Prey selection by larvae of *Prochilodus lineatus* (Pisces: Curimatidae): indigenous zooplankton versus veligers of the introduced bivalve *Limnoperna fortunei* (Bivalvia: Mitilidae)

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Abstract We studied experimentally the feeding selectivity of larvae of *Prochilodus lineatus* (Pisces), with particular emphasis on the role of veligers of the exotic bivalve *Limnoperna fortunei*. Three concentrations of veligers were offered to three developmental stages of *P. lineatus.* Veliger concentrations were: (1) higher than in the field ("enriched", 0.09 ind. ml^{-1}), (2) unmodified from field conditions ("normal", 0.06 ind. ml^{-1}), and (3) lower than in the field ("low", 0.02 ind. ml^{-1}). Fish developmental stages were protolarvae (approx. 10 days old), mesolarvae (17 days), and metalarvae (25 days). Proportions (in terms of numbers and biomass) and selectivity values were calculated for each prey item evaluated: veligers, small cladocerans + nauplii, medium-sized cladocerans, copepodits, and large cladocerans + copepods. Protolarvae and mesolarvae consumed veligers almost exclusively (88–90%, both in numbers and in biomass) when offered prey enriched in veligers, whereas for metalarvae veligers represented only 16.0% of the food consumed. At lower veliger concentrations, only protolarvae preferred *Limnoperna* veligers, whereas older fishes switched gradually to crustacean plankton. We conclude that veligers are preferred by the early fish developmental stages, and we speculate that this may be because their slower swimming makes them easier to capture than planktonic crustaceans. However, as fish larvae grow larger, veligers become too small a prey for their energetic needs, and they switch to larger items like cladocerans and copepods. We anticipate that this new and abundant food resource has an important impact on the survival and growth of *P. lineatus.*

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Introduction

One of the most important and extensively studied effects of the introduction of freshwater exotic bivalves is their impact on trophic relations and food web structures (Karatayev et al. 2002; Feyrer et al. 2003; Barnard et al. 2006). Interactions with the plankton have two opposing components: on one

hand the bivalve's high grazing rates affect plankton abundance and composition, on the other it contributes to planktonic communities with its free-swimming veliger larvae. These veligers often outnumber crustacean and rotifer zooplankton (Karatayev et al. 2007), and they represent an abundant food resource for native species (Paolucci et al. 2007). While assessment of the final overall influence of these new interactions is elusive, both adverse (French and Bur 1996; Nagelkerke and Sibbing 1996; Pothoven and Madenjian 2008) and positive consequences for the communities involved have been described (Strayer et al. 2004; Boltovskoy et al. 2006; Sylvester et al. 2007).

The Asian mytilid bivalve *Limnoperna fortunei* (Dunker 1857)*, introduced in the Río de la Plata watershed around 1990, was found to be an important prey item for at least 17 adult and 11 larval fishes, including some of the most ecologically and economically important species (Boltovskoy et al. 2006; Paolucci et al. 2007; Cantanhêde et al. 2008). Some of them, like the larval stages of *Prochilodus lineatus* (sábalo), *Pseudoplatystoma* spp. (surubí), *Sorubim lima* (cucharón), and *Iheringichthys labrosus* (bagre trompudo) were found to feed on veligers of *L. fortunei*, while others, like *Micropogonias furnieri* (corvina rubia) and *Potamotrygon brachyura* (raya), were reported to consume adult mussels. Furthermore, several species (e.g., *Leporinus obtusidens* and *Schizodon borellii*—boga*, Pterodoras granulosus*—armado, *Pimelodus maculatus*—bagre amarillo, *P. albicans*—bagre blanco) feed on planktonic veligers during their larval stages, whereas their adults graze on the benthic adults of the mussel.

The incidence of *Limnoperna* veligers in the diet of larval fishes is particularly high, often accounting for up to 100% of the gut contents, as reported by Paolucci et al. (2007) for protolarvae of *P. lineatus* in the Middle and Lower Paraná River. These high values suggest that larval fishes exert a positive selectivity on mussel veligers over other prey types (positively selected prey items are the ones whose proportion in the diet is greater than the proportion in the available prey), but actual evidence is scarce and restricted to two field studies (Rossi 2001; Paolucci et al. 2007). Paolucci et al. (2007) concluded that protolarvae of sábalo favor *Limnoperna* veligers over cladocerans and copepods. The sábalo is the most abundant and economically important fish in the

Paraná-Uruguay watershed: this migratory species accounts for over half of the overall fish biomass (Quirós and Cuch 1989; Sverlij et al. 1993). However, so far the study of the effects of these new food item has been restricted in scope and degree of detail as only a small period of *P. lineatus* larval development and low concentrations of *L. fortunei* veligers have been considered.

In order to assess the importance of this new food item on the feeding behavior of *P. lineatus* larvae, we performed a laboratory study encompassing several developmental stages of the fish. Fish larvae were fed a natural zooplankton assemblage with different proportions of *L. fortunei* veligers. The objectives of our survey are (1) to assess the importance of mussel veligers in the diet of *P. lineatus* larvae, (2) to test the selectivity of *P. lineatus* larvae for indigenous vs. introduced prey items, and (3) to analyze changes in selectivity with fish larval development and *L. fortunei* veliger concentration. Coupled with previous data on the feeding of *P. lineatus* larvae in natural conditions (Rossi 1992; Paolucci et al. 2007), these results allow key aspects of the changes brought about in this system by the invasion of *L. fortunei* to be addressed. For this fish species, as well as for the many others that consume veligers, the availability of a new trophic item at such an early developmental stage, when vulnerability is highest, seems particularly significant.

