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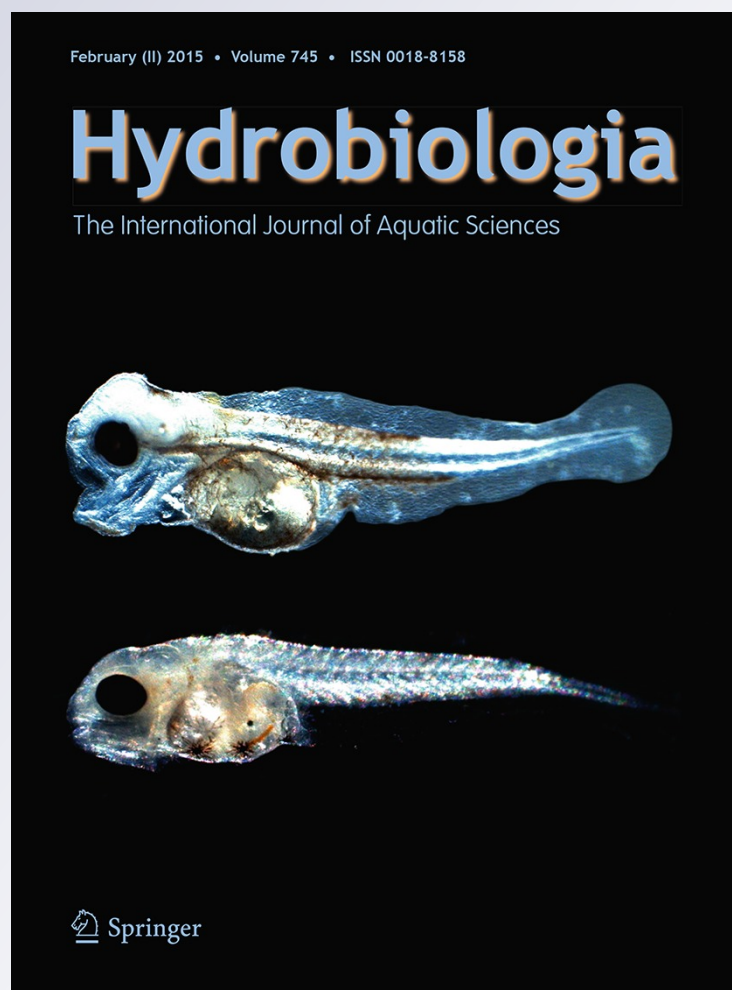
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Native fish larvae take advantage of introduced mussel larvae: field evidence of feeding preferences on veligers of the introduced freshwater bivalve *Limnoperna fortunei*

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Abstract Previous work has shown that the invasive bivalve *Limnoperna fortunei* has had a measurable impact on local food webs, but knowledge of the trophic interactions involved is still very limited. On the basis of samples collected along the lower Paraguay-middle Paraná rivers, we studied the feeding behavior and selectivity of larval fish, with emphasis on veligers of the introduced bivalve *L. fortunei*. Among feeding larvae (i.e., without a yolk sac), 16.5% had only *Limnoperna* veligers in their guts, while 15.6% had veligers and some other prey. Half of the fish taxa recorded (8 out of a total of 16) consumed

Limnoperna veligers. The Paraguay and Paraná rivers differed strongly in the proportions of fish larvae that consumed veligers: 14 and 68%, respectively. This difference paralleled the availability of veligers in the water column, which was significantly lower in the Paraguay ($0.8 \pm 0.5 \text{ ind. l}^{-1}$) than in the Paraná River ($5.5 \pm 2.3 \text{ ind. l}^{-1}$). Conversely, cladocerans, originally the staple food of fish larvae, were more abundant in the Paraguay (consumed by 48% of the individuals) than in the Paraná River (26%). These results indicate that, when widely available, *Limnoperna* veligers largely replace the original prey of fish larvae, especially in their younger stages (protolarvae).

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Introduction

Limnoperna fortunei, an Asian mytilid bivalve, was first discovered in the Río de la Plata estuary, Argentina, around 1990 (Pastorino et al., 1993). Two decades later, *Limnoperna* had colonized practically all the Río de la Plata basin, including areas in Argentina, Bolivia, Paraguay, Uruguay, and Brazil (Darrigran, 2002; Boltovskoy et al., 2006). On suitable substrata, *Limnoperna* beds can reach very high

densities, up to over 200,000 ind. m⁻² (Sylvester et al., 2007), resulting in high veliger concentrations in the water column (occasionally up to over 100 larvae l⁻¹, normally around 7–20 larvae l⁻¹ (Boltovskoy et al., 2009; Nakano et al., 2010). Local fishes have rapidly adjusted to this new resource: at least 50 species have been observed to feed on adult mussels (Boltovskoy & Correa, 2014), and 11 larval fishes on *Limnoperna* veligers (Paolucci et al., 2007). Laboratory experiments indicate that predation pressure of fish larvae on *Limnoperna* veligers depends on the relative availability of the prey (Paolucci et al., 2010a, b), which has been shown to vary greatly, both in time and in space (Boltovskoy et al., 2009; Darrigran et al., 2011; Oliveira et al., 2011; Boltovskoy et al., 2013). However, in wild communities, the implications of these experimental results have not been investigated.

Here, we estimate dietary differences in indigenous larval fishes exposed to dissimilar densities of *Limnoperna* veligers, analyzing the mechanisms involved in the responses of native predators, and interpreting the impacts on both prey and predators. The objectives of our study were: (1) To compare the importance of mussel veligers in the diet of fish larvae from the Lower Paraguay and the Middle Paraná rivers; (2) To analyze changes in the trophic selectivity of larval fishes as a function of their developmental stage and veliger availability; (3) To assess the selectivity of the fish larvae for indigenous versus introduced prey items; and (4) To analyze temporal differences in the impact of veligers on the diet of larval fishes in the Parana River by comparing these results with a similar database collected 5 years earlier.

