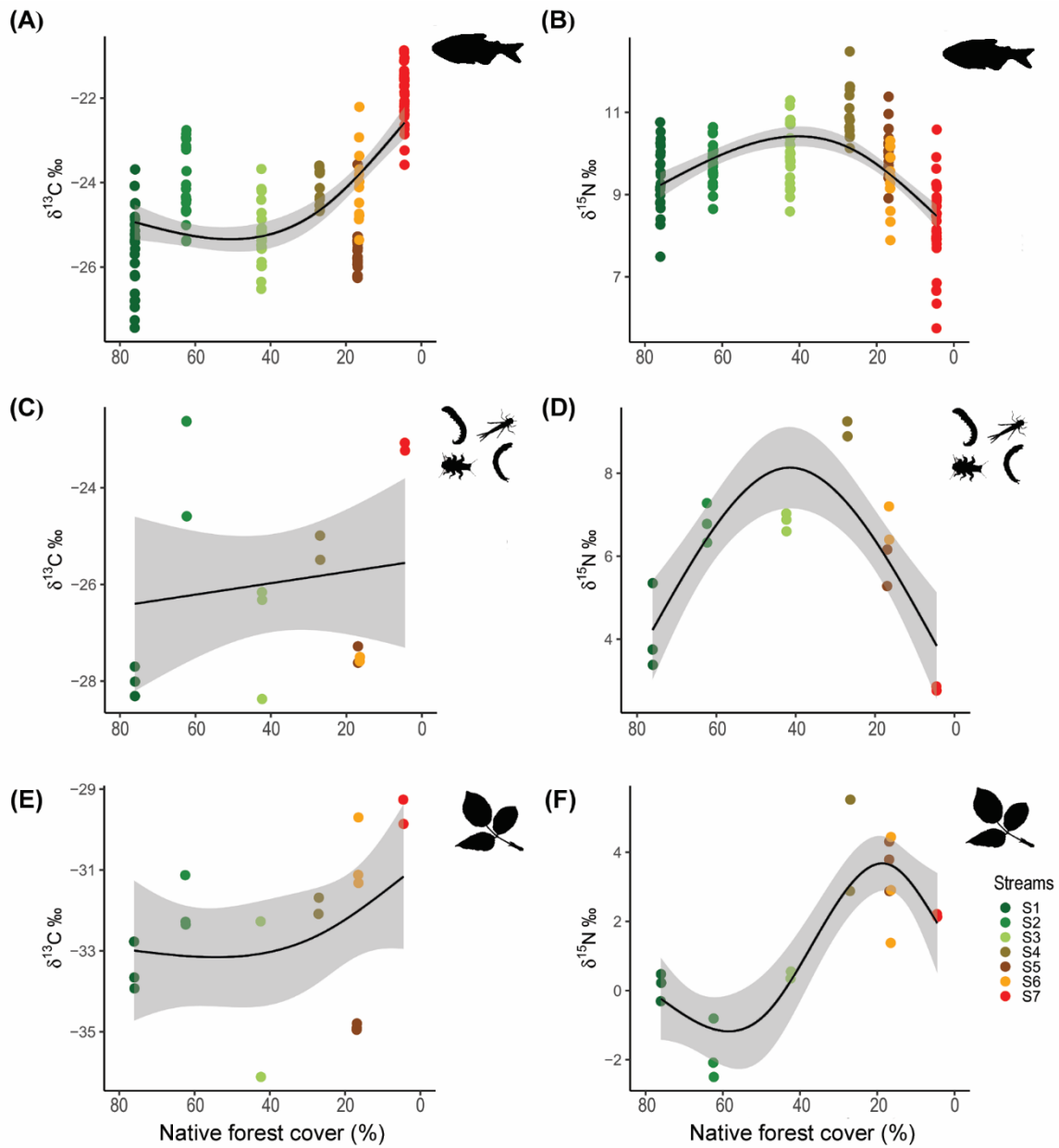
## 322 **Results**

### 323 *Isotopic composition of omnivorous fishes, macroinvertebrate prey, and basal resources*

324 In agreement with our first hypothesis, human disturbances altered the stable isotope values  
325 of basal resources, macroinvertebrate prey, and omnivorous fishes. The streams with lower  
326 proportion of native forest cover were associated with an increase in  $\delta^{13}\text{C}$  values of  
327 omnivorous fishes (GAM;  $R^2 = 0.62$ ,  $F_{[6,148]} = 58.6$ ,  $p = < 0.001$ ; Fig. 1A). There was also a  
328 nonlinear relationship between native forest cover and fish  $\delta^{15}\text{N}$  values (GAM;  $R^2 = 0.46$ ,  
329  $F_{[6,148]} = 30.8$ ,  $p < 0.001$ ; Fig. 1B). There was no evidence that native forest cover affected  
330  $\delta^{13}\text{C}$  values of aquatic macroinvertebrates (GAM;  $R^2 = 0.51$ ,  $F_{[6,17]} = 2.93$ ,  $p = 0.07$ ; Fig.  
331 1C). However, there was strong evidence for the effects of the native forest cover on  $\delta^{15}\text{N}$

332 values of macroinvertebrates (GAM;  $R^2 = 0.77$ ,  $F_{[6,17]} = 11.8$ ,  $p < 0.001$ ; Fig. 1D).  
333 Similarly, there was no evidence that the native forest cover influenced the  $\delta^{13}\text{C}$  values of  
334 basal resources (GAM;  $R^2 = 0.51$ ,  $F_{[6,18]} = 1.41$ ,  $p = 0.32$ ; Fig. 1E), but there was strong  
335 evidence of nonlinear effect on the  $\delta^{15}\text{N}$  values of basal resources (dicotyledons; GAM;  $R^2$   
336  $= 0.85$ ,  $F_{[6,18]} = 19.1$ ,  $p < 0.001$ ; Fig. 1F). Aquatic macroinvertebrates and fishes were  
337 enriched in  $\delta^{15}\text{N}$  ( $6.0 \pm 1.9\text{‰}$ ;  $9.5 \pm 1.1\text{‰}$ , respectively), especially in streams at  
338 intermediate values of the native forest cover (%) and where there were agricultural  
339 activities (S3, S4 and S5; Fig. 1; Fig. S4). For native vegetation (dicotyledons), the mean  
340  $\delta^{15}\text{N}$  value was  $1.6 \pm 2.2\text{‰}$  and the highest values were in streams with the lowest coverage  
341 of forest cover.





342

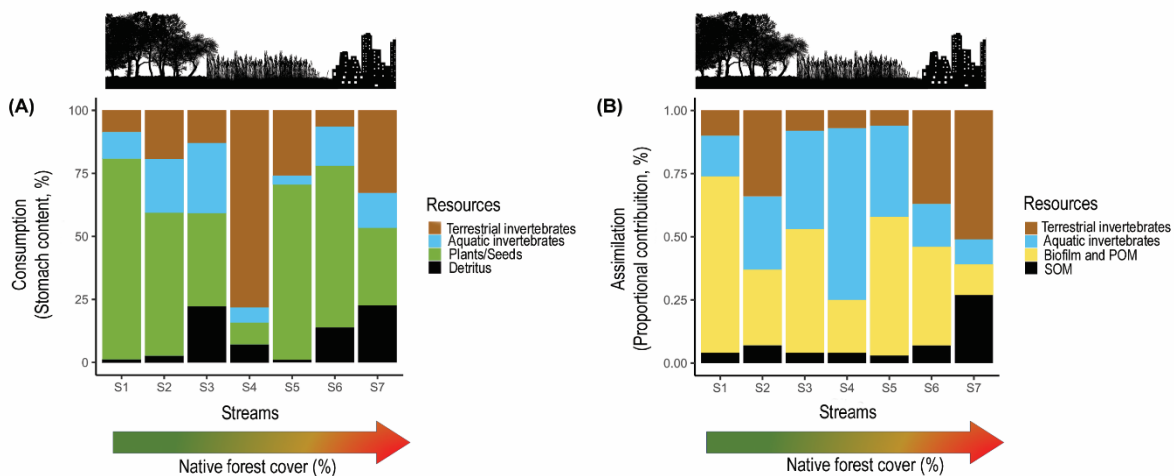
343 **Fig. 1.** The nonlinear effects of native forest cover (%) on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  
 344 omnivorous fishes (A-B), aquatic macroinvertebrate prey (C-D) and basal resources  
 345 (dicotyledons) (E-F). Black regression lines and shaded 95% confidence intervals predicted  
 346 from GAMs. The GAM relationships were evaluated by the significance of the smooth  
 347 terms.