Materials and methods

Prey selection experiments were performed during the reproductive season of *P. lineatus* (December 2006–January 2007). Fish larvae were collected in December 27, 2006 in the lower Paraná River, near the city of Zárate (34° 6′11.39″S–59° 0′25.06″W) using a 0.35 m mouth diameter, 1 m long conical plankton net and 300 μm mesh. Larvae were transported within 3 h to the laboratory and transferred to 50 l aquaria placed in a temperature-controlled chamber at 22 ± 1°C (field water temperatures at the time of sample collection were 21–22°C), under natural illumination, gently aerated (oxygen saturation, checked daily, was maintained at 80–90% saturation), and fed daily with *Artemia persimilis*. Half of the volume of the aquaria was replaced with fresh water in identical conditions (dechlorinated, aereated) daily.

Table 1 Prey densities for enriched, normal and low-veliger treatments used in the *P. lineatus* feeding experiments (means for the five trials of each test)

	Mean size [range] (mm)	Concentration (ind. L^{-1}) $\pm$ 1 SD			P
		Enriched	Normal	Low	
Protolarvae					
Veligers	0.09 [0.07–0.18]	78.0 $\pm$ 10.7	57.6 $\pm$ 9.3	13.6 $\pm$ 5.0	<0.001
Small cladocerans	0.26 [0.05–0.34]	11.2 $\pm$ 4.3	8.8 $\pm$ 2.0	14.0 $\pm$ 4.3	0.292
Medium cladocerans	0.39 [0.35–0.44]	18.8 $\pm$ 6.0	9.6 $\pm$ 2.0	16.6 $\pm$ 6.7	0.188
Copepodites	0.19 [0.26–0.40]	16.4 $\pm$ 4.0	17.6 $\pm$ 4.5	20.8 $\pm$ 5.3	0.746
Larger cladocerans and copepods	0.82 [0.45–1.57]	35.2 $\pm$ 6.7	45.6 $\pm$ 9.0	51.2 $\pm$ 9.6	0.229
Total		159.6 $\pm$ 28	139.2 $\pm$ 28.1	116.2 $\pm$ 39.1	0.326
Mesolarvae					
Veligers	0.09 [0.07–0.18]	109.6 $\pm$ 15	81.4 $\pm$ 12	38 $\pm$ 7.3	<0.001
Small cladocerans	0.26 [0.05–0.34]	1.2 $\pm$ 0.2	13 $\pm$ 3.7	1.6 $\pm$ 1.0	0.089
Medium cladocerans	0.39 [0.35–0.44]	5.2 $\pm$ 0.4	14.8 $\pm$ 3.0	5.0 $\pm$ 2.7	0.178
Copepodites	0.19 [0.26–0.40]	19.2 $\pm$ 3.0	16.2 $\pm$ 4.0	38.4 $\pm$ 10.3	0.146
Larger cladocerans and copepods	0.80 [0.45–1.57]	6.2 $\pm$ 1.1	7.8 $\pm$ 0.2	9.8 $\pm$ 4.2	0.435
Total		141.4 $\pm$ 23.3	133.2 $\pm$ 29.6	92.8 $\pm$ 32.1	0.218
Metalarvae					
Veligers	0.08 [0.07–0.18]	80.0 $\pm$ 13.0	53.6 $\pm$ 17.1	17.6 $\pm$ 6.5	<0.001
Small cladocerans	0.27 [0.05–0.34]	8.0 $\pm$ 1.0	4.8 $\pm$ 2.0	20.0 $\pm$ 8.4	0.125
Medium cladocerans	0.39 [0.35–0.44]	10.4 $\pm$ 1.0	19.2 $\pm$ 5.0	17.6 $\pm$ 5.2	0.275
Copepodites	0.19 [0.26–0.40]	30.4 $\pm$ 1.5	57.6 $\pm$ 13.0	34.4 $\pm$ 4.6	0.151
Larger cladocerans and copepods	0.85 [0.45–1.44]	28.8 $\pm$ 4.3	31.2 $\pm$ 11.0	29.6 $\pm$ 3.5	0.962
Total		157.6 $\pm$ 31.2	166.4 $\pm$ 31.1	119.2 $\pm$ 25.6	0.213

P values refer to one-way ANOVA for significance of difference between the three diets for each prey item

Zooplankton was collected on the same day of each selectivity experiment (January 2, 9 and 17) from the Río de la Plata estuary, off the city of Buenos Aires (34° 32′50.26″S–58° 25′48.55″W) using a 0.35 m mouth diameter, 1 m long conical plankton net with 25 μm mesh and transported within 30 min to the laboratory. At the time of zooplankton collection, the water temperature was 23–25°C. A fraction of the sample was frozen for subsequent zooplankton biomass determinations (see later), and the remainder was kept at 22 $\pm$ 1°C until use.

A total of 45 experimental runs with one fish larva each were performed: 9 experimental treatments (three larval fish development stages, each with three different veliger concentrations) with 5 replicates each. The larval fish developmental stages utilized were protolarvae (no rays in pectoral fins, approximately 10 days old, mean length: 7.7 mm),

mesolarvae (with rays in pectoral fins, approximately 17 days old, mean length: 10.0 mm), and metalarvae (well-developed rays in all median fins, approximately 25 days old, mean length: 13.1 mm) (cf. Snyder 1983). Each experiment consisted of feeding the fishes with a natural zooplankton assemblage at one of three different concentrations of *L. fortunei* veligers (Table 1): "enriched" where proportions of veligers were artificially enhanced (target density = 0.09 ind. ml^{-1}; see later); "normal" where veligers concentrations were unmodified from concentrations encountered during field sampling (typically 0.06 ind. ml^{-1}), and "low" where veligers concentrations were diluted (target density = 0.02 ind. ml^{-1}). Absolute densities of the prey items were within naturally occurring ranges reported for the Paraná River (Boltovskoy and Cataldo 1999; Cataldo and Boltovskoy 2000).