Methods

The study area included the Lower Paraguay River, from the city of Asunción, Paraguay (25°15'S, 57°38'W) to its confluence with the Paraná River (27°17'S, 58°37'W); and the Middle Paraná River from its confluence with the Paraguay River to around 32°18'S, 60°41'W (Fig. 1). This ca. 1,000 km stretch forms part of one of the largest lotic systems in the world, discharging around 20,000 m³ s⁻¹ of water into the Río de la Plata estuary. In both rivers, *L. fortunei* colonized areas upstream of our sampling sites since at least 1997 (Boltovskoy et al., 2006). The Paraguay and Paraná rivers host over 400 fish species

(Quirós et al., 2007), including several large migratory forms such as *Prochilodus lineatus*, *Leporinus obtusidens*, *Schizodon fasciatus*, *Pimelodus maculatus*, *P. albicans*, *Sorubim lima*, *Pseudoplatystoma fasciatum*, *P. corruscans*, *Salminus brasiliensis*, and others. Mature adults of these species migrate upstream along the Paraguay-Paraná system, spawn (chiefly between October–March), and larvae drift passively downstream until they reach a marginal wetland where they feed and grow for 1–2 years (de Resende, 2003). Young adults return to the main river channel and drift downstream until sexual maturation, when the upstream migration begins (de Resende, 2003). Unlike the Paraguay-Paraná system, fish migrations between the Middle and the Upper Paraná rivers are interrupted by a major barrier: the Yacretá dam (Fig. 1).

Daytime samples of drifting ichthyoplankton (2–3 per site) and zooplankton (1–2 per site) were collected at 15 locations along the Paraguay and Paraná rivers (Fig. 1). All samples were collected daily during a cruise of the “Dr. Leloir” research vessel (Prefectura Naval Argentina—Argentine Coast Guard) between 28 October and 9 November 2005. For the ichthyoplankton, flow-metered stationary nets (conical, 0.35 m mouth diameter, 1 m-long, 300 µm-mesh) were deployed for periods of ~10 min in open areas with ~0.3–1.0 m s⁻¹. Zooplankton samples were obtained filtering 100 l of bucket-collected surface water through a 25 µm-mesh net. Immediately after collection samples were fixed with 5% formaldehyde.

Larval fish and zooplankton (*Limnoperna* veligers, cladocerans, copepods, and insect larvae) were counted and measured under a binocular microscope equipped with an eyepiece micrometer. Counts were based on the entire volume retrieved (when total numbers of organisms were below 50) or, for larger samples, on subsamples obtained with a Folsom splitter (McEwen et al., 1954) containing at least 50 organisms. In order to better account for the larger (and scarcer) organisms, most feeding fish larvae were removed from the sample prior to subsampling (the abundance figures reported account for this method). This procedure allowed analyzing ca. 100% of the fish larvae with gut contents present in the materials collected.

Identifications of the fish larvae were performed according to Nakatani et al. (2001). Developmental stages were assigned to larval fish as follows: proto-larvae (no median fins); mesolarvae (with rays in some

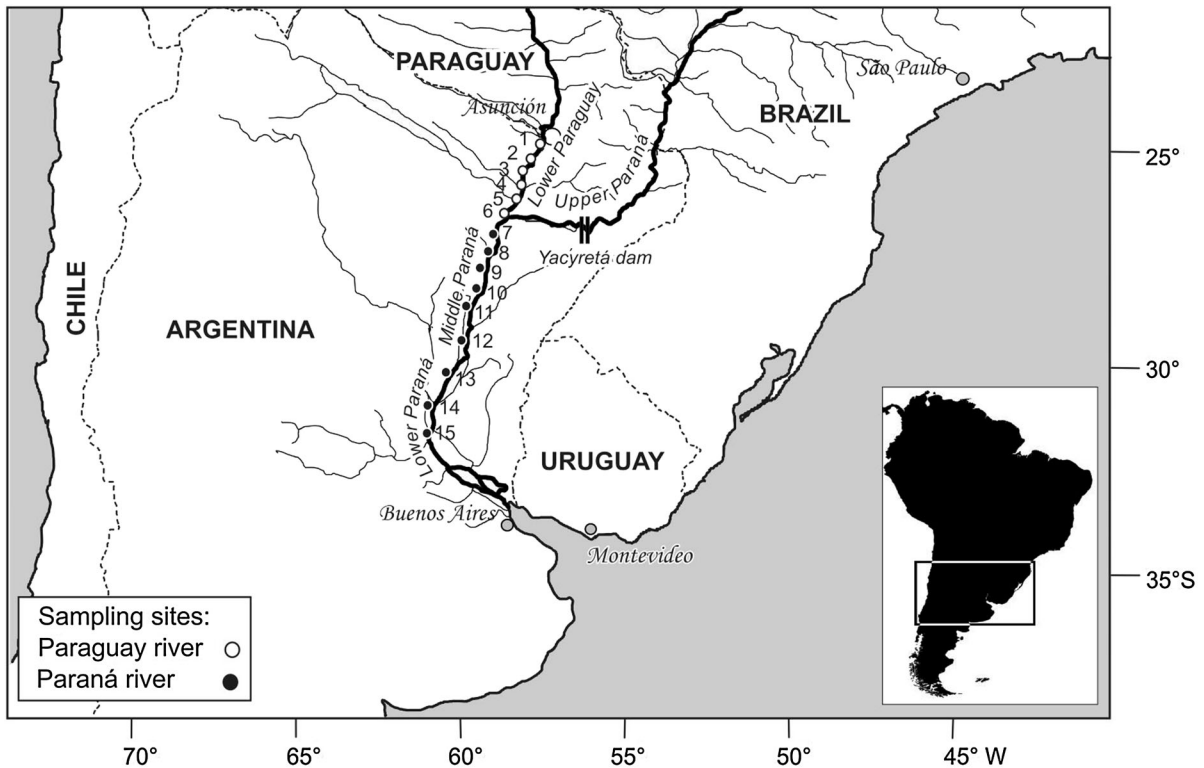


Fig. 1 Location of sampling sites along the Paraguay (*empty circles*; Stations 1–6) and Paraná (*filled circles*; Stations 6–15) rivers

median fins); and metalarvae (well-developed rays in all median fins) (Snyder, 1983).

Larval fish were dissected, and their gut contents examined under a binocular microscope. Food items were identified, measured, quantified, and assigned to one of six categories: *Limnoperna* veligers, cladocerans, copepods, immature insects, fish larvae, and algae. Unidentifiable remains were combined into an “Unidentified material” category. Food items with clear signs of having been captured shortly before or during sample collection (undigested and located in the mouth or anterior section of the digestive tract), were excluded from the analyses in order to avoid the bias resulting from accidental predation after capture (Fuentes & Quiroga, 2012).