348

349 ***Diet consumption and assimilation of a widely distributed omnivorous fish***

350 In agreement with our second hypothesis, the proportion of native forest cover affected the  
351 trophic ecology of a dominant omnivorous species. The diet of *P. bifasciatus*, a widely  
352 distributed species across all focal streams, was different in each stream (Fig. 2A; Table  
353 S5). This species consumed a high proportion of terrestrial leaves, seeds, and aquatic  
354 insects (Ephemeroptera, Trichoptera, Plecoptera) in streams with higher native forest cover  
355 (Fig. 2A; Table S5). However, the diet shifted toward a higher consumption of terrestrial  
356 insects (Coleoptera, Hymenoptera, Diptera) and detritus in streams with low native forest  
357 cover (Table S5). Moreover, biofilm, particulate organic matter (POM) and aquatic  
358 invertebrates became less assimilated into the fish tissues in the most disturbed urban  
359 streams (Fig. 2B; Table S10). As hypothesized, in these disturbed streams, the most  
360 assimilated resources were terrestrial invertebrates and sedimentary organic matter (SOM,  
361 Fig. S10, Table S10).

362



364 **Fig. 2.** The relative contribution (%) of main food resources consumed and assimilated by  
365 *P. bifasciatus* in seven streams along a gradient of native forest cover (%). **(A)** Proportions  
366 of consumed resources in diet were estimated by volume from stomach content analysis.  
367 **(B)** The assimilated diet contributions were estimated using a MixSIAR Bayesian mixing  
368 model. SOM denotes sedimentary organic matter and POM denotes particulate organic  
369 matter.

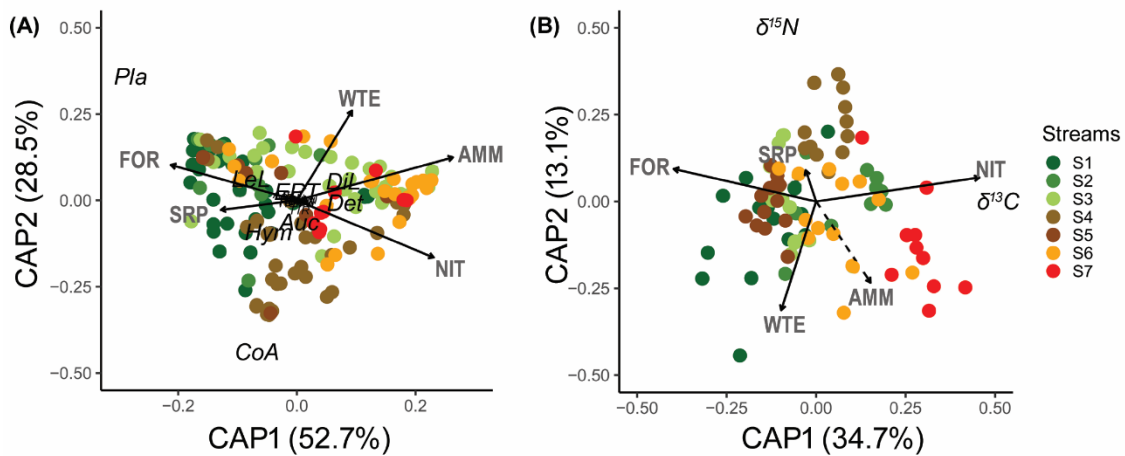
370

371 The environmental characteristics associated with human disturbances affected both  
372 resource consumption (dbRDA, Fig. 3A) and isotopic composition (Fig. 3B) of the  
373 dominant omnivorous fish. There were strong patterns of associations between  
374 environmental variables and food items ingested by *P. bifasciatus* (dbRDA,  $F_{[5,164]} = 4.64$ ,  
375  $p = 0.001$ ). In the CAP1 axis ( $F_{[1,164]} = 12.22$ ,  $p = 0.001$ ), higher consumption of plant  
376 resources was associated with higher proportions of native forest cover ( $F_{[1,164]} = 7.80$ ,  $p =$   
377  $0.001$ ) while higher consumption of detritus and Diptera larvae was associated with higher  
378 concentrations of ammonium ( $F_{[1,164]} = 3.00$ ,  $p = 0.003$ ) and nitrate ( $F_{[1,164]} = 3.30$ ,  $p =$   
379  $0.002$ ). In the CAP2 axis ( $F_{[1,164]} = 6.61$ ,  $p = 0.001$ ), negative scores suggested that the  
380 higher consumption of Coleoptera adult and other terrestrial insects was related to higher  
381 concentrations of SRP ( $F_{[1,164]} = 2.21$ ,  $p = 0.01$ ). Higher consumption of aquatic insects was  
382 positively associated with higher water temperature ( $F_{[1,164]} = 6.91$ ,  $p = 0.001$ ).

383 There were also strong patterns of associations between environmental variables  
384 and the isotopic composition of *P. bifasciatus* (dbRDA,  $F_{[5,83]} = 15.06$ ,  $p = 0.001$ , Fig. 3B).  
385 In the CAP1 ( $F_{[1,86]} = 56.87$ ,  $p = 0.001$ ), negative scores indicated that lower  $\delta^{13}\text{C}$  values ( $-$   
386  $25.836 \pm 0.9\text{‰}$ ) were associated with streams characterized by higher proportions of native

387 forest cover ( $F_{[1,83]} = 35.43$ ,  $p = 0.001$ ). In contrast, *P. bifasciatus* had higher  $\delta^{13}\text{C}$  values (-  
 388  $22.051 \pm 0.6\text{‰}$ ) in urban streams with high nitrate concentrations ( $F_{[1,86]} = 17.87$ ,  $p =$   
 389  $0.001$ ). In the CAP2 ( $F_{[1,86]} = 21.17$ ,  $p = 0.001$ ), water temperature ( $F_{[1,83]} = 9.37$ ,  $p = 0.001$ )  
 390 was negatively associated with  $\delta^{15}\text{N}$  values of *P. bifasciatus*. On this axis, *P. bifasciatus*  
 391 had slightly elevated  $\delta^{15}\text{N}$  values ( $11.0 \pm 0.6\text{‰}$ ) in streams with higher SRP concentrations  
 392 ( $F_{[1,83]} = 9.47$ ,  $p = 0.001$ ) and with agricultural influence than in streams with higher native  
 393 forest cover ( $\delta^{15}\text{N}$  values,  $9.4 \pm 0.8\text{‰}$ ).