Enrichment and dilution of natural zooplankton with *Limnoperna* larvae was accomplished by pouring the net-concentrated field-collected plankton in darkened glass cylinders (50 cm long, 3 cm diameter) illuminated from the top. Due to the different swimming capabilities and photo- and geotropic behavior of the veligers and the crustaceans, most veligers tended to settle on the bottom, whereas crustaceans migrated to the top. After 15 min, the upper half of the water column was separated from the bottom, thus obtaining two samples with different concentrations of *Limnoperna* larvae (Table 1). The two fractions were then split in half with the aid of a Folsom plankton sample splitter; one half was used in the feeding experiment, while the other was immediately preserved with 5% formaldehyde in order to assess initial prey concentrations.

Composition of the zooplanktonic assemblages collected for feeding experiments was similar throughout the experimental period. Numerically dominant components were *L. fortunei* veligers, calanoid copepods (*Boeckella bergi*, *Notodiaptomus* sp., *Argyrodiaptomus* sp.), copepodites, and cladocerans (*Diaphanosoma birgei*, *Moina micrura*, *Bosmina longirostris*, *Ceriodaphnia* sp.). Most (>90%) of the food items supplied to the fish larvae were smaller than 0.7 mm; larger organisms were represented by some copepods and a few large cladocerans, none of which were found in the guts of the experimental fish larvae. Low-veliger prey contained 12–41% *L. fortunei* veligers, normal prey contained 32–60%, and enriched prey contained 49–78%. The prey supplied to mesolarvae was particularly rich in *L. fortunei* veligers. Conversely, the zooplankton fed to protolarvae had higher proportions of cladocerans and lower of copepods than that supplied to metalarvae. Despite these differences, in all cases selective fractioning of the raw zooplankton sample allowed attaining three prey compositions with significantly different densities of mussel veligers (ANOVA, $P < 0.01$; Table 1).

For each prey treatment, five *P. lineatus* larvae of the same developmental stage were chosen at random, fasted for 24 h, and used in the prey selection experiments. Experiments were conducted at 22°C, in 1.5 l clear plastics jars with 1 l of dechlorinated tap water and one fish larva each, using five replicates per treatment (enriched, normal, and low). Plastic jars were illuminated from all sides,

including top and bottom, and prey items did not show a tendency to concentrate at the top (crustacean zooplankton) or the bottom (veligers) of the jar. Fish larvae were allowed to feed for 1 h, after which time the content of the jars was preserved with 50 ml of formaldehyde. The total length and the mouth width of the fish larvae were measured, and their gut contents analyzed. All items in the prey offered and in the gut contents of the experimental fish larvae were identified, counted, and measured under a binocular microscope. Prey items were classified into five categories: *L. fortunei* veligers (0.09–0.20 mm), small cladocerans + nauplii (0.05–0.34 mm), copepodits (0.20–0.40 mm), medium-sized cladocerans (0.35–0.44 mm), and large cladocerans and copepods (0.45–0.66 mm). Despite some size overlap, copepodits were considered separately from the cladocerans because of their higher swimming and predation-avoidance capabilities (see below). Small items, such as rotifers, as well as organisms larger than 0.7 mm were not considered because previous results have indicated that they are not consumed by *P. lineatus* larvae (Rossi 1992; Paolucci et al. 2007).

Frozen aliquots of the three zooplankton samples used for the feeding experiments (see above) were thawed, mixed, and veligers and crustaceans were isolated manually for biomass estimates. For the veligers, we isolated three sets with 500 individuals each; these sets were dried to constant weight at 60°C and then ashed at 500°C for 4 h. Because the size of veligers varied very little, the averaged ash-free dry weight values derived from these three sets of 500 individuals were applied to all subsequent density to biomass conversions. For cladocerans and copepods, a similar drying and ashing procedure was applied using 300–500 individuals of each group. However, because the sizes of these organisms were variable, dry weight figures were calculated for every experiment separately applying the equations proposed by Dumont et al. (1975) and Bottrell et al. (1976) to all the individuals present in the prey offered and in the gut contents; these dry weight values were subsequently corrected for ash contents using the ash weight to dry weight proportions for cladocerans and copepods estimated earlier.

Selectivity values were assessed with the aid of a Chi-square-based index (Pearre 1982):

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

where

$a = a_d + a_e$

$b = b_e + b_d$

$d = a_d + b_d$

$e = a_e + b_e$

$n = a + b$

where a_d and a_e are the number of specimens of prey item a in the diet and in the environment, respectively, at the start; b_d and b_e, are the same numbers for all the other prey items summed.

This index ranges between $+1$ and -1; index values significantly different from zero indicate that the item is preferentially preyed upon (positive values), or rejected (negative values); zero indicates neutral selection. This expression was selected because it is not affected by the relative abundance of rare prey items. In addition, it allows statistical estimates of significance for any sample size (Pearre 1982; Lazzaro 1987), which is particularly important for the scarcest prey items. In all cases when the expected frequency of a given prey item was above 5%, the statistical significance of the corresponding selectivity value was assessed with the χ^2-test using Yate's correction for continuity (Pearre 1982; Zar 1999). Spearman's (non-parametric) correlation test was used to compare total fish larva length with mouth width. Differences in prey concentrations were assessed with one-way ANOVA ($\alpha = 0.05$) and Duncan's multiple contrasts (Zar 1999).