For each prey item, frequency of occurrence (FO) was estimated as the number of stomachs containing one or more individuals of the corresponding prey as a proportion of all stomachs with some gut contents (Hyslop, 1980). For cladocerans, copepods, and larval insects, the size-to-weight conversions of Dumont et al. (1975), Bottrell et al. (1976) and

Masaferró et al. (1991) were used to estimate biomass. Mean dry weight of *Limnoperna* veligers was based on 400 straight-hinged and unbanded veligers oven-dried at 60°C to constant weight. For the most abundant fish larvae (*Prochilodus lineatus* and Pimelodidae) found in the stomach contents of other fish larvae, the dry weight was estimated in a similar manner using a total of 383 individuals grouped into twenty 0.5 mm length categories. Fish larvae were classified on the basis of the biomass of the main prey items consumed.

Estimates of the food available to the fish larvae at each site were based on the zooplankton samples, except for larval fish (as prey for other larvae), for which the ichthyoplankton samples were used.

Selectivity values for each prey item were assessed using a Chi-square-based index (Pearre, 1982):

$$C = \pm \left[\frac{(|a_d \cdot b_e - a_e \cdot b_d| - (n/2))^2}{(a \cdot b \cdot e \cdot d)} \right]^{1/2}$$

Table 1 General information on the diet of fish larvae and zooplankton recorded in the Paraguay and Paraná rivers. Biomass values are in μg dry weight

Variable	River		Mann–Whitney <i>U</i> test	
	Paraguay (Stations 1–6)	Paraná (Stations 7–15)	Z value	<i>P</i> value
Larvae analyzed	1,275	806		
Total larvae with yolk sac [1]	673 [52.8%]	286 [35.5%]		
Total feeding fish larvae	602	520		
Larvae without gut content [2]	448 [74.4%]	443 [85.2%]		
Larvae with gut contents [2]	154 [25.6%]	77 [14.8%]	−0.94	0.3458
Protolarvae [3]	73 [47.1%]	55 [71.4%]	−1.30	0.1949
Mesolarvae [3]	62 [40.5%]	15 [19.5%]	1.47	0.1407
Metalarvae [3]	19 [12.4%]	7 [9.1%]	–	–
Zooplankton (ind. $l^{-1} \pm \text{SD}$)			Mann–Whitney <i>U</i> test	
			Z value	<i>P</i> value
Veligers of <i>L. fortunei</i>	0.8 \pm 0.5	5.5 \pm 2.3	−3.97	0.0001
Cladocerans	0.5 \pm 0.3	0.3 \pm 0.4	2.86	0.0043
Copepods	0.6 \pm 0.4	0.5 \pm 0.5	1.31	0.1891
Larval fish ($\times 10^{-2}$)	2.4 \pm 1.8	0.8 \pm 0.4	1.65	0.0990
Insects	0.6 \pm 0.9	0.1 \pm 0.1	0.95	0.3410
Frequency of occurrence			<i>t</i> test (<i>df</i> = 13)	
			<i>t</i> value	<i>P</i>
<i>L. fortunei</i> [3]	22 [14.2%]	53 [68.4%]	−3.57	0.0034
Cladocerans [3]	74 [48.0%]	20 [26.3%]	3.42	0.0046
Copepods [3]	17 [11.0%]	2 [2.6%]	2.34	0.0356
Larval fish [3]	27 [17.5%]	6 [7.8%]	1.41	0.1818
Insects [3]	24 [15.5%]	6 [7.8%]	3.17	0.0074
Mean biomass (μg dry weight)			Mann–Whitney <i>U</i> test	
			Z value	<i>P</i>
<i>L. fortunei</i> [4]	21.4 [1.4%]	112.5 [19.8%]	−2.24	0.0251
Cladocerans [4]	93.8 [4.5%]	12.3 [2.1%]	2.65	0.0080
Copepods [4]	43.5 [2.1%]	2.0 [0.3%]	1.94	0.0518
Larval fish [4]	1896.3 [92.1%]	441.0 [77.5%]	0.94	0.3458
Insects [4]	2.0 [0.1%]	0.4 [0.1%]	1.65	0.9896

[1] Proportion of totals analyzed at each site; [2] Proportion of all feeding fish larvae; [3] Proportion of all non-empty guts analyzed at each site; and [4] Proportion of total food biomass at each site

Where:

