Supplementary material

Article

Integrative Analysis to Manage Aquatic Resources Based on Fish Feeding Patterns in Neotropical Rivers

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Supplementary Material S1: Sampling area

A rapid bioassessment protocol was applied to characterize the four sample sites.

Table S1 Description of the physical features of four sites sampled in the Verde River.

Sampling sites	Width (m)	Depth (m)	Riparian vegetation integrity	Riverbank stability	Riverbed complexity
Headwater	5.83±0.94	1.28±0.14	Comprised by steppe-type vegetation, it is relatively preserved and in accordance with Brazilian laws for riparian vegetation width (30 m in both riverbanks).	Riverbanks are stable, presenting more than 90% of plant cover. There are several individual plants in the land-water boundary with submerged structures (*e.g.* roots, branches and leaves).	Riverbed is composed by rock-outcrops covered by sand and plant debris from riparian vegetation (*e.g.* leaves, branches and trunks).
Upper middle stretch	6.04±1.99	1.33±0.30	Formed by steppe-type and Mixed Ombrophilous Forest (MOF) vegetations, it is relatively preserved. Shrub layer is disrupted in MOF. Riparian vegetation width is lesser than Brazilian laws in both margins.	Riverbanks are stable, presenting 70-90% of plant cover. Riverbank soils are mainly comprised by rocky outcrops. In some portions, they are less shallow and support plants with submerged structures in the land-water boundary.	Riverbed is heterogeneous and structured in riffle-pool sequences. Riffles are associated with rocky substrates and cobbles, presenting aquatic plants attached to them. Pools are found in the outsides of the river bends and rifles, presenting gravels, coarse sand and plant debris.
Lower middle stretch	10.03±0.75	0.41±0.13	Comprised by MOF, it is few preserved, showing disrupted canopy and shrub layers. Its width is lesser than Brazilian laws in both margins.	Riverbanks are unstable, presenting less than 50% of plant cover, with exposed and eroded soils. There are individual plants and tree debris in the land-water boundary that accumulate garbage in their branches.	Riverbed varies from heterogeneous to homogeneous, presenting stretches in advanced siltation process. Riffle stretches are short, presenting rock outcrops, cobbles, gravels, and sand. There are few stretches with aquatic plants. Pool stretches are sandy covered, presenting gravels and plant debris. In lentic stretches, substrates are sludge.
River mouth	13.08±2.19	0.87±0.41	Formed by MOF, it is degraded, presenting disrupted canopy and absence of shrub layer. Despite of it, riparian vegetation width is in accordance with Brazilian laws in both margins.	Riverbanks are unstable, presenting less than 50% of plant cover, with exposed and eroded soils. There are few individual plants alive in the land-water boundary, which accumulate debris and garbage in their branches.	Riverbed is unstable and homogeneous, in advanced siltation process. Riverbed presenting great amounts of sludge. Riffles, pools, and aquatic plants are absent.

Supplementary Material S2: PERMANOVA

The sets of %O_{fi} values were subjected to variability analysis under interspecific, spatial and temporal aspects were by means of a three-way PERMANOVA (Euclidian' distance; 9,999 permutations) using arcsine data transformation. PERMANOVA was performed using the function *adonis* (*vegan* package in R-statistical software (R 3.5.3 version). From the PERMANOVA results, *VR* fish community showed substantial differences in diet trends linked to interspecific variations ($F = 7.5733$; $p < 0.0001$) and to species-sites interactions ($F = 1.6531$; $p = 0.0111$), but not related to temporal variation (Table 2SM).

Table S2 Results of permutational multivariate analysis of variance (PERMANOVA) examining interspecific, spatial, and temporal differences in the diet of the fish community from the Verde River. Df = degrees of freedom; SS = sum of squares; MS = mean square; R^2 = variance explained by the model. * Indicate statistical significance (* for $p \leq 0.05$; *** for $p \leq 0.001$)

Source	**Df**	**SS**	**MS**	**F value**	**R²**	***p*-value**
Species	6	76.588	12.7646	7.5733	0.31144	0.0001***
Sites	3	7.384	2.4615	1.4604	0.03003	0.0886
Months	11	24.704	2.2458	1.3325	0.10046	0.0727
Species:Sites	8	22.290	2.7863	1.6531	0.09064	0.0111*
Species:Months	37	64.421	1.7411	1.0330	0.26196	0.4317
Sites:Months	20	31.990	1.5995	0.9490	0.13008	0.6230
Residuals	11	18.540	1.6855		0.07539	
Total	96	245.918			1.00000	