Results

On average, veliger-enriched prey had 1.2–1.6 times more *L. fortunei* veligers than "normal" prey treatments, and the latter had 1.5–3.5 times more veligers than "low" treatments (Table 1). Total densities of prey items ranged between 116.2–159.6 ind. l^{-1} (assays with protolarvae), 92.8–141.4 ind. l^{-1}, (mesolarvae), and 119.2–166.4 ind. l^{-1} (metalarvae) (Table 1). None of the differences involved were statistically significant (one-way ANOVA, $P > 0.05$;

Table 1). Furthermore, the lack of correlation between total prey density and selectivity for veligers ($r^2 = 0.005$, $P = 0.788$) suggests that differences in selectivity were not influenced by the overall concentration of food in the experiments.

The mean total length of the fish larvae used in the experiments varied with their ontogenetic stage, protolarvae: 7.3 ± 0.5 mm, mesolarvae: 10.0 ± 0.8 mm, and metalarvae: 13.0 ± 1.9 mm (Table 2). Their mouth width ranged from 0.52 to 1.7 mm, increasing linearly with their total length (6.3–17 mm) (Spearman $r = 0.917$, $P < 0.01$; Fig. 1). All the larvae utilized in our experiments (45) had prey in the guts at the end of the assays, with older larvae generally consuming more items than younger ones (Table 2). The proportion of veligers in the diets increased from protolarvae (45%) to mesolarvae (69%) dropping sharply (15%) in metalarvae. Table 2 shows the size and mean ash-free dry weight of the prey items found in the guts, indicating that veliger biomass is proportionally over four times greater than that of similarly sized crustaceans.

Veligers were present in the gut content of all developmental stages, regardless of their concentration in the prey offered (Fig. 2). For the protolarvae, average proportions of veligers were always higher in the gut contents than in the prey offered (Fig. 2), indicating that their selectivity toward this item was positive and, in the case of the veliger-enriched and normal prey, statistically significant (Chi-square $P < 0.01$ and $P < 0.05$, respectively) (Fig. 3). The other food items strongly favored by the protolarvae were small cladocerans and nauplii, which always yielded positive and statistically significant selectivity indices (Fig. 3). Of the other three food items, only medium-sized cladocerans in the veliger-low prey showed a positive (albeit non significant) selectivity value, while all others were proportionally more abundant in the prey offered than in the gut contents, suggesting the inability of protolarvae to prey upon them efficiently (Figs. 2, 3).

Mesolarvae preyed on veligers selectively only when these were very abundant in the medium (veliger-enriched prey, Fig. 2); however, when veliger concentrations dropped (normal and low treatments), they were consumed less selectively, with gut contents yielding lower proportions of veligers than the prey offered (Fig. 2). This developmental stage was especially efficient at consuming small and

Table 2 Size and biomass of the prey categories recorded in the gut content of the experimental fish larvae and calculated average ingestion rates

Categories	N	Mean size [range] (mm)	Ash-free dry weight (mean ± 1 SD, µg)	Ingestion rate (mean ± 1 SD, ind. h^{-1})
Protolarvae	15	7.3 [6.3–8.4]		
Veligers	42	0.11 [0.09–0.18]	0.21 ± 0.05	3.13 ± 0.05
Small cladocerans + nauplii	49	0.24 [0.05–0.34]	0.13 ± 0.04	2.07 ± 0.04
Medium-sized cladocerans	12	0.40 [0.35–0.44]	0.46 ± 0.05	0.67 ± 0.05
Copepodits	–	–	–	–
Copepods + large cladocerans	–	–	–	–
Total	103			6.90 ± 2.34
Mesolarvae	15	10.0 [8.8–11.2]		
Veligers	82	0.11 [0.09–0.18]	0.21 ± 0.05	6.47 ± 0.05
Small cladocerans + nauplii	49	0.25 [0.05–0.34]	0.14 ± 0.03	2.13 ± 0.03
Medium-sized cladocerans	6	0.40 [0.35–0.44]	0.46 ± 0.05	1.40 ± 0.05
Copepodits	1	0.39	0.57	0.27
Copepods + large cladocerans	1	0.52	0.84	0.07
Total	139			9.26 ± 4.42
Metalarvae	15	13.0 [12.0–17.2]		
Veligers	24	0.11 [0.09–0.18]	0.21 ± 0.05	1.60 ± 0.05
Small cladocerans + nauplii	74	0.25 [0.05–0.34]	0.14 ± 0.02	4.13 ± 0.02
Medium-sized cladocerans	18	0.40 [0.35–0.44]	0.46 ± 0.05	2.47 ± 0.05
Copepodits	25	0.26 [0.20–0.40]	0.31 ± 0.06	0.70 ± 0.06
Copepods + large cladocerans	17	0.59 [0.45–0.66]	1.10 ± 0.22	1.80 ± 0.22
Total	158			10.53 ± 4.22

medium-sized cladocerans, whose selectivity indices were almost invariably positive and statistically significant (Fig. 3). Small cladocerans + nauplii, in particular, peaked from 3 to 14% in the prey offered to 36–56% in the guts. The two largest items, copepodits and copepods + large cladocerans, were often absent altogether from the guts and never represented the choice food item (Figs. 2, 3).