$$e = a_e + b_e$$

$$a = a_d + a_e$$

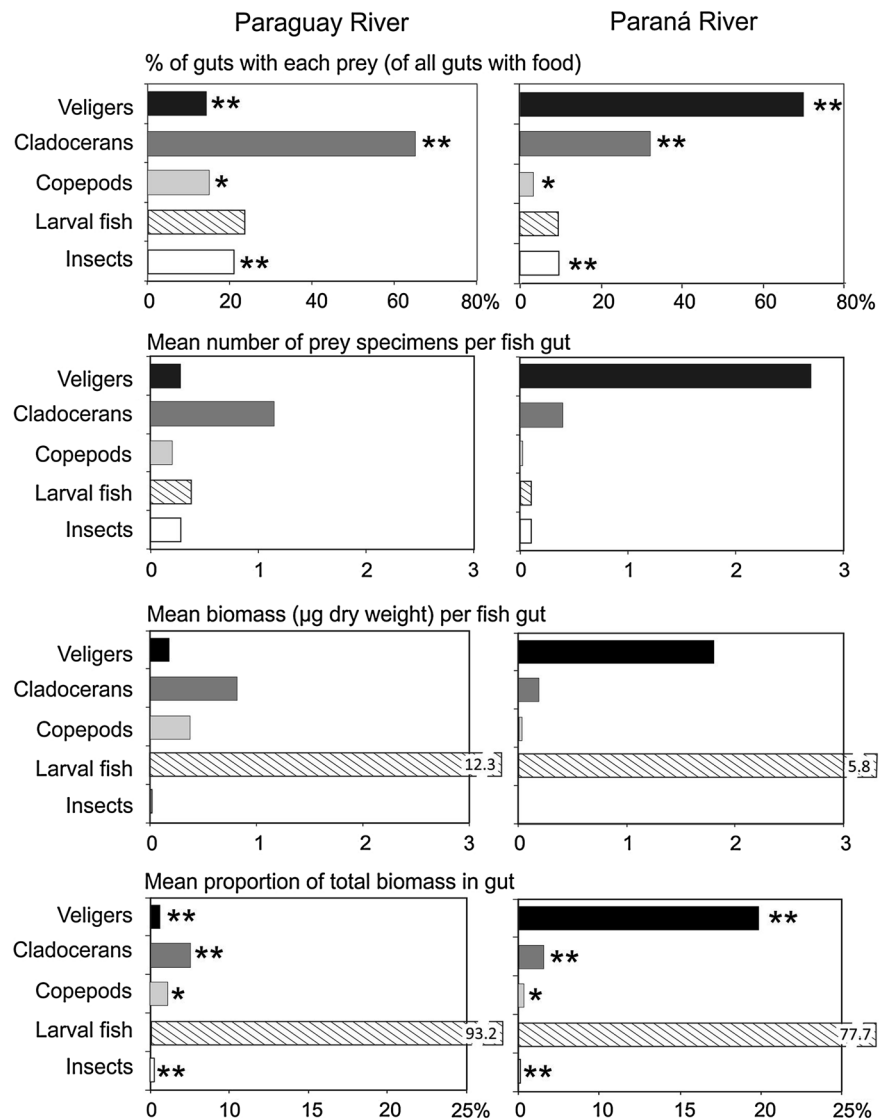
$$n = a + b$$

$$b = b_e + b_d$$

where a_d and a_e are the numbers of specimens of prey a in the diet and in the environment, respectively; b_d and b_e , are the summed numbers

$$d = a_d + b_d$$

Fig. 2 Mean contribution of the five main food items to the diet of fish larvae recorded in the Paraguay and Paraná rivers. Statistically significant differences between the two rivers are denoted with asterisks: * $P < 0.05$ or ** $P < 0.01$, t test, and Mann–Whitney test for FO and biomass, respectively



of all other prey items in the diet and in the environment, respectively. This index ranges between 1 and -1; with significant positive and negative values indicating that the item is preferentially preyed upon or preferentially rejected, respectively. Zero indicates lack of selectivity. This expression was chosen because it is not affected by the relative abundance of uncommon prey items. In addition, it allows statistical estimates of significance for any sample size, which is especially important for the scarcest prey items (Lazzaro, 1987; Pearre, 1982). When the expected frequency of a given prey item was above 5%, the

corresponding selectivity value was statistically assessed using χ^2 test and Yate's correction for continuity (Pearre, 1982; Zar, 1999).

Differences in zooplankton densities, frequency, and biomass of prey items between rivers and between developmental stages were tested using t tests after arc-sin transformation of the frequency data (Sokal & Rohlf, 1979). When the assumptions for parametric tests were not fulfilled, the differences were assessed with non-parametric techniques (Mann–Whitney test) (Daniel, 1978). A significance level of 0.05 or 0.01 was used for all statistical tests.

The large proportion of yolk sac fish larvae, as well as, fish larvae with empty guts recorded by this and previous studies (de Resende, 2003; Paolucci et al., 2007) in the main channel of the Paraná and Paraguay rivers indicates that the most abundant fish species feed chiefly in the marginal environments (Welcomme, 1979; Fuentes & Espinach Ros, 1998). In contrast, the main channel is an important nursery habitat for several large migratory species (Rossi, 2001; Rossi et al., 2007), but since their proportion of all fishes is low, even large sample sizes yield few feeding larvae. In our samples, only ~10% of the >2,000 fish larvae analyzed contained some gut contents (see below). In order to assess whether our main conclusions could have been biased by these low sample sizes, we repeated all statistical analyses on a reduced dataset where 50% of the data had been eliminated at random. The same procedure (randomly eliminating different subsets of data) was replicated 10 times for each analysis (See online Resource 1 and 2). The results of this exercise showed that all major statistically significant differences obtained with the reduced datasets paralleled those based on the entire dataset, suggesting that the trends described are strong and consistent, and were not affected by sample size.

Results

Of the 2081 fish larvae analyzed (~140 per site), 959 (46%) were feeding endogenously (yolk sac), and 891 (43%) had empty guts. In most analyses, these larvae were only considered in estimates of fish larvae as prey of other fish larvae, but not in those aimed at assessing larval fish diets and selectivity (Table 1). Among the 1,122 larvae without a yolk sac, proportions of those that had some gut contents varied widely between species, ranging from over 50% (in Siluriformes), to below 2% (some Characiformes) (Table 2).

Of the 231 larval fish with gut contents, 38 (16.5%) fed exclusively on *Limnoperna* veligers, and 36 (15.6%) had veligers, and some other prey in their guts. Veligers were found in 32% of the feeding larvae analyzed, whereas cladocerans were recorded in 40%. Biomass of veligers was first in importance in 66% (24 cases) of the specimens that consumed *Limnoperna* veligers and any other food item (36 larval fish).

On the basis of the biomass of the different prey items consumed, the 16 fish taxa recorded were

classified into three groups (Table 2). The largest group, encompassing seven taxa (*P. lineatus*, *Apareiodon affinis*, *Plagioscion* sp, *Catathyrnidium jenymsii*, protolarae of Anostomidae and Pimelodidae, and other small unidentified siluriformes), had a diet mostly composed of cladocerans and/or veligers, and occasionally copepods and insects (Table 2). The second group, with six taxa (*Iheringichthys labrosus*, *S. lima*, *Luciopimelodus pati*, *Pimelodus* sp., members of the Fam. Characidae, and other small unidentified Characiformes), consumed chiefly copepods and/or larval fish, and to a lesser extent cladocerans, veligers, and occasionally other items such as insects (Table 2). The third group, with three taxa (*S. brasiliensis*, *Pseuplatystoma* sp., and *Rhaphiodon vulpinus*), was largely piscivorous consuming larvae of *P. lineatus* and Pimelodidae.

Paraguay versus Paraná rivers

The Paraguay River yielded higher abundances and diversities of fish larvae than the Paraná (Tables 1 and 2), but the proportions of larvae with gut contents did not differ significantly between the two rivers (Table 1). On the other hand, the composition of the zooplankton and larval fish diets was strikingly different (Table 1; Figs. 2 and 3). On average, veligers were over 6 times more abundant in the zooplankton of the Paraná than in the Paraguay River ($P < 0.0001$, Mann–Whitney U test; Table 1; Fig. 3). Cladocerans, copepods, larval fish, and insects were more abundant in the Paraguay River, but only for cladocerans, were these differences statistically significant (Table 1).