394



395

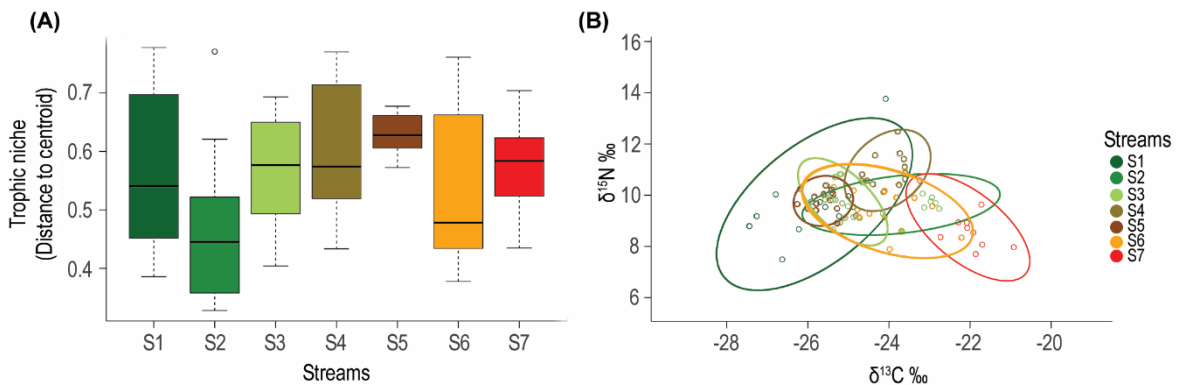
396 **Fig. 3.** Distance-based redundancy analysis (dbRDA) illustrating the association between  
 397 **(A)** food items consumed and **(B)** isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of *P. bifasciatus*  
 398 and the key environmental variables for all streams (FOR: native forest cover, WTE: water  
 399 temperature, AMM: ammonium, SRP: soluble reactive phosphorus, NIT: nitrate). Solid  
 400 arrows indicate statistically significant associations. Food items code: Det: detritus, Pla:  
 401 plants/seeds, AIR: aquatic insect remains, HemN: Hemiptera nymph, EPT: Ephemeroptera,  
 402 Plecoptera and Trichoptera larvae, OdoN: Odonata nymph, CoL: Coleoptera larvae, DiL:  
 403 Diptera larvae, LeL: Lepidoptera larvae, TIR: terrestrial insect remains, Hym:

404 Hymenoptera, Auc: Auchenorrhyncha, HemA: Hemiptera adult, CoA: Coleoptera adult,  
405 LepA: Lepidoptera adult, PleA: Plecoptera adult, Ara: Araneae, Cru: Crustacea, Oli:  
406 Oligochaeta.

407

#### 408 *Trophic niche of dominant omnivorous fish species*

409 In agreement with our third hypothesis, the differences in the niche dispersion of *P.*  
410 *bifasciatus* among streams indicated that this species had more restricted diets in impacted  
411 streams than in streams with higher proportion of native forest cover, suggesting  
412 intraspecific responses to different land use types (PERMDISP;  $F_{[6,151]} = 2.50$ ,  $p < 0.002$ ;  
413 Fig. 3A; Tables S6 and S7). However, there was no evidence that these differences were  
414 related to the proportion of native forest cover (GAM;  $R^2 = 0.21$ ,  $F_{[1,6]} = 1.33$ ,  $p = 0.30$ ).



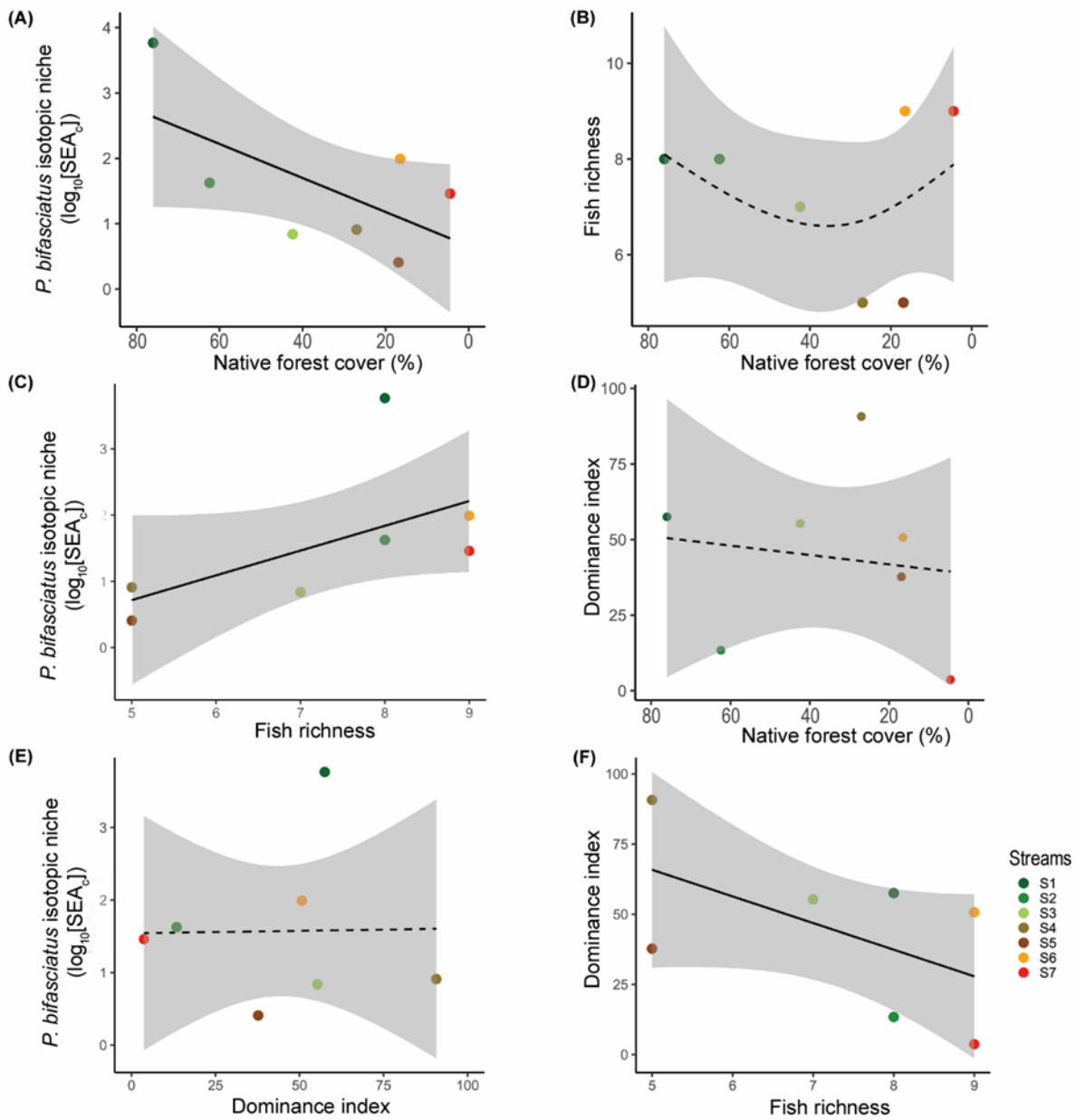
415

416 **Fig. 4.** Differences in trophic niche (A) measured as a distance to centroid (using  
417 PERMDISP) and (B) isotopic niches evaluated by the ellipse-based metric (SEA<sub>c</sub>) of *P.*  
418 *bifasciatus* in seven streams along a gradient of native forest cover (S1 to S7). Box lower  
419 and upper endpoints represent the 25<sup>th</sup> and 75<sup>th</sup> quartiles, respectively and the horizontal bar  
420 inside each box represents median trophic niche (for summary see Tables S6 and S7).