As opposed to younger larvae, metalarvae never selected veligers, regardless of their concentration in the prey offered; at the end of each experiment veliger proportions were always higher in the medium than in the gut contents (Figs. 2, 3). As with the mesolarvae, mealarvae clearly favored small and medium-sized cladocerans, whereas copepodits and copepods + large cladocerans, although often present in the gut contents, were always proportionally more abundant in the prey offered than in the stomachs (Figs. 2, 3).

Overall, copepodites, copepods, and larger cladocerans were never the choice food item, and in 7

(of a total of 9) experiments, they were significantly underrepresented in the gut contents of the fish larvae as compared with the medium (Fig. 3). Differences between their availability and their consumption were particularly high for the smaller fish larvae, and somewhat lower (but still negative and significant) for the metalarvae.

In terms of biomass, veligers were the most important food for proto- and mesolarvae, accounting for an average of 62% of food consumed. Highest proportions of veliger biomass (up to 95%) were recorded in the guts of fishes fed veliger-enriched diets. However, when veliger biomass in the medium was low (normal and, especially, low-veliger treatments), small and medium-sized cladocerans became the dominant food (Fig. 4). For metalarvae, on the other hand, veliger biomass was never first in importance, regardless of their concentration in the medium. This stage favored larger prey items, chiefly cladocerans. Nevertheless, the contribution of veliger biomass to the diet of metalarvae was always sizable,
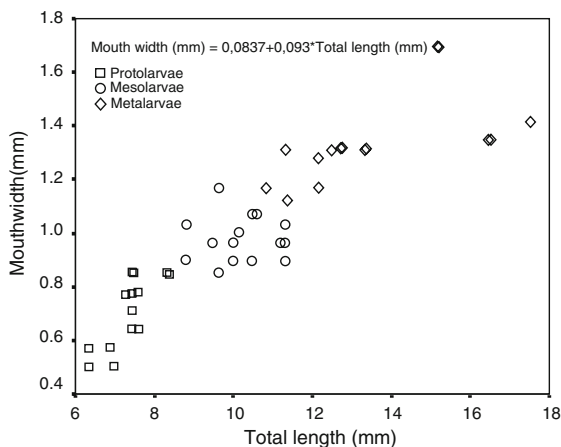
Fig. 1 Correlation between total length and mouth width of the *Prochilodus lineatus* fish larvae used in the selectivity experiments ($R = 0.917$; $P < 0.01$; $n = 45$)

representing around 10–20% of the ash-free organic matter recorded in their guts (Fig. 4).

Discussion

Absolute prey densities and proportions of *Limnoperna* veligers used in our experiments are generally representative of natural conditions for South American lotic and lentic water bodies invaded by the mussel. Concentrations of crustacean zooplankton utilized varied between 32 and 112 ind. (cladocerans + copepods) per liter (Table 1). These figures are roughly comparable to the range of values reported for the Paraná River, as well as for some reservoirs colonized by *Limnoperna* in the last decade (Bonetto and Martinez de Ferrato 1966). Proportions of *Limnoperna* veligers used in the experiments ranged from 12 to 78% of overall zooplankton (Fig. 2). In the field, veligers of *Limnoperna* (the only mussel with free-swimming, planktonic larvae in this system) account for 0% of veligers + crustaceans (during the winter, when reproduction of the bivalve is minimal), to over 95% (during the summer peaks, when up to over 100 larvae per liter are found in the water column) (Boltovskoy and Cataldo 1999; Cataldo and Boltovskoy 2000). Thus, we anticipate that the experimental results obtained are a reasonable proxy of the trophic relationships in the wild. These results clearly highlight the importance of the planktonic larvae of *L. fortunei* during the early development of *P. lineatus* and allow pinpointing variations in their contribution to the diet of the fish under different conditions.

For protolarvae and mesolarvae, veligers were usually the most important prey item, in terms of both numbers and biomass. Their contribution to the diet was particularly important at high concentrations in the medium, when they represented around 90% of the food consumed by the fish larvae. When the relative abundance of veligers in the medium dropped, their proportions in the guts also decreased, and they were partly replaced by cladocerans. Although metalarvae favored larger food items, like cladocerans, they also consumed *Limnoperna* veligers in sizable proportions (Figs. 2, 4).

Our laboratory-based results are in agreement with those of a previous field study. Paolucci et al. (2007) compared the diet of larval *P. lineatus* collected in two dissimilar environments of the lower Paraná River: the main river channel and marginal floodplain lagoons. In the main channel, where from spring to fall *Limnoperna* veligers are 8–9 times more abundant than copepods and cladocerans combined (Karatayev et al. 2007; our unpublished data), the relative importance of mussel larvae in the guts analyzed was much higher (75% in numbers, ca. 100% in biomass) than in the marginal lagoons (20% in numbers, 16% in biomass), whose zooplankton contained only 4% of *Limnoperna* veligers. Highest positive selectivity values were also associated with the highest proportions of veligers in the zooplankton. Nevertheless, even in the low-veliger environment (the marginal floodplain lagoons), selectivity toward the mussel larvae was positive (4% in the plankton vs. 20% in the guts).