Both pooled data and average values per fish species indicate that veligers were ca. 5 times more common in fishes caught in the Paraná (68.4% of which consumed *Limnoperna*) than in the Paraguay River (14.2%) ($P < 0.01$, t test; Fig. 2; see Online Resources 1 and 2 for results based on reduced dataset). Concomitantly, veliger biomass represented a higher proportion of overall gut contents in fishes from the Paraná (mean: 19.8%) than in those from the Paraguay River (mean: 1.4%; Fig. 2; $P < 0.01$, Mann–Whitney U test; see Online Resources 1 and 2). As opposed to veligers, cladocerans were more frequently consumed and represented a higher proportion of the gut contents in the Paraguay River ($P = 0.0046$, t test, and $P = 0.0080$, Mann–Whitney U test for FO and biomass, respectively; Table 1;

Table 2 Diet of the ichthyoplankton recovered in the Paraguay and Paraná rivers

	Larvae retrieved [%]	Larvae analyzed		Frequency of occurrence [4]				Mean biomass [%]																					
		Paraguay River [1]	Paraná River [1]	N [2]	With gut contents [3]	<i>L. fortunei</i>	Cladocerans	Copepods	Larval fish	Insects	Unidentif material	<i>L. fortunei</i>	Cladocerans	Copepods	Larval fish	Insects													
Group 1: main diet <i>Limnoperna</i> larvae and/or cladocerans																													
<i>Prochilodus lineatus</i>	30.2	4.79	4.44	625	7	5	0	0	1	1	0	0	0	1	3.3	0.0	0.7	0.0	0.0	0.0	[0.0]	[83.5]	[0.0]	[16.5]	[0.0]	[0.0]	[0.0]		
<i>Apareitodon affinis</i>	0.4	0.05	0.06	9	3	0	1	0	0	0	0	0	0	3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	[100.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	
Anostomidae	31.9	9.25	0.96	404	2	1	0	0	0	0	0	0	0	1	0.7	0.0	0.0	0.0	0.0	0.0	0.0	[100.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	
Siluriformes NI	2.8	0.43	0.39	54	9	0	1	0	0	0	0	1	8	0.0	0.3	0.0	0.0	0.0	0.0	0.1	0.0	[100.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.1]	
Pimelodidae NI	3.5	0.66	0.46	208	76	46	29	0	4	0	0	8	13	89.8	22.2	5.4	0.0	0.0	0.5	0.0	0.0	[78.9]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[21.1]	
<i>Plagioscion</i> sp	0.2	0.07	-	17	4	0	4	0	0	0	0	0	0	0	0.0	12.4	0.0	0.0	0.0	0.0	0.0	[18.8]	[76.2]	[4.6]	[0.0]	[0.0]	[0.0]	[0.4]	
<i>Catathridium jerynsii</i>	0.1	0.02	0.01	7	4	1	1	0	0	0	0	0	0	0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	[100.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	
Group 2: main diet Copepods and/or larval fish																													
Fam.	0.4	0.13	-	20	5	0	2	0	2	2	0	2	1	0.0	1.6	0.0	10.6	0.0	0.6	0.0	0.0	[86.5]	[13.5]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]
Characidae		[0.3]		[17]	[29.4]	[0.0]	[40.0]	[0.0]	[40.0]	[40.0]	[0.0]	[40.0]	[0.0]	[20.0]	[0.0]	[12.5]	[82.5]	[0.0]	[4.9]			[0.0]	[0.0]	[12.5]	[0.0]	[0.0]	[0.0]	[4.9]	
Characiformes NI	28.6	7.31	1.13	527	9	1	3	1	2	2	1	2	2	5.4	1.2	4.3	36.3	0.1	0.1	0.0	0.0	[88.8]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	
<i>Pimelodus</i> spp.	0.1	0.03	0.01	16	11	1	9	0	1	1	3	3	1	0.7	18.2	3.3	191.9	0.2	0.2	0.0	0.0	[18.0]	[18.0]	[9.3]	[0.0]	[0.0]	[0.0]	[0.3]	
<i>Iheringichthys labrosus</i>	0.5	0.1	0.04	72	54	17	34	0	5	5	12	8	7	33.5	269.5	8	730.5	0.5	0.5	0.0	0.0	[90.0]	[0.0]	[10.0]	[0.0]	[0.0]	[0.0]	[0.1]	
<i>Sorubim lima</i>	0.1	0.03	0.001	13	8	0	4	0	2	2	0	0	6	0.0	2.7	7.9	0.0	0.0	0.0	0.0	0.0	[62.9]	[14.8]	[0.8]	[0.0]	[0.0]	[0.0]	[0.0]	
<i>Luciopimelodus pati</i>	0.2	0.05	-	28	15	2	4	0	2	2	0	5	5	2.6	2.9	4.3	0	0.2	0.0	0.0	0.0	[50.0]	[0.0]	[25.7]	[0.0]	[0.0]	[0.0]	[0.0]	
Group 3: main diet fish larvae																													
<i>Pseudoplatystoma</i> spp.	0.2	0.06	-	27	5	0	0	0	0	0	4	0	1	0.0	0.0	0.0	181.0	0.0	0.0	0.0	0.0	[20.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	
<i>Rhaphiodon vulpinus</i>	0.2	0.07	-	32	12	0	1	0	0	0	9	0	3	0.0	0.3	0.0	472.5	0	0.0	0.0	0.0	[8.3]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	

Table 2 continued

	Larvae retrieved [%]		Density (larvae m ⁻³)		Larvae analyzed		Frequency of occurrence [4]						Mean biomass [%]							
	Paraguay River [1]	Paraná River [1]	N [2]	With gut contents [3]	L. fortunei	Cladocerans	Copepods	Larval fish	Insects	Unidentified material	L. fortunei	Cladocerans	Copepods	Larval fish	Insects	L. fortunei	Cladocerans	Copepods	Larval fish	Insects
<i>Salminus brasiliensis</i>	0.4	0.06	22	7	0	0	0	6	0	1	0.0	0.0	0.0	441.1	0.0	0.0	0.0	0.0	441.1	0.0
	[0.1]	[0.3]	[12]	[58.3]	[0.0]	[0.0]	[0.0]	[85.7]	[0.0]	[14.2]	[0.0]	[0.0]	[0.0]	[100.0]	[0.0]	[0.0]	[0.0]	[0.0]	[100.0]	[0.0]

[1] Proportions of larvae retrieved in each river; [2] Total feeding larvae; [3] Proportions of all feeding larvae; and [4] Proportions of all non-empty guts analyzed. Biomass values are in µg dry weight

Fig. 2). This trend between rivers was observed for 5 of the 6 commonest fish species recorded on this survey (Fig. 4).