421

422           There was moderate evidence suggesting that isotopic niche (measured as the size  
423 of the ellipse for each stream,  $SEA_c$ ) of *P. bifasciatus* was influenced not only by the  
424 proportion of native forest cover (GLM;  $R^2 = 0.62$ ,  $F_{[1,5]} = 2.19$ ,  $p = 0.03$ ; Fig. 4B and 5A),  
425 but also by the richness of the fish species (GLM;  $R^2 = 0.73$ ,  $F_{[1,4]} = 0.68$ ,  $p = 0.003$ ; Fig.  
426 5C). Variation in isotopic niche appeared to be related to the availability of resources and to  
427 the co-occurrence of other tetra fish species. High isotopic niche variation of *P. bifasciatus*  
428 was observed when it co-occurred with *B. ikaa* and *P. aff. gymnodontus* (Fig. 6A-B),  
429 whereas lower isotopic variation was observed when it co-occurred with *P. aff. paranae*  
430 (Fig. 6C-E).

431



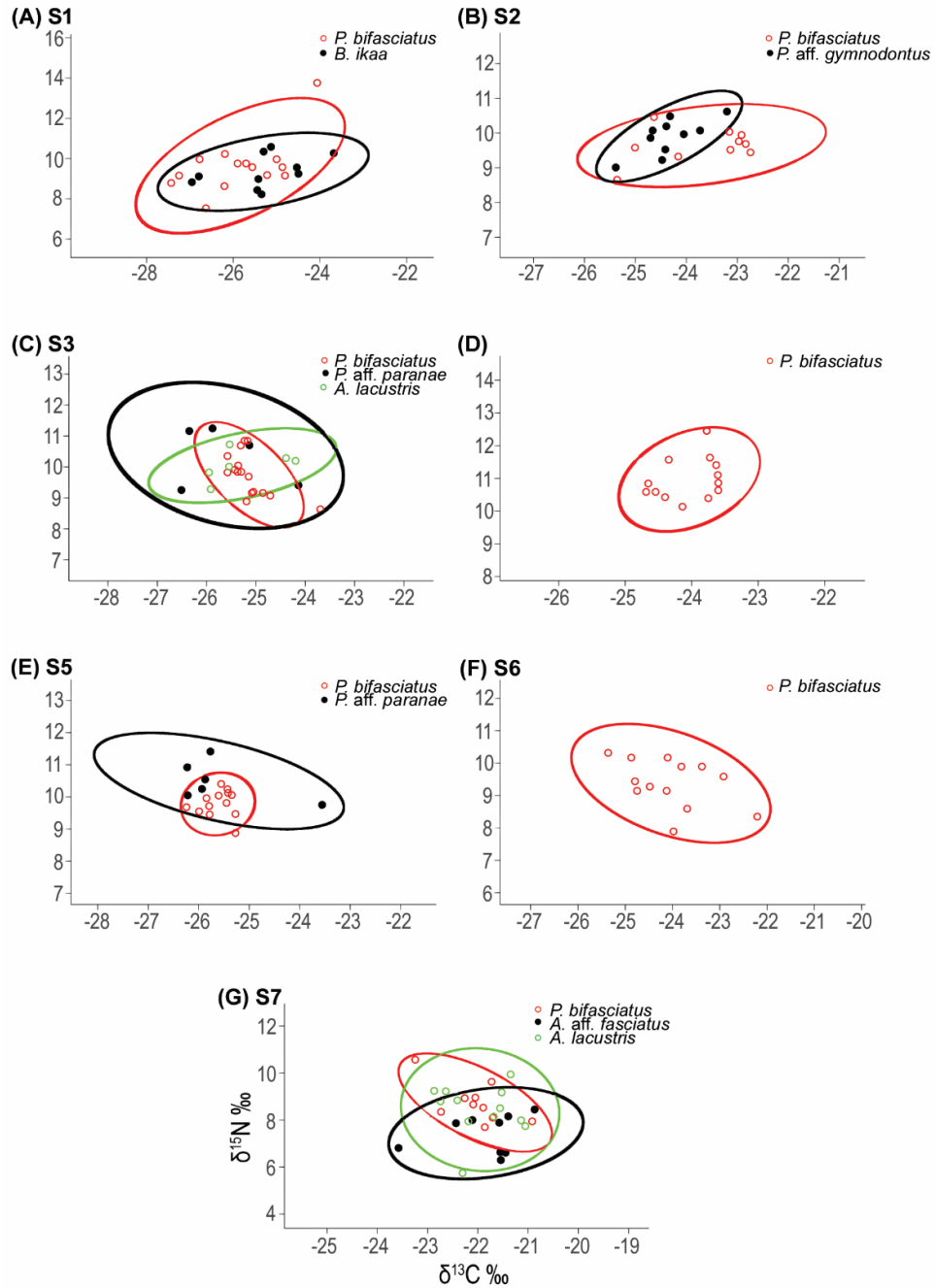
432

433 **Fig. 5.** The relationships between the native forest cover (%) and *P. bifasciatus* isotopic  
 434 niche ( $\log_{10}[\text{SEAc}]$ ), fish community richness and *P. bifasciatus* dominance index (A, B,  
 435 D). Relationship among fish community richness, *P. bifasciatus* isotopic niche  
 436 ( $\log_{10}[\text{SEAc}]$ ) and dominance index (C, E, F). Black lines and shaded areas represent  
 437 predicted GAMs and GLMs fits and 95% confidence intervals. Solid black line indicates a

438 significant relationship and dotted black line indicates a non-significant relationship. The  
439 GAM relationships were evaluated by the significance of the smooth terms.

440





441

442 **Fig. 6.** Omnivorous tetra fish species coexisting in each of seven streams and their isotopic  
 443 niches, illustrated as size of ellipses (SEAc). The points represent the δ<sup>13</sup>C and δ<sup>15</sup>N isotopic  
 444 values of each individual of omnivorous species sampled in each stream. Colors illustrate  
 445 different species in each stream.

446 The isotopic niche of *P. bifasciatus* was broadest in low-impacted streams with high  
447 proportion of native forest cover and high richness of fish assemblages. Conversely,  
448 isotopic niche was compressed in streams with agricultural influence and low fish richness  
449 (Figs. 4B and A) indicating a more selective diet. Although both native forest cover and  
450 fish richness were important factors influencing the isotopic niche of the dominant species,  
451 there was no evidence that native forest cover had an effect on fish richness (GAM;  $R^2 =$   
452  $0.36$ ,  $F_{[1,4]} = 0.40$ ,  $p = 0.71$ ; Fig. 5B), indicating that the effects of these variables were  
453 largely independent of each other.

#### 454 ***Relationship among native forest cover, fish diversity and trophic niche of dominant fish*** 455 ***species***

456 In agreement with our third hypothesis, there was strong evidence for the dominance index  
457 being negatively associated with the fish species richness indicating that *P. bifasciatus*  
458 dominance decreased with increasing richness of the fish assemblages (GLM;  $R^2 = 0.66$ ,  
459  $F_{[1,5]} = 14.17$ ,  $p < 0.001$ ; Fig. 5F). However, there was no evidence that the native forest  
460 cover affected either the *P. bifasciatus* dominance (GLM;  $R^2 = 0.02$ ,  $F_{[1,5]} = 0.10$ ,  $p = 0.76$ ;  
461 Fig. 5D) or the breath of an isotopic niche (GLM;  $R^2 = 0.001$ ,  $F_{[1,5]} = 0.36$ ,  $p = 0.87$ ; Fig.  
462 5E).