Rossi (1992) analyzed the feeding preferences of *P. lineatus* larvae before *Limnoperna* was present in South America, concluding that small cladocerans and nauplii were the main prey items of the fish larvae. Our laboratory results and available field data (Paolucci et al. 2007) indicate that small crustaceans have been largely replaced by veligers, especially when the latter are abundant. This diet switch, however, is largely restricted to the earliest larvae. As fishes grow larger, the relative importance of large crustaceans increases. This is especially noticeable for metalarvae, whose consumption of the three
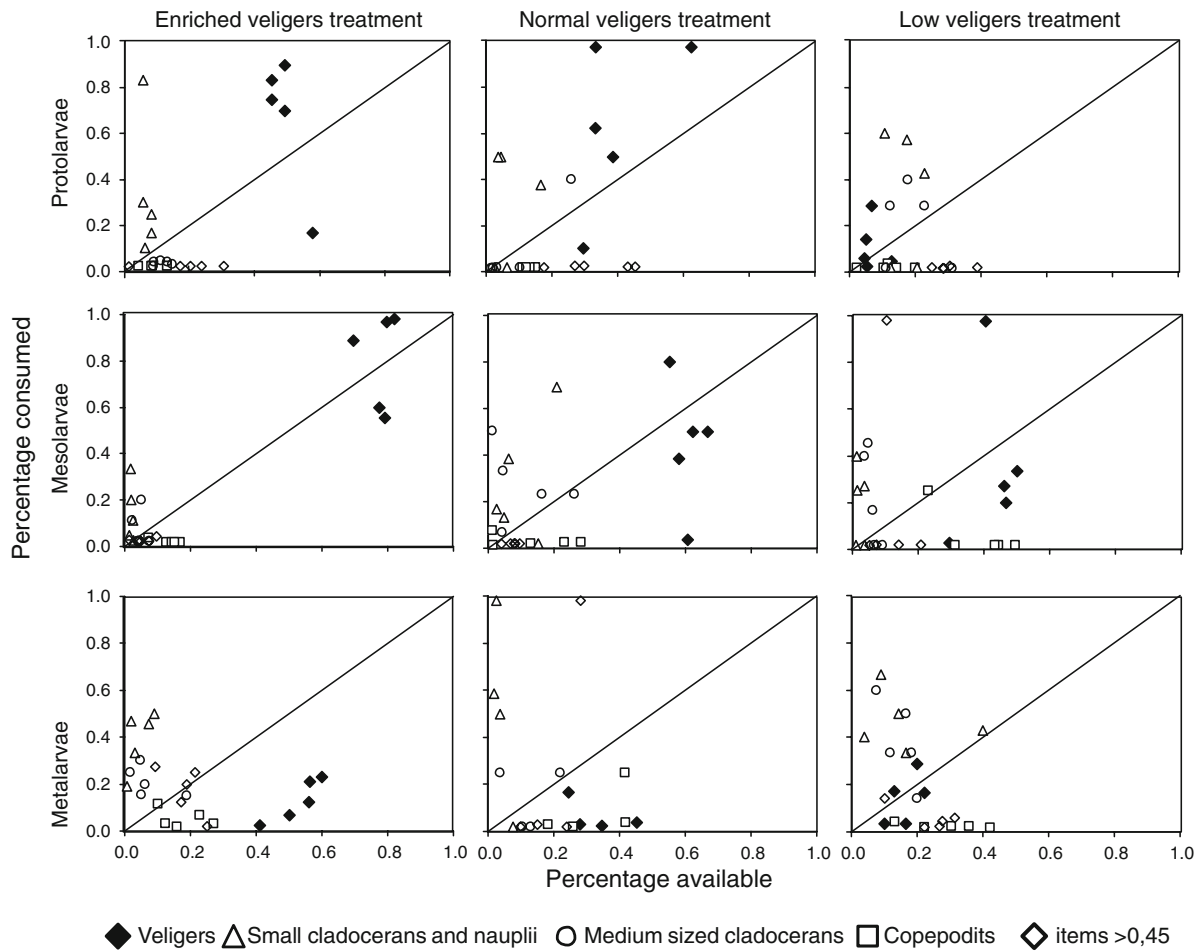
Fig. 2 Numeric percentages consumption of prey plotted in relation to percentage availability for laboratory selectivity experiments with protolarvae (*n* = 15), mesolarvae (*n* = 15) and metalarvae (*n* = 15) in the three experimental settings (enriched, normal and low veligers concentrations). *Points above the diagonal line indicate* prey items that are consumed in higher proportion than their availability in the plankton. In cases where points representing percent prey items consumed overlapped completely with others points, one of these were offset 1*x*-axis unit (*a percentage point*) to the *right*

largest prey categories (14%) is over twice as large as those in proto (5%) and mesolarvae (7%) (Fig. 2).

We speculate that the replacement of crustaceans by *Limnoperna* veligers in the diet of larval fishes is chiefly because veligers are poor swimmers, with limited neuromuscular coordination and have a less efficient predator-avoidance behavior compared to crustacean zooplankton. Cladocerans, in turn, are slower and less agile than copepods. This gradient in predator-avoidance capabilities seems the main prey selection factor during the earliest life stages of the fish. Indeed, mollusk larvae have been reported to be preferred over crustaceans (Lehtiniemi et al. 2007;

Pepin and Penney 1997; our data), and cladocerans are generally preferred over copepods (Cooper and Goldman 1980; Vanderploeg et al. 1982; Clarke et al. 2004), sometimes regardless of prey size (Werner 1974).