The higher incidence of veligers (in terms of both numbers and biomass) in fishes from the Paraná River was particularly noticeable among the early developmental stages. Proto- and mesolarval stages of fishes from the Paraná River displayed significantly higher values of frequency of occurrence than those collected in the Paraguay River ($P < 0.03$ and $P < 0.04$, Mann–Whitney U test for proto and mesolarval fish, respectively; Fig. 5). In both rivers, the relative FO and biomass of food items consumed varied with fish size: small larvae (5–6 mm in length) relied chiefly on veligers, whereas larger ones (> 11.3 mm) gradually shifted to cladocerans and fish larvae (Fig. 5).

Differences between the two rivers surveyed were also strongly reflected by the alimentary selectivity of their fish larvae (Fig. 6). For most fishes, selectivity toward *Limnoperna* larvae was positively associated with the absolute abundance of veligers in the water column (Fig. 3 and 6); thus, the more veligers were available, the more fishes relied on them for their feeding. In the Paraguay River, none of the fishes assayed showed statistically significant positive selectivity for veligers, whereas in the Paraná, significant positive figures were found for *I. labrosus* and Pimelodidae. In the Paraguay River, cladocerans were usually favored over other prey, often accounting for significantly higher proportions of all items in the stomachs than in the water column. In contrast, in the Paraná, higher proportions in the diet than in the water column were almost invariably shown by veligers (Fig. 6).

Discussion

A salient result of our survey is that the importance of veligers of *Limnoperna fortunei* in the diet of larval fishes significantly differs between the Middle Parana and the Lower Paraguay River. On average for all the fish that had food in their alimentary tract, veligers were 5 times more important as a food resource in the Paraná. With the only exception of *S. lima*, which never consume veligers, all of the most abundant fishes present in both rivers had a noticeably higher incidence of veligers in the Paraná than in the Paraguay River (Fig. 4). Between-river differences

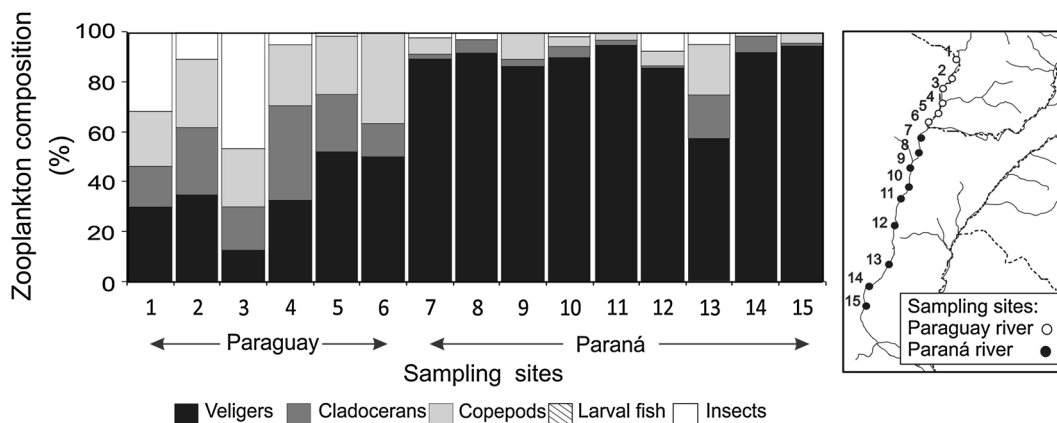


Fig. 3 Proportions of the main zooplankton components (in numbers) along the surveyed area (Paraguay River, stations 1–6, and Paraná River, stations 6–15)

in the taxonomic composition of the ichthyoplankton were minor (Table 2) and, therefore, unlikely to explain this contrast. Proportions of protolarval fish stages, which feed preferentially on veligers (Table 1), were 1.5 times higher in the Paraná, but this difference was too low to account for the dissimilarity in the diet.