463

## 464 **Discussion**

465 Here we showed how differences in native forest cover, associated with human land use  
466 changes, altered the trophic ecology of omnivorous fishes. There was strong evidence for  
467 the non-linear effects of the native forest cover on the  $\delta^{15}\text{N}$  values of native vegetation,

468 aquatic macroinvertebrates, and omnivorous fishes, with the highest  $\delta^{15}\text{N}$  values observed  
469 in streams with an intermediate cover of native forest, and agricultural influences with high  
470 concentrations of SRP and ammonium. Native forest cover affected  $\delta^{13}\text{C}$  values of  
471 omnivorous fishes without changes to their prey, indicating the fishes modified their use of  
472 resources. As the native forest cover decreased, fishes reduced their consumption and  
473 assimilation of high-quality autochthonous resources and increased consumption of low-  
474 quality SOM and allochthonous terrestrial invertebrates with higher  $\delta^{13}\text{C}$  values. Because  
475 deforestation can negatively influence species richness (in rural streams) or increase species  
476 richness through introduction of exotic species (in urban streams), the isotopic niche of *P.*  
477 *bifasciatus* compressed with deforestation but expanded with richness of other fish species  
478 (Table S11). In addition, deforestation tends to make the environment more homogeneous  
479 and reduce the diversity of food items and, consequently, result in more restricted niche  
480 breadths and greater competitive pressure for food resources. This scenario illustrates how  
481 species interactions can mediate changes in the isotopic niche of dominant omnivorous  
482 consumer. These findings also indicate that the persistence of *P. bifasciatus* in disturbed  
483 habitats may be facilitated by plasticity in diet use and the trophic niche expansion in  
484 presence of other competitors.

485 The impacts of riparian deforestation were related to the transference of solar energy to  
486 the aquatic ecosystems, the interception of nutrients and sediments that enter the streams,  
487 and the exchange of organic material between the terrestrial and aquatic realms (Casatti et  
488 al. 2010). In addition to replacing the native forest composed mainly of C3 plants with C4  
489 plants such as corn, wheat, and soybeans in agricultural areas, and by grasses in urban areas  
490 (Magioli et al. 2019), the greater solar incidence in the exposed aquatic environment

491 promotes increased productivity of algae, which can lead to eutrophication (Hill et al. 1995;  
492 Hill et al. 2008). In this study, the effects of land use changes surrounding streams were  
493 reflected in the isotopic composition of omnivorous fish tissues. Fishes had higher  $\delta^{13}\text{C}$   
494 values in streams with lower proportion of native forest cover, reflecting the changes in  
495 carbon source due to land use changes. Shifts in algal productivity associated with land use  
496 changes for the same streams were previously reported by Larentis et al. (2022), suggesting  
497 bottom-up effects on these food webs. In agricultural streams with high concentrations of  
498 ammonium (Table S1), fishes also had higher  $\delta^{15}\text{N}$  values. The fertilizers and other  
499 chemicals such as heavy metals and animal sewage, are commonly used in agricultural  
500 activities and, in ecosystems with the reduced riparian vegetation, can more easily enter  
501 aquatic ecosystems via runoff (Kautza et al. 2015; Price et al. 2019). Studies have shown  
502 the effects of agriculture on the concentrations of nutrients in aquatic environments (Riseng  
503 et al. 2011; Carvalho et al. 2019) and suggested that these activities increase the  $\delta^{15}\text{N}$   
504 values. Artificial fertilizers have relatively low  $\delta^{15}\text{N}$  (-2 to +2.5‰, Rapisarda et al. 2010).  
505 However, the increased  $\delta^{15}\text{N}$  values likely resulted from higher N-cycling and  
506 transformation (Anderson and Cabana 2005), especially due to denitrification. Strong  
507 fractionation during the denitrification process often leaves the residual  $\text{NO}_3$  pool highly  
508 enriched (Clément et al. 2003; Sigman and Casciotti 2001), which could explain the high  
509  $\delta^{15}\text{N}$  values observed in our study. Our findings highlighted how the changes in carbon  
510 source and input of nitrogen can reverberate through the food web up to omnivorous fishes.

511           Deforestation of riparian vegetation can alter the habitat complexity, primary  
512 productivity, and aquatic-terrestrial inputs (Sweeney et al. 2004; Wohl 2006; Price et al.  
513 2019; Effert-Fanta et al. 2022). Aquatic habitats tend to become more homogeneous with

514 reduced riparian cover and macroinvertebrate abundance and diversity tends to decline  
515 (Kautza et al. 2015). In addition, these changes are often associated with higher  
516 sedimentation and silting loads. Thus, with the lower availability of autochthonous  
517 resources (i.e., macroinvertebrates), and greater input of sediments and terrestrial insects,  
518 particularly during the rainy season (the study area is rainy in summer), omnivorous fishes  
519 may be able to sustain their populations through shifting to these more abundant resources.  
520 Fishes can show spatiotemporal shifts in diet and trophic niche but not shifts in trophic  
521 position (Neves et al. 2021; Flood et al. 2023). Our analyses showed that tetra fish shifted  
522 from autochthonous to allochthonous resources across the forested-rural-urban gradient  
523 without changing their trophic position or condition factor (see details in supplementary  
524 material, Fig. S5-S7), indicating how feeding plasticity can promote their persistence.

525         It has been long recognized that riparian vegetation contributes large amounts of  
526 allochthonous detritus especially to headwaters (Vannote et al. 1980). However, recent  
527 studies have suggested that food webs in many headwater streams, especially in the tropics,  
528 rely heavily on autochthonous within-stream derived resources (Brett et al. 2017; Ceneviva-  
529 Bastos et al. 2017; Reis et al. 2020; Wilkinson et al. 2021). Anthropogenic disturbances  
530 alter the relative contribution of aquatic and terrestrial invertebrate subsidies to tropical  
531 streams and can be used to determine ecosystem responses to disturbances with  
532 implications for conservation, management, and monitoring programs (Kautza et al. 2015).  
533 It is also important to consider the effects of reduction of canopy cover on water  
534 temperature, and consequently, on the fish metabolism. Experimental work has suggested  
535 that warming can alter carbon incorporation into soft tissues, with increased values of  $\delta^{13}\text{C}$   
536 in muscle and liver at higher temperatures (Martino et al. 2019).

537 In agreement with Neves et al. (2021), we also observed that plants and seeds are  
538 consumed but not assimilated into the fish tissues. This incongruence between consumption  
539 and assimilation may be governed by several mechanisms. For instance, individual food  
540 items can differ in their digestibility and nutritional quality (Bowen et al. 1995). There may  
541 also be a lack of specialization in the digestive tract of tetra fishes to facilitate the  
542 breakdown, digestion, and assimilation of plant material (Pelster et al. 2015), or this  
543 incongruence can be caused by the higher nutritional quality of animal prey (Gerking  
544 1994). Possible ecological and behavioral explanations such as opportunistic feeding  
545 strategy and accidental consumption (Bastos et al. 2017; Bonato et al. 2018) have also been  
546 proposed. Alternatively, ingested plant material may include a biofilm, which is the main  
547 resource assimilated into fish tissues. Finally, we also need to consider the differences  
548 between the stomach content and stable isotopes analysis which focus on trophic processes  
549 operating over different time scales (Nielsen et al. 2018). In addition to the incongruence  
550 between consumption and assimilation of plant resources, the disagreement between  
551 stomach contents and stable isotopes for stream S4 (high consumption of Coleoptera, but  
552 assimilation of aquatic invertebrates) may represent the sporadic supply of resources.  
553 During the sampling campaign, we observed animal carcasses on the banks of this stream  
554 which could be related to the increased availability of decomposing beetles. This short-term  
555 coleopteran consumption close to the sampling campaign might not have been reflected yet  
556 in the stable isotope analyses.

557 Trophic niche of species can be influenced by the availability and diversity of resources  
558 but also by species interactions such as interspecific competition (Costa-Pereira et al. 2017;  
559 Perkins et al. 2018). Although there was no clear relationship between the breadth of

560 trophic niche and the proportion of native forest cover, we showed an intriguing positive  
561 association between isotopic niche area of *P. bifasciatus* and richness of other co-occurring  
562 fish species. In rural streams with low richness of fish species, *P. bifasciatus* had the most  
563 compressed isotopic niche indicating more specialized resource exploitation. Similar results  
564 were observed by Perkins et al. (2018) for trout in temperate streams, who found trout,  
565 when dominant, had a contracted isotopic niche. By contrast, Carvalho et al. (2019) showed  
566 a narrower trophic niche for a small and invasive characid fish in streams influenced by  
567 pasture activities. Our findings indicated that both deforestation and fish richness can alter  
568 the trophic niche of a widely distributed species.