Another reason for the active consumption of veligers is probably associated with their comparatively high biomass to size ratio, which is ca. 4 times higher than that of the crustaceans (Table 2). Thus, when compared with cladocerans or copepods of similar dimensions, mussel larvae yield 3–4 times more energy than crustaceans (Sprung 1993; Akopian et al. 2001; Gonzalez et al. 2008).
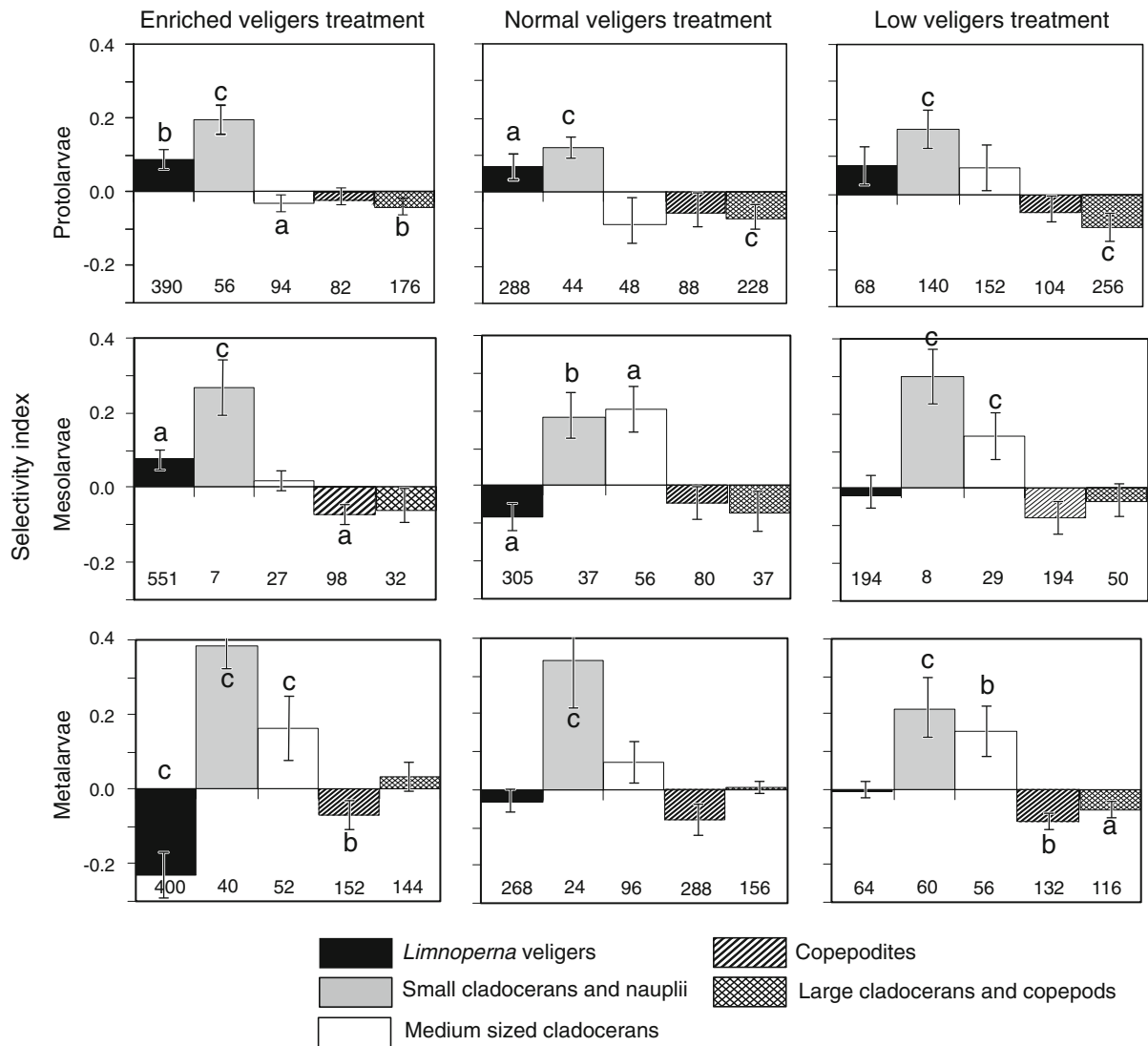
Fig. 3 Average selectivity index for the prey item assessed by protolarvae ($n = 15$), mesolarvae ($n = 15$) and metalarvae ($n = 15$). *Letters* denote significant differences between the offered prey and the gut contents at $P < 0.05$ (**a**), $P < 0.01$ (**b**) or $P < 0.001$ (**c**) (Chi-square tests). *Error bars* denote confidence intervals at $P < 0.05$. *Numbers inside each graph* indicate the total number of each prey item offered

As larvae grow in size they consume fewer veligers. This shift to larger preys seems to respond to both the changing foraging capacities and bioenergetic needs of the predators. Their improved mobility and swimming speed, in combination with a larger mouth gape (Fig. 1), allow them to take advantage of larger and faster prey (Lazzaro 1987; Michaletz et al. 1987; Pryor and Epifanio 1993). At the same time, their higher feeding requirements make small prey less profitable in terms of the

balance between energy invested in prey capture vs. energy obtained.