This result was partially expected, because fish species in VR naturally showed longitudinal distribution governed by several geomorphological factors, as follows:

At VR headwater and upper middle stretch there is a large cascade (about 10 m height) that does not allow fish upstream displacements. In these areas (including the lower middle stretch too), smaller cascades and canyons favored the colonization and maintenance of several benthic and nektobenthic fishes, including species of Loricariidae, Callichthyidae, Heptapteridae, Parodontidae, Characidae, and others. This pattern of occurrence was also influenced by river capture events (RCE) among VR and neighboring basins. Mixture events after RCE were responsible for the conservative molecular patterns found in populations of *G. brasiliensis* and *Hoplias* cf. *malabaricus* in VR and Ribeira River basin (RRB). Molecular evidence supported that *P. paranae* is endemic from VR and showed the occurrence of *Apareiodon* sp.

Another important factor influencing fish diversity in VR is its good connectivity with the main channels of the Pitangui and Tibagi rivers. It allowed species inhabiting these rivers, such as *P.* aff. *fasciatus*, to reach UPRE headwaters during their reproductive displacements. This scenario confirms the strong influence of the palaeogeomorphology in the longitudinal distribution and structure of VR fish community, reflecting directly in its feeding ecology and interspecific variability. The current environment state had created a longitudinal gradient in VR, affecting the fish distribution due to differences in the connectivity among sampling sites and their structural features, also affecting fish diet.

Supplementary Material S3: fish species size structure

In Verde River (25°01'S – 25°07'S; 49°59'W – 50°09'W, Brazil, Neotropical region), seven fish species presented a minimal number of five sampled stomachs with food. Among them, *Apareidon* sp. summed 58 individuals (body length range 7.30-15.30 cm; mean = 11.79 cm), *Psalidodon* aff. *fasciatus* included seven specimens (8.00-12.20 cm; mean = 9.37 cm), *P. paranae* summed 198 individuals (4.60-13.40 cm; mean = 9.41 cm), *Corydoras ehrhardti* included 44 specimens (2.70-7.20 cm; mean = 5.34 cm), *Geophagus brasiliensis* summed 59 individuals (6.60-22.80 cm; mean = 11.18 cm), *Hypostomus strigaticeps* included seven specimens (12.70-39.20 cm; mean = 21.48 cm) and *Rhamdia quelen* included five individuals (12.00-29.50 cm; mean = 18.85 cm) (Figure SM1).

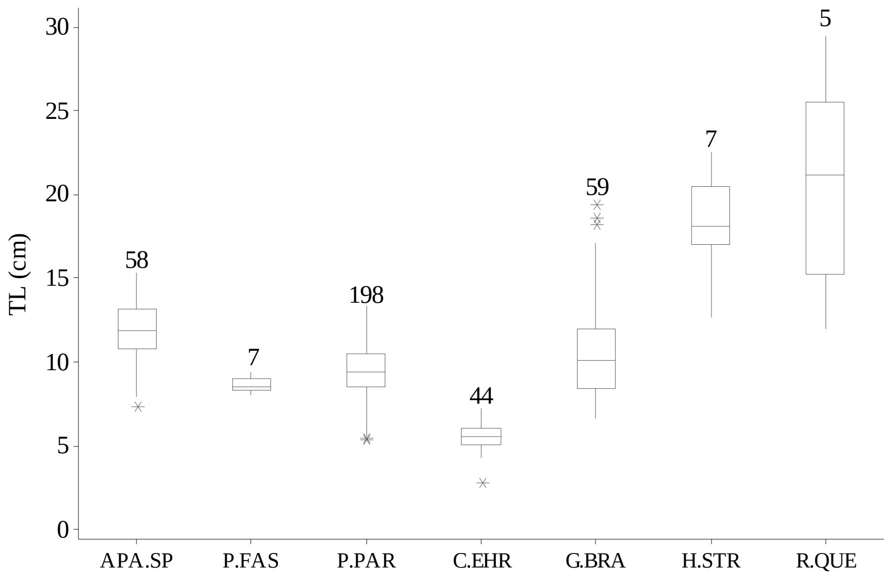

Figure S1. Fish species caught in the Verde River that presented at least five stomachs with food. APASP = *Apareidon* sp., P.FAS = *Psalidodon* aff. *fasciatus*, P.PAR = *P. paranae*, C.EHR = *Corydoras ehrhardti*, G.BRA = *Geophagus brasiliensis*, H.STR = *Hypostomus strigaticeps*, and R.QUE = *Rhamdia quelen* (* indicates outliers).