569 The Niche Variation Hypothesis predicts that weaker competitive pressure leads to a  
570 niche expansion through greater between-individual variation while individual niche  
571 breaths remain constant (Van Valen 1965). In contrast to these predictions, in rural streams  
572 with low fish richness, we found contracting isotopic niche of *P. bifasciatus* while in  
573 forested and urban streams with high fish species richness we found a niche expansion for  
574 this species. The mechanism that promotes the expansion of trophic niche trophic of  
575 widespread species, such as *P. bifasciatus*, in presence of more competitor species needs to  
576 be further investigated. Although the richness of fish species was high in both forest and  
577 urban streams, it is important to highlight that the composition of fish fauna was different  
578 than in relatively unimpacted streams with high forest cover. Moreover, some native and  
579 endemic tetra fish species, such as *P. aff gymnodontus* and *B. ikaa*, only occurred in the  
580 relatively unimpacted streams with high forest cover.

581 Delariva et al. (2018) report lower fish richness in rural streams, suggesting that the  
582 physical-chemical alterations caused by agricultural activities play an important role in

583 environmental filtering of sensitive species. In urban streams, the richness of fish species  
584 increased due to the presence of species indicative of poor environmental quality, tolerant to  
585 hypoxia and anthropogenic stressors, such as *Synbrachus marmoratus* and *Hypostomus*  
586 *ancistroides* (Table S11). These streams are also more susceptible to introduced fish species  
587 such as *Gymnotus sylvius* and *Oreochromius niloticus* (Larentis et al. 2022). The  
588 relationships among native forest cover, trophic niche, and fish richness rely strongly on the  
589 most pristine site S1, the only stream in a Private Reserve of Natural Heritage (Salto Portão  
590 Park), reinforcing the role of protected areas for conservation of biodiversity.

591 Our findings showed how anthropogenic disturbances alter the trophic ecology of  
592 omnivorous fish species and reinforce the fact that omnivory is not a static trait, but its  
593 magnitude changes across time and space (Kratina et al. 2012; Gutgesell et al. 2022).  
594 Through changes in habitat structure and the diversity of resources, deforestation can alter  
595 energy fluxes for consumers at high trophic levels. These findings also indicated that the  
596 wide distribution of dominant omnivores in streams with a gradient of native forest cover can  
597 be explained by shifts in diet from high quality autochthonous resources to poor quality and  
598 allochthonous resources, and, by expansion of the trophic niche in streams with high richness  
599 of fish species. Despite the challenging field work and financial resources in many tropical  
600 areas, more detailed insights into the mechanisms observed here would be gained in future  
601 studies by including more sites and species across other Neotropical biomes under increased  
602 human influence. Our findings are of particular importance in Brazil, where changes to the  
603 Forest Code in 2012 made regulation around minimum permanent preservation areas size,  
604 such as riparian buffers, more flexible, with negative effects ignored or justified for economic  
605 advancement (Alves et al. 2020). This study showed that the effects of native forest cover on



606 omnivorous fishes needs to be investigated within a food web context, to improve our  
607 understanding of wider effects on ecosystem functioning and to devise mitigation and  
608 conservation measures in highly threatened ecosystems.

609

## 610 **References**

- 611 Abell, R., M. L. Thieme, C. Revenga, M. Bryer, M. Kottelat, N. Bogutskaya, ... and P.  
612 Petry. 2008. Freshwater ecoregions of the world: a new map of biogeographic units  
613 for freshwater biodiversity conservation. *Bioscience* 58: 403–414.  
614 doi:10.1641/B580507
- 615 Alves, G. H. Z., R. M. Tófoli, J. L. Rodrigues-Filho, P. A. Sacramento, V. M. Cionek, B.  
616 R. Figueiredo, and E. V. Couto. 2020. Brazil's vegetation ravage may be encouraged  
617 by law. *Biodiversity Conserv.* 29: 1105–1107. doi:10.1007/s10531-020-01933-7
- 618 Anderson, C., and G. Cabana. 2005.  $\delta^{15}\text{N}$  in riverine food webs: effects of N inputs from  
619 agricultural watersheds. *Can. J. Fish. Aquat. Sci.* 62: 333–340. doi:10.1139/f04-191
- 620 Anderson, M. J. 2006. Distance based tests for homogeneity of multivariate dispersions.  
621 *Biometrics* 62: 245–253. doi:10.1111/j.1541-0420.2005.00440.x.
- 622 Apha. 2005. Standard methods for the examination of water and waste water. Amer. Pub.  
623 Health Assoc. Inc.
- 624 Bastos, R. F., F. Corrêa, K. O. Winemiller, and A. M. Garcia. 2017. Are you what you eat?  
625 Effects of trophic discrimination factors on estimates of food assimilation and  
626 trophic position with a new estimation method. *Ecol. Indic.* 75: 234–241.  
627 doi:10.1016/j.ecolind.2016.12.007
- 628 Bonato, K. O., E. D. Burrell, C. B. Fialho, and J. W. Armbruster. 2018. Resource  
629 partitioning among syntopic Characidae corroborated by gut content and stable  
630 isotope analyses. *Hydrobiologia* 805: 311–324. doi:10.1007/s10750-017-3314-0
- 631 Bowen, S. H., E. V. Lutz, and M. O. Ahlgren. 1995. Dietary protein and energy as  
632 determinants of food quality: trophic strategies compared. *Ecology* 76: 899–907.  
633 doi:10.2307/1939355

634 Brett, M. T., S. E. Bunn, S. Chandra, A. W. Galloway, F. Guo, M. J. Kainz, ... and J. D.  
635 Wehr. 2017. How important are terrestrial organic carbon inputs for secondary  
636 production in freshwater ecosystems?. *Freshw. Biol.* 62: 833–853.  
637 <https://doi.org/10.1111/fwb.12909>

638 Burdon, F. J., A. R. McIntosh, and J. S. Harding. 2019. Mechanisms of trophic niche  
639 compression: evidence from landscape disturbance. *J. Anim. Ecol.* 89: 730–740.  
640 doi:10.1111/1365-2656.13142

641 Carvalho, D. R., D. M. P. Castro, M. Callisto, A. J. M. M. Chaves, Z. Moreira, and P. S.  
642 Pompeu. 2019. Stable isotopes and stomach content analyses indicate omnivorous  
643 habits and opportunistic feeding behavior of an invasive fish. *Aquat. Ecol.* 53: 365–  
644 381. doi:10.1007/s10452-019-09695-3

645 Casatti, L. 2010. Alterações no Código Florestal Brasileiro: impactos potenciais sobre a  
646 ictiofauna. *Biota Neotrop.* 10: 31–34. doi:10.1590/S1676-06032010000400002

647 Ceneviva-Bastos, M., C. G. Montaña, C. M. Schalk, P. B. Camargo, and L. Casatti. 2017.  
648 Responses of aquatic food webs to the addition of structural complexity and basal  
649 resource diversity in degraded Neotropical streams. *Austral Ecol.* 42: 908–919.  
650 doi:10.1111/aec.12518

651 Clément, J. C., R. M. Holmes, B. J. Peterson, and G. Pinay. 2003. Isotopic investigation of  
652 denitrification in a riparian ecosystem in western France. *J. Appl. Ecol.* 40: 1035–  
653 1048. doi:10.1111/j.1365-2664.2003.00854.x