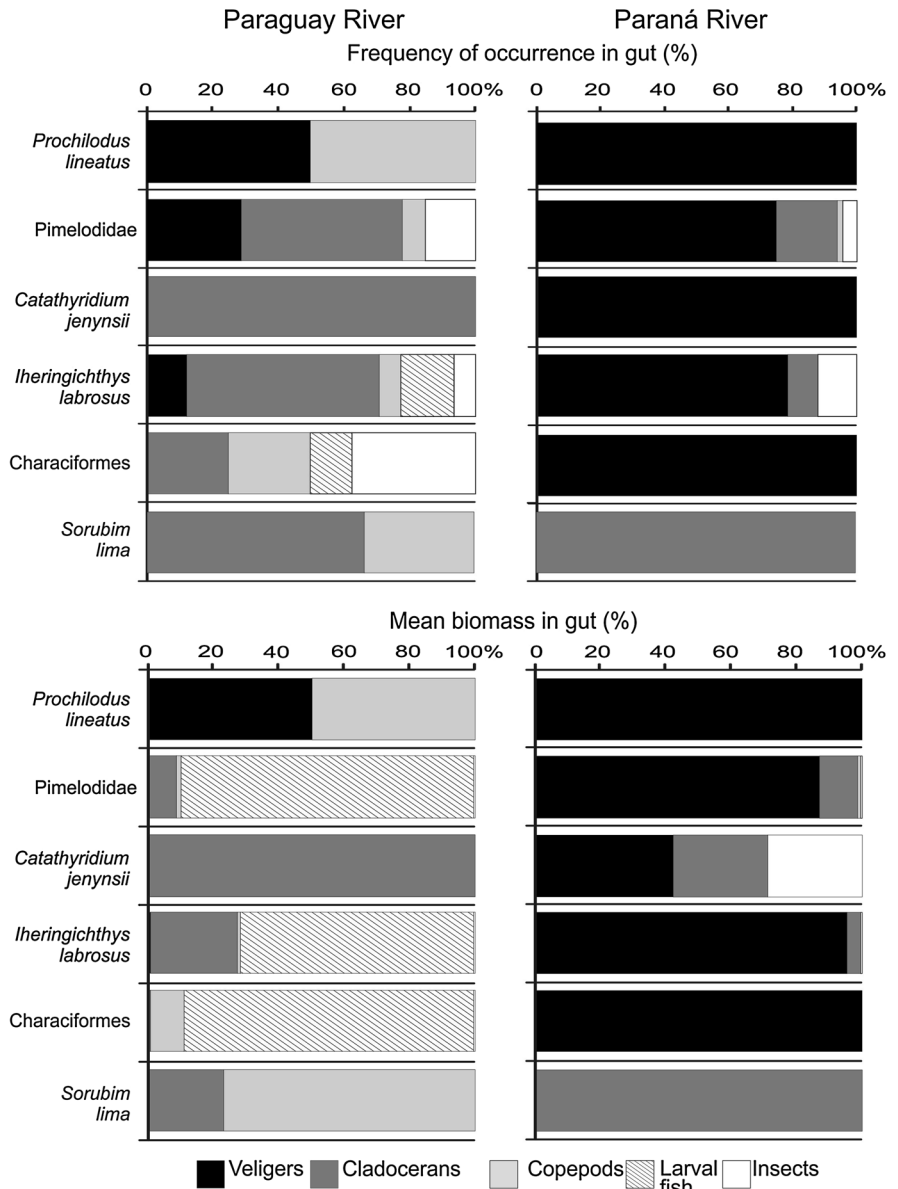
Conversely, the density of veligers (and, therefore, their availability as a food resource) was almost 7 times higher in the Paraná than in the Paraguay River (Table 1; Fig. 3). This contrast seems to be a permanent feature of the area: mean annual larval densities in the Paraná are 6–7 larvae/l, whereas for the Paraguay, they are ca. ten times lower (Boltovskoy et al., 2009; Oliveira et al., 2011). This difference has been attributed to less suitable conditions in the Paraguay River, including reduced availability of hard substrates for adult mussels (Amsler & Drago, 2009), lower phytoplankton and calcium concentrations (Oliveira et al., 2010a, 2011), and recurrent extensive anoxic events leading to mass mortalities of sessile adults (Oliveira et al., 2010b). We contend that these higher densities of veligers in the Paraná are most probably responsible for the dissimilar feeding strategies of fish larvae in the two rivers.

Experimental results with fish larvae of *Prochilodus lineatus* indicate that increasing the proportion of veligers in the plankton strongly enhances their consumption and selectivity by the fish (Paolucci et al., 2010a). Prey density plays a central role in the selectivity of the predators, mainly due to an increase

in the rate of predator–prey encounters (Deudero & Morales-Nin, 2001; Graeb et al., 2004; Fulford et al., 2006). In fish larvae, this effect can be strong enough to shift the selectivity behavior from neutral or even negative—at low prey densities, to positive—when prey abundance is increased (Dou, 2000; Islam & Tanaka, 2008). Thus, our results are likely the expression of this phenomenon, where larvae of the same fish species avoid feeding on *Limnoperna* veligers when these are scarce (Paraguay River), but select them over other prey when they become abundant.

Investigation of predator–prey relationships can offer insightful perspectives of evolutionary mechanisms (Abrams, 2000). In this context, analyses involving introduced species, either as predators or as prey, constitute unique opportunities for testing hypotheses and furthering our understanding of co-evolutionary processes (Sax et al., 2007; Carlsson & Strayer, 2009). Current theory recognizes several mechanisms by which natives may adapt to introduced species (e.g., learning, phenotypic plasticity, and contemporary evolution), modulating the strength, or even direction of the interactions (Carlsson & Strayer, 2009). However, most work on the impact of introduced species on indigenous communities has been centered on the effects of introduced predators on indigenous prey, while the effects of introduced prey on population traits of their indigenous predators are perceived as a less acute problem, and therefore, has received less attention (Carlsson et al., 2009). Yet

Fig. 4 Average frequency of occurrence and mean biomass of prey items of the commonest larval fish species recorded



examples of interactions between species that do not share an evolutionary history resulting in a positive outcome for the indigenous one, also termed evolutionary release, are not uncommon (Carroll et al. 1997, 1998; Schlaepfer et al., 2005; Strauss et al., 2006). In some cases, adjustment of the predator to the new prey involves an adaptive lag (Carlsson & Strayer, 2009), but in others, including the one discussed here, replacement of the original diet is very fast (Mills et al., 1992; Coulas et al., 1998; MacIsaac et al., 1999).

The introduction of *Limnoperna* in the Paraguay-Paraná watershed is a relatively recent phenomenon: colonization of the Pantanal, in the Upper Paraguay River, occurred around 2000 (Boltovskoy et al., 2006). Comparison of our current results, based on samples collected in 2005, with a similar study carried out on materials collected in the Middle and Lower Paraná in 2000–2001 (Paolucci et al., 2007), allows assessing whether the incidence of veligers on the diet of larval fishes has increased (for example, because of