This behavior confirms earlier results for a wide range of animals, sometimes referred to as the "optimal foraging theory" (Schoener 1971; Stephens and Krebs 1986), whereby survival success is maximized when predators rely on the more abundant prey of higher alimentary value, which yields a higher ratio of energy obtained vs. effort invested in capture and digestion (Stephens and Krebs 1986;
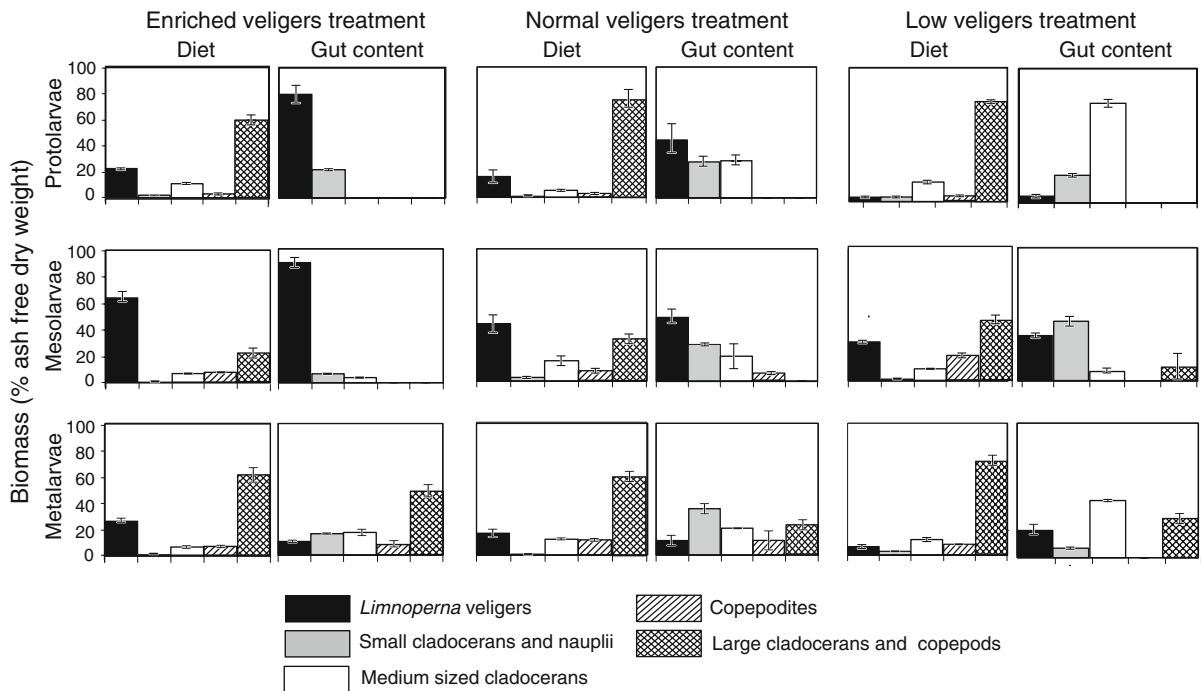
Fig. 4 Biomass percentages of the prey items in the offered prey and in the gut contents of protolarvae, mesolarvae, and metalarvae in the three experimental settings (enriched, normal, and low concentration of veligers). *Error bars* denote confidence intervals at $P < 0.05$

Fryxell and Lundberg 1998). These relationships have often been analyzed in an evolutionary context, where the development of optimal foraging strategies requires the co-evolution of predator-prey interactions (Abrams 2000). However, our results indicate that rapid behavioral changes in the presence of alternative more profitable resources are not necessarily preceded by evolutionary adjustments. In this respect, invasive species represent invaluable natural experiment opportunities for testing these predictions. Similar results were also observed with other invasive species elsewhere. For example, Ponto-Caspian cercopagid cladocerans introduced in parts of Europe and North America have been shown to swiftly become the choice prey of local fish species (Mills et al. 1992; Coulas et al. 1998; MacIssac et al. 1999).

An issue of major interest in association with these trophic relationships is the impact of the predators on *Limnoperna* and the possibility that this grazing impact curtails growth of mussel populations. Sylvester et al. (2007) reported that benthic populations of the (adult) mussel, whose densities range around

10,000 and up to 200,000 ind. m^{-2}, loose yearly over 90% of their biomass to predation, chiefly by fish. A similar estimate for the veligers is more complicated because we are still missing several key elements of this equation, in particular the mussel's reproductive output. Assuming conservative densities of 1000 ind. m^{-3} for the veligers (Boltovskoy et al. 2009) and around 3 ind. m^{-3} for the fish larvae (Rossi et al. 2007), and an ingestion rate of 2 veligers h^{-1} (this work, Table 2), one could speculate that on a steady-state basis *P. lineatus* consumes daily between 10 and 20% of the standing stock of veligers. This figure should probably be doubled to account for all the other fish larvae that consume veligers (Paolucci et al. 2007). On the other hand, the fact that *Limnoperna*'s reproductive season (around September to April) is much longer than that of most fishes (between November and December and February and March; Rossi et al. 2007) must decrease the long-term impact very significantly. Thus, although these estimates are very rough, they support the conclusion of Sylvester et al. (2007) who concluded that this predation impact on the geographic spread of the species is probably

minor, and the contribution of predation to the control of the bivalve seems limited. Comparison of these results with data on other invasive bivalves indicates that the magnitude of these trophic impacts is highly variable. In North America, very dissimilar results have been reported, ranging from a negligible impact of predators on the zebra mussel, *Dreissena polymorpha*, to the effective control of the mussel's population growth (e.g. Stewart et al. 1998; Bartsch et al. 2005). Conversely, in some cases predators of the mussel showed increased productivity and growth as a result of the new food supply (Poddubnyi 1966), whereas in other cases foraging on the invader has had negative effects on the predators (French and Bur 1996).

On the other hand, the impact of this new resource on fish populations is probably very important. Our results suggest that *P. lineatus* (and probably the other ten fish species whose larvae have been observed to feed on the veligers, Paolucci et al. 2007) have greatly benefited from *Limnoperna*'s planktonic stages. The fact that the earliest fish larvae are the most active consumers of veligers is particularly significant because they usually represent the most vulnerable life stage where mortality rates are highest (Leiby 1984; Fortier and Leggett 1985; Li Sifa and Mathias 1987). Temporal overlap between fish and mussel reproduction periods (see above), thus providing a stable food supply for the larval fishes is also a key factor for this relationship. Comparisons with *D. polymorpha* suggest that its much shorter reproductive period (Nichols 1996) may be among the reasons for its considerably lower presence in the