654 Costa-Pereira, R., L. E. Tavares, P. B. de Camargo, and M. S. Araujo. 2017. Seasonal  
655 population and individual niche dynamics in a tetra fish in the Pantanal wetlands.  
656 *Biotropica* 49: 531–538. doi:10.1111/btp.12434

657 Delariva, R. L., and M. P. Neves. 2020. Morphological traits correlated with resource  
658 partitioning among small characin fish species coexisting in a Neotropical river.  
659 *Ecol. Freshwater Fish* 29: 640–653. doi:10.1111/eff.12540

660 Delariva, R. L., M. P. Neves, C. Larentis, B. C. K. Kliemann, M. C. Baldasso, and L. L.  
661 Wolff. 2018. Fish fauna in forested and rural streams from an ecoregion of high  
662 endemism, lower Iguaçu River basin, Brazil. *Biota Neotrop.* 18: e20170459.  
663 doi:10.1590/1676-0611-bn-2017-0459

664 Doi, H. 2009. Spatial patterns of autochthonous and allochthonous resources in aquatic  
665 food webs. *Popul. Ecol.* 51: 57–64. doi:10.1007/s10144-008-0127-z

666 Effert-Fanta, E. L., R. U. Fischer, and D. H. Wahl. 2022. Riparian and watershed land use  
667 alters food web structure and shifts basal energy in agricultural streams. *Aquat. Sci.*  
668 84(4): 61. doi: 10.1007/s00027-022-00895-y

669 Etard, A., A. L. Pigot, and T. Newbold. 2022. Intensive human land uses negatively affect  
670 vertebrate functional diversity. *Ecol. Lett.* 25: 330–343. doi:10.1111/ele.13926

671 Flood, P. J., W. F. Loftus, and J. C. Trexler. 2023. Fishes in a seasonally pulsed wetland  
672 show spatiotemporal shifts in diet and trophic niche but not shifts in trophic position.  
673 *Food Webs* 34: e00265. doi:10.1016/j.fooweb.2022.e00265

674 Fricke, R., W. N. Eschmeyer, and R. van der Laan (eds). 2023. Eschmeyer's catalog of  
675 fishes: genera, species, references.  
676 (<http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.as>).  
677 Electronic version accessed 12 September 2023.

678 Garcia, A. M., M. C. Claudino, R. Mont'Alverne, P. E. R. Pereyra, M. Copertino, and J. P.  
679 Vieira. 2017. Temporal variability in assimilation of basal food sources by an  
680 omnivorous fish at Patos Lagoon Estuary revealed by stable isotopes (2010–2014).  
681 *Mar. Biol. Res.* 13: 98–107. doi:10.1080/17451000.2016.1206939

682 Gerking, S. D. 1994. Feeding ecology of fishes. Academic Press, San Diego, CA.

683 Gutgesell, M. K., K. S. McCann, G. Gellner, K. Cazelles, C. J. Greyson-Gaito, C. Bieg, ...  
684 and B. C. McMeans. 2022. On the dynamic nature of omnivory in a changing world.  
685 *BioScience* biab144. doi:10.1093/biosci/biab144

686 Hellawell, J. M., and R. Abel. 1971. A rapid volumetric method for the analysis of the food  
687 of fishes. *J. Fish Biol.* 3: 29–37. doi:10.1111/j.1095-8649.1971.tb05903.x

688 Hill, W. R., M. G. Ryon, and E. M. Schilling. (1995). Light limitation in a stream  
689 ecosystem: responses by primary producers and consumers. *Ecology* 76: 1297–1309.  
690 <https://doi.org/10.2307/1940936>

691 Hill, W. R., S. E. Fanta, and B. J. Roberts. (2008). <sup>13</sup>C dynamics in benthic algae: effects of  
692 light, phosphorus, and biomass development. *Limnol. Oceanogr.* 53: 1217–1226.  
693 <https://doi.org/10.4319/lo.2008.53.4.1217>

694 Hoffman, J. C., M. E. Sierszen, and A. M. Cotter. 2015. Fish tissue lipid-C: N relationships  
695 for correcting  $\delta^{13}\text{C}$  values and estimating lipid content in aquatic food-web studies.  
696 Rapid Commun. Mass Spectrom. 29: 2069–2077. doi:10.1002/rcm.7367

697 Hyslop, E. J. 1980. Stomach content analysis: a review of methods and their application. J.  
698 Fish Biol. 17: 411–429. doi:10.1111/j.1095-8649.1980.tb02775.x

699 Ingram, T., R. Svanbäck, N. J. Kraft, P. Kratina, L. Southcott, and D. Schluter. 2012.  
700 Intraguild predation drives evolutionary niche shift in threespine stickleback.  
701 Evolution. 66: 1819–1832. doi:10.1111/j.1558-5646.2011.01545.x

702 Jackson, A. L., R. Inger, A. C. Parnell, and S. Bearhop. 2011. Comparing isotopic niche  
703 widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in  
704 R. J. Anim. Ecol. 80: 595–602. doi:10.1111/j.1365-2656.2011.01806.x

705 Kautza, A., and S. M. P. Sullivan. 2015. Shifts in reciprocal river-riparian arthropod fluxes  
706 along an urban-rural landscape gradient. Fresh. Biol. 60: 2156–2168.  
707 doi:10.1111/fwb.12642

708 Kemp, V. A., J. Grey, D. Hemprich-Bennett, S. J. Rossiter, O. T. Lewis, C. L. Wilkinson,  
709 ... and P. Kratina. 2023. Changes in trophic ecology of mobile predators in response  
710 to rainforest degradation. J. App. Ecol. 60: 1139–1148. doi:10.1111/1365-  
711 2664.14396

712 Kratina, P., R. M. LeCraw, T. Ingram, and B. R. Anholt. 2012. Stability and persistence of  
713 food webs with omnivory: is there a general pattern? Ecosphere 3: 50.  
714 doi:10.1890/ES12-00121.1

715 Larentis, C., B. C. K. Kliemann, M. P. Neves, and R. L. Delariva. 2022. Effects of human  
716 disturbance on habitat and fish diversity in Neotropical streams. Plos One 17:  
717 e0274191. doi:10.1371/journal.pone.0274191

718 Legendre, P., and M. J. Anderson. 1999. Distance-based redundancy analysis: testing  
719 multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69:  
720 1–24. doi:10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2

721 Lu, X., C. Gray, L. E. Brown, M. E. Ledger, A. M. Milner, R. J. Mondragón, ... and A. Ma.  
722 2016. Drought rewires the cores of food webs. Nat. Clim. Change 6: 875–878.  
723 doi:10.1038/nclimate3002

724 MacArthur, R. H. 1972. Geographical ecology: patterns in the distribution of species.  
725 Harper & Row.

726 Mackereth, F. J. H., J. Heron, and J. F. Talling. 1978. Water analysis: some revised  
727 methods for limnologists. Freshwater Biological Association.