Supplementary Material S4: frequency of occurrence of consumed foods

Analysis of the 483 stomach contents provided 30 food categories in the fish community of Verde River (Table 3SM). Diptera was the most recurrent consumed food. *Psalidodon paranae* showed omnivore feeding behaviour with a pattern mixing plants (aquatic and terrestrial angiosperms) and invertebrates (Diptera, Coleoptera, Hymenoptera and fragments). *Psalidodon* aff. *fasciatus* and *R. quelen* mainly consumed invertebrates (highlighting Diptera and Coleoptera), indicating insectivory at the water column. *Apareiodon* sp., *C. ehrhardti*, *H. strigaticeps* and *G. brasiliensis* mainly consumed invertebrates (Diptera) and sediments, indicating insectivore feeding behaviours related to benthophagy.

Table S3 (first part). Diet of the fish community from the Verde River. *P.fas* = *Psalidodon* aff. *fasciatus*; *P.par* = *P. paranae*; *Apa.sp* = *Apareiodon* sp.; *C.ehr* = *Corydoras ehrhardti*; *H.str* = *Hypostomus strigaticeps*; *R.que* = *Rhamdia quelen*; *G.bra* = *Geophagus brasiliensis*. $\%O_{fi}$: frequency of occurrence; Bold values highlighted the most consumed food categories ($\%O_{fi} > 30\%$).

Food categories	***P.fas*** $\%O_{fi}$	***P.par*** $\%O_{fi}$	***Apa.sp*** $\%O_{fi}$	***C.ehr*** $\%O_{fi}$	***H.str*** $\%O_{fi}$	***R.que*** $\%O_{fi}$	***G.bra*** $\%O_{fi}$
Cyanobacteria		2.3	1.5		11.1		
Bacillariophyta	6.7	11.4	13.2	4.5	33.3		1.3
Plantae							
Bryophyta		1.4	1.5				
Aquatic angiosperm	20.0	**42.9**	7.4	1.1			
Terrestrial angiosperm	20.0	**41.6**	10.3	7.9		12.5	28.0
Plant fragments	20.0	12.8	7.4	1.1	11.1		1.3
Mollusca							
Gastropoda		0.5					
Annelida							
Oligochaeta		3.2					1.3
Crustacea							
Isopoda	6.7	0.5	1.5				
Chelicerata							
Acarina				2.2			6.7
Araneae	6.7	2.7					
Insecta							
Blattodea		0.5					
Coleoptera	**33.3**	**40.6**	2.9	4.5		**37.5**	26.7
Diptera	**86.7**	**41.1**	**77.9**	**87.6**	**55.6**	**75.0**	**77.3**
Ephemeroptera		15.5	5.9	2.2	11.1		
Hemiptera		14.6					2.7
Hymenoptera	**40.0**	**37.9**	1.5			**37.5**	8.0
Isoptera		0.9					
Lepidoptera		6.8				12.5	
Neuroptera		0.5					
Odonata	6.7	5.0				12.5	
Orthoptera		0.5				25.0	1.3
Trichoptera	6.7	7.8	11.8	5.6			4.0
Other invertebrates							
Invertebrate eggs		2.7					1.3
Invertebrate fragments	26.7	**37.9**	1.5	24.7			17.3
Undetermined invertebrates	**46.7**	8.2	7.4	1.1			5.3

Table S3 (second part). Diet of the fish community from the Verde River. *P.fas* = *Psalidodon* aff. *fasciatus*; *P.par* = *P. paranae*; *Apa.sp* = *Apareiodon* sp.; *C.ehr* = *Corydoras ehrhardti*; *H.str* = *Hypostomus strigaticeps*; *R.que* = *Rhamdia quelen*; *G.bra* = *Geophagus brasiliensis*. $\%O_{fi}$: frequency of occurrence; Bold values highlighted the most consumed food categories ($\%O_{fi} > 30\%$).

Food categories	***P.fas*** $\%O_{fi}$	***P.par*** $\%O_{fi}$	***Apa.sp*** $\%O_{fi}$	***C.ehr*** $\%O_{fi}$	***H.str*** $\%O_{fi}$	***R.que*** $\%O_{fi}$	***G.bra*** $\%O_{fi}$
Vertebrata							
Teleostei	6.7	18.3	1.5	1.1		12.5	8.0
Other sources							
Plastic		0.5					
Sediments	13.3	21.9	**91.2**	**36.0**	**100**		**32.0**
Undetermined matter	6.7	4.6	1.5	1.1			1.3
Number of analyzed stomachs	15	219	68	89	9	8	75

Supplementary Material S5: feeding profile of ten identified feeding trends

The ten *FT*s were differentiated by relatively high consumptions of some food types highlighted by box plots (Figure S2).

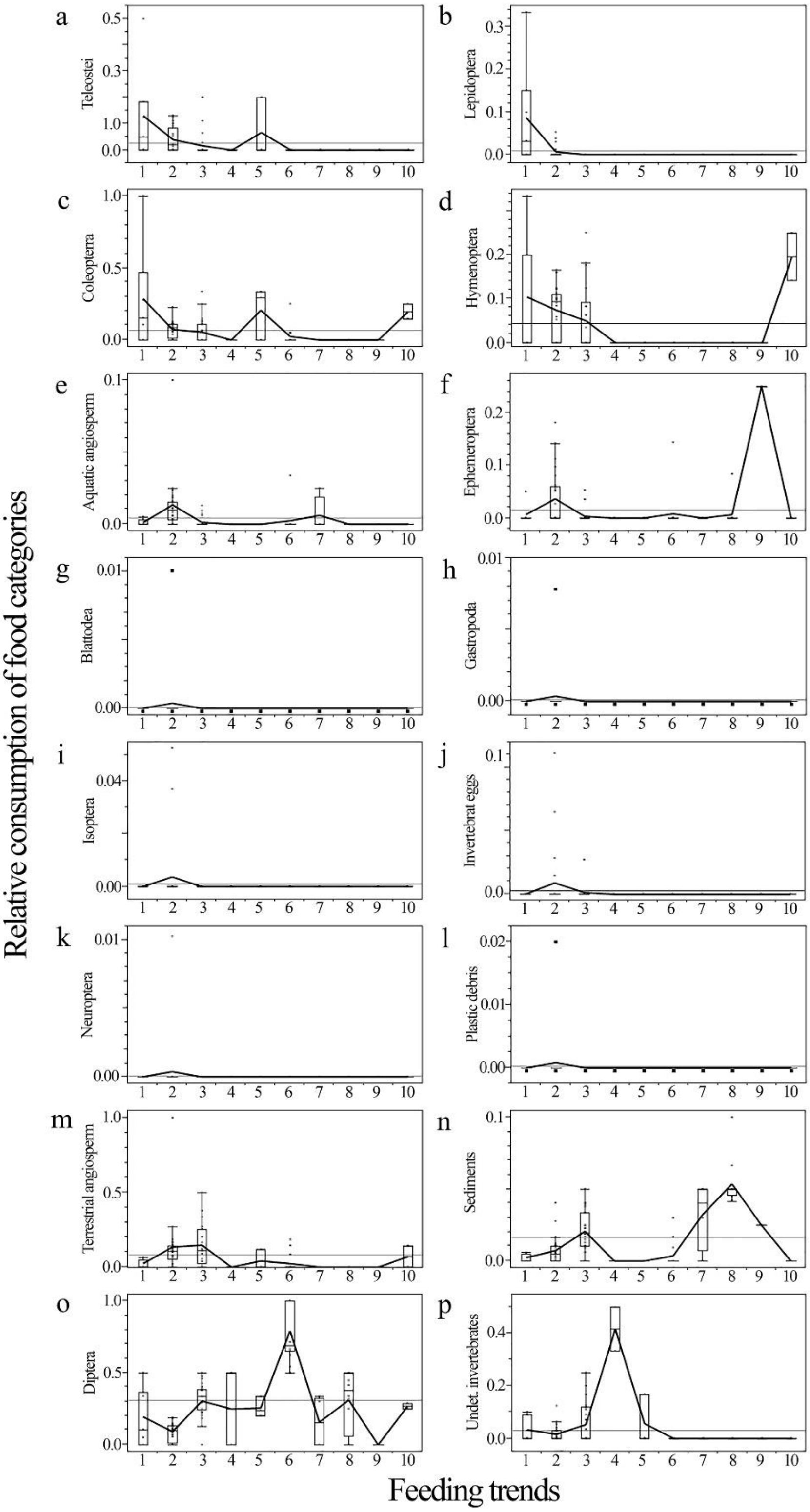

Figure S2 (first part). Composition and the recurrence of food categories by feeding trends for the fish community from the Verde River.

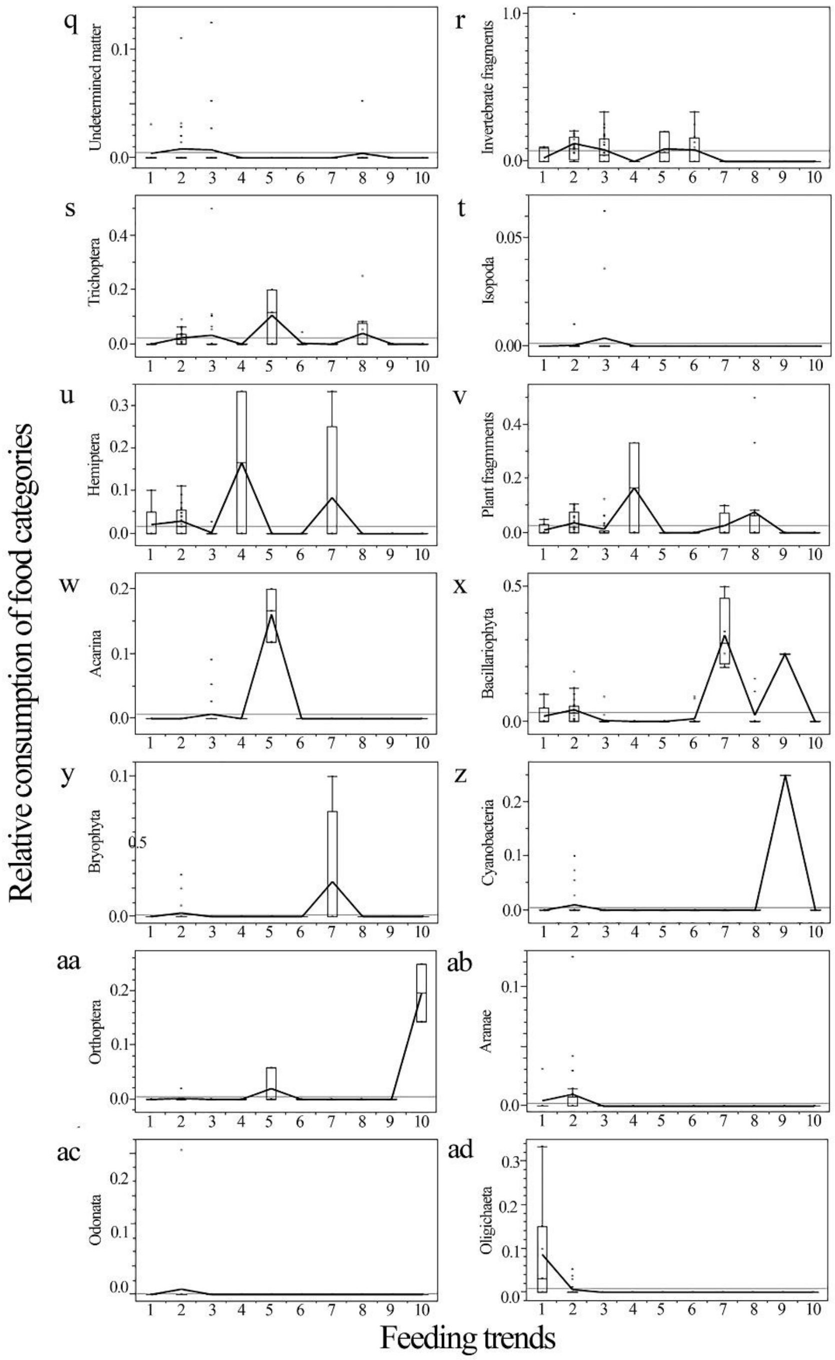

Figure S2 (second part). Composition and the recurrence of food categories by feeding trends for the fish community from the Verde River.