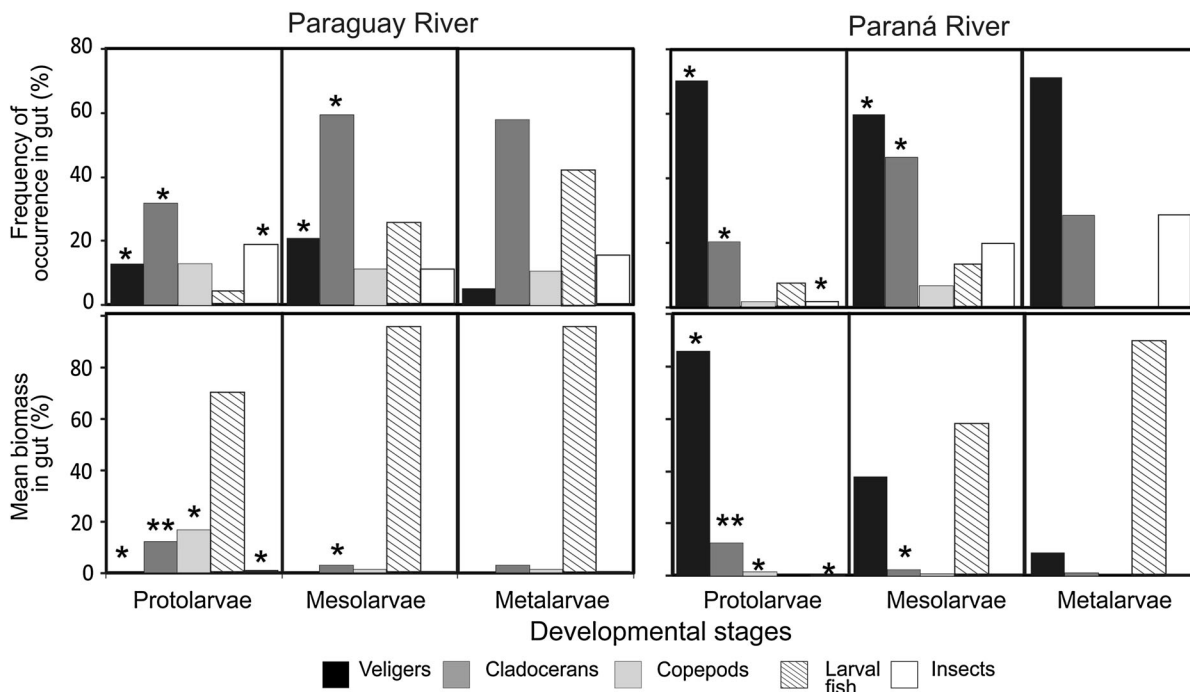


Fig. 5 Average frequency of occurrence and biomass of prey items found in fish protolarvae, mesolarvae, and metalarvae. Asterisks denote significant feeding differences between rivers

for the same developmental stage at $*P < 0.05$ or $**P < 0.01$, Mann–Whitney U test

expanding mussel distribution), or if it has already stabilized. In 2000–2001, the proportions of fish larvae with veligers in their guts (56.0%) were similar to those recorded in 2005 (68.4%). The biomass of veligers, as a proportion of all food items, was also very similar in 2000–2001 (20.3%) and in 2005 (19.8%). With the only exception of *C. jenynsii*, which was not recorded at all in 2000–2001, the inventory of taxa that consume veligers was identical in the two periods. This result underscores the feeding plasticity and speed in the response of these native predators to the new trophic resource represented by *Limnoperna* larvae.

In agreement with previous data (Boltovskoy et al., 2006; Paolucci et al. 2007, 2010a, b), these results suggest that the presence of *Limnoperna* has favored native fishes. However, consumption of veligers by fish larvae is but one of many changes brought about by the invasion, many of which may have impacts as large as, or even larger, than the supply of veligers as a food

resource. For example, although *Limnoperna* larvae may conceivably have decreased fish predation pressure on other zooplankton, veligers and adult mussels may compete for food with native zooplankton. Furthermore, adult mussels have been shown to consume some of the zooplankters that their veligers have replaced in the fish diets analyzed (Rojas Molina et al., 2009). Significant indirect effects may also play key roles in these interactions. For example, it has been shown that *Limnoperna* boosts toxic cyanobacterial blooms (Cataldo et al., 2012), which can cause large-scale fish mortalities. In Japan, invasion by *Limnoperna* has been associated with the introduction of new fish parasites (Ogawa et al., 2004; Urabe et al., 2008). Thus, while natural experiments created by invasions are useful for investigating the role of evolution in community assembly (Strauss et al., 2006), modifications associated with species introductions are often too many and too intricate to be amenable to straightforward cause–effect interpretations.

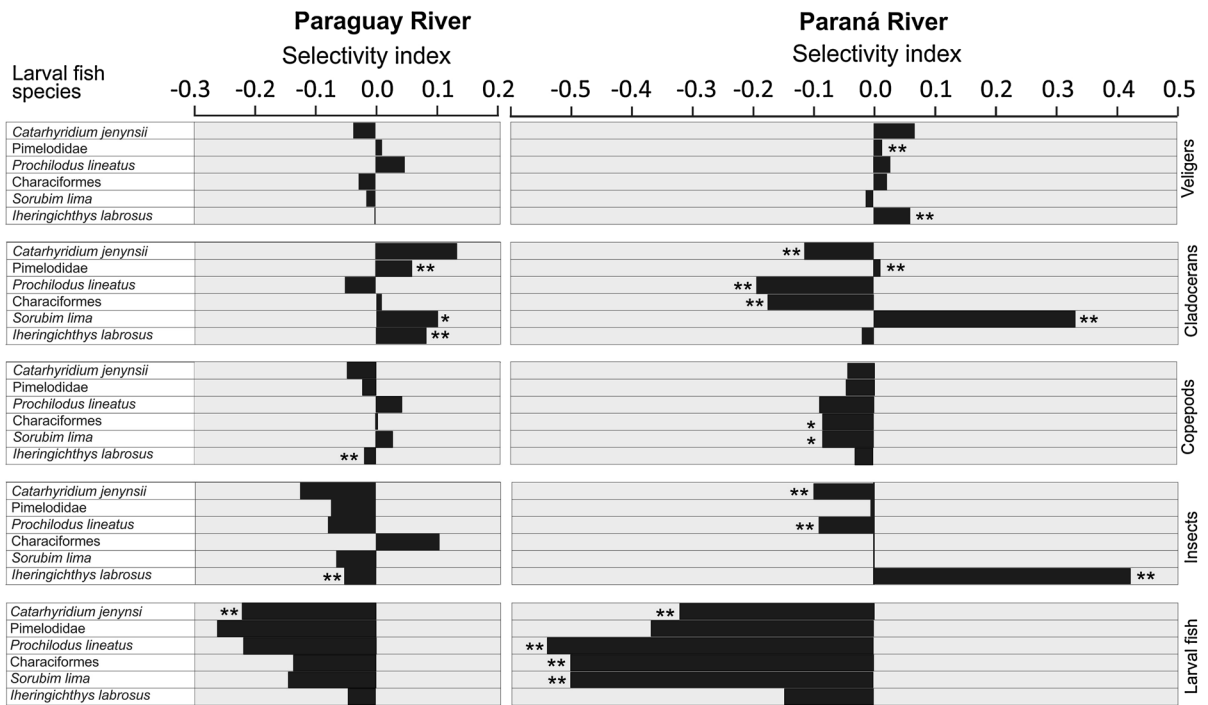


Fig. 6 Mean selectivity indices for the prey items assessed by the most abundant species present in the Paraguay and Paraná rivers. Asterisks denote significant differences between

proportions of the corresponding prey in the water column and in the gut content at $*P < 0.05$ or $**P < 0.01$ (Chi square tests)

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