728 Magioli, M., M. Z. Moreira, R. C. B. Fonseca, M. C. Ribeiro, M. G. Rodrigues, and K. M.  
729 P. M. Barros. 2019. Human-modified landscapes alter mammal resource and habitat  
730 use and trophic structure. *Proc. Natl. Acad. Sci. U.S.A.* 116: 18466–18472.  
731 doi:10.1073/pnas.1904384116

732 MapBiomas Project. 2021. Coleção 6 (1985-2020) da Série Anual de Mapas de Uso e  
733 Cobertura da Terra do Brasil. <https://mapbiomas.org/>

734 Martino, J. C., Z. A. Doubleday, and B. M. Gillanders. 2019. Metabolic effects on carbon  
735 isotope biomarkers in fish. *Ecol. Indic.* 97: 10–16. doi:10.1016/j.ecolind.2018.10.010

736 McCutchan, J. H. Jr., W. M. Lewis Jr., C. Kendall, and C. C. McGrath. 2003. Variation in  
737 trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102: 378–  
738 390. doi:10.1034/j.1600-0706.2003.12098.x

739 Mohebalian, P. M., L. N. Lopez, A. B. Tischner, and F. X. Aguilar. 2022. Deforestation in  
740 South America's tri-national Paraná Atlantic Forest: Trends and associational factors.  
741 *For. Policy Econ.* 137:102697. doi:10.1016/j.forpol.2022.102697

742 Moi, D. A., M. Barrios, G. Tesitore, M. Burwood, G. Q. Romero, R. P. Mormul, ... and F.  
743 T. Mello. 2023. Human land-uses homogenize stream assemblages and reduce  
744 animal biomass production. *J. Anim. Ecol.* 92: 1176–1189. doi:10.1111/1365-  
745 2656.13924

746 Navarro, A. B., M. Magioli, J. A. Bogoni, M. Z. Moreira, L. F. Silveira, E. R. Alexandrino,  
747 ... and K. M. P. M. B. Ferraz. 2021. Human-modified landscapes narrow the isotopic  
748 niche of neotropical birds. *Oecologia* 196: 171–184. doi:10.1007/s00442-021-04908-  
749 9

750 Neres-Lima, V., E. F. Brito, F. A. Krsulovic, A. M. Detweiler, A. E. Hershey, and T. P.  
751 Moulton. 2016. High importance of autochthonous basal food source for the food  
752 web of a Brazilian tropical stream regardless of shading. *Int. Rev. Hydrobiol.* 101:  
753 132-142. doi:10.1002/iroh.201601851

754 Neves, M. P., P. Kratina, R. L. Delariva, J. I. Jones, and C. B. Fialho. 2021. Seasonal  
755 feeding plasticity can facilitate coexistence of dominant omnivores in Neotropical  
756 streams. *Rev. Fish Biol. Fish.* 31: 417–432. doi:10.1007/s11160-021-09648-w

757 Nielsen, J. M., E. L. Clare, B. Hayden, M. T. Brett, and P. Kratina. 2018. Diet tracing in  
758 ecology: Method comparison and selection. *Methods Ecol. Evol.* 9: 278–291.  
759 doi:10.1111/2041-210X.12869

760 Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlim, ... and H.  
761 Wagner. 2020. The vegan package. *Community Ecology Package*. R package version  
762 2.5-5.

763 Pelster, B., C. M. Wood, B. Speers-Roesch, W. R. Driedzic, V. Almeida-Val, and A. Val.  
764 2015. Gut transport characteristics in herbivorous and carnivorous serrasalmid fish  
765 from ion-poor Rio Negro water. *J. Comp. Physiol.* 185: 225–241.  
766 doi:10.1007/s00360-014-0879-z

767 Perkins, D. M., I. Durance, F. K. Edwards, J. Grey, A. G. Hildrew, M. Jackson, ... and G.  
768 Woodward. 2018. Bending the rules: exploitation of allochthonous resources by a  
769 top-predator modifies size-abundance scaling in stream food webs. *Ecol. Lett.* 21:  
770 1771–1780. doi:10.1111/ele.13147

771 Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, ... and E. J.  
772 Ward. 2014. Best practices for use of stable isotope mixing models in food-web  
773 studies. *Can. J. Zool.* 92: 823–835. doi:10.1139/cjz-2014-0127

774 Price, E. L., M. Sertić Perić, G. Q. Romero, and P. Kratina. 2019. Land use alters trophic  
775 redundancy and resource flow through stream food webs. *J. Anim. Ecol.* 88: 677–  
776 689. doi:10.1111/1365-2656.12955

777 R Core Team. 2022. R: A language and environment for statistical computing. R  
778 Foundation for Statistical Computing, Vienna, Austria. [http://www.r-](http://www.r-project.org/index.html)  
779 [project.org/index.html](http://www.r-project.org/index.html)

780 Rapisarda, P., F. Camin, S. Fabroni, M. Perini, B. Torrisi, and F. Intrigliolo. 2010.  
781 Influence of different organic fertilizers on quality parameters and the  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ ,  
782  $\delta^2\text{H}$ ,  $\delta^{34}\text{S}$ , and  $\delta^{18}\text{O}$  values of orange fruit (*Citrus sinensis* L. Osbeck). *J. Agric. Food*  
783 *Chem.* 58: 3502–3506. <https://doi.org/10.1021/jf903952v>

784 Reis, A. D. S., M. P. Albrecht, and S. E. Bunn. 2020. Food web pathways for fish  
785 communities in small tropical streams. *Freshwater Biol.* 65: 893–1907.  
786 doi:10.1111/fwb.13471

787 Riseng, C. M., M. J. Wiley, R. W. Black, and M. D. Munn. 2011. Impacts of agricultural  
788 land use on biological integrity: a causal analysis. *Ecol. Appl.* 21: 3128–3146.  
789 doi:10.1890/11-0077.1

790 Sigman, D. M., K. L. Casciotti. 2001. Nitrogen isotopes in the ocean. *Encyclopedia of*  
791 *ocean sciences*, 3<sup>rd</sup> Edition, 3: 1884–1894. doi: /10.1016/B978-0-12-409548-  
792 9.11605-7

793 Stock, B. C., and B. X. Semmens 2016. Unifying error structures in commonly used  
794 biotracer mixing models. *Ecology* 97: 2562–2569. doi:10.1002/ecy.1517

795 Sweeney, B. W., T. L. Bott, J. K. Jackson, L. A. Kaplan, J. D. Newbold, L. J. Standley, ...  
796 and R. J. Horwitz. 2004. Riparian deforestation, stream narrowing, and loss of stream  
797 ecosystem services. *Proc. Natl. Acad. Sci.* 101(39): 14132–14137. doi:  
798 10.1073/pnas.0405895101

799 Van Valen L. 1965. Morphological variation and width of ecological niche. *Am. Nat.* 99:  
800 377–390. doi:10.1086/282379

801 Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980.  
802 The river continuum concept. *Can. J. Fish. Aquat. Sci.* 37: 130–137.  
803 doi:10.1139/f80-017

804 Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag.

805 Wilkinson, C. L., K. W. Chua, R. Fiala, J. H. Liew, V. Kemp, A. Hadi Fikri, ... and D. C.  
806 Yeo. 2021. Forest conversion to oil palm compresses food chain length in tropical  
807 streams. *Ecology* 102: e03199. doi:10.1002/ecy.3199

808 Wohl, E. 2006. Human impacts to mountain streams. *Geomorphology* 79(3-4): 217–248.  
809 doi: 10.1016/j.geomorph.2006.06.020

810 Wood, S., and F. Scheipl. 2017. *gamm4: Generalized Additive Mixed Models using 'mgcv'*  
811 *and 'lme4'*. R package version 0.2-5.

812

813 **Acknowledgements**

814 We thank the Universidade Estadual do Oeste do Paraná for support and infrastructure  
815 during field campaigns. We are also indebted to the members of the LIEB, S. Pini and J. F.  
816 Neves for assistance with field campaigns, the Laboratório de Ictiologia Universidade  
817 Federal do Rio Grande do Sul for help with taxonomic identification, C. Larentis performed  
818 nutrients analyses, the Conselho Nacional de Desenvolvimento Científico e Tecnológico  
819 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for a PhD scholarship  
820 (CNPq Proc. 152847/2016-2, CAPES Proc. 88887.363419/2019-00, to MPN), the  
821 Programa de Pós-Graduação em Biologia Animal/UFRGS, and L. Nash for comments on  
822 the manuscript. P.K. gratefully acknowledges funding from the Royal Society (grant  
823 NAF\R2\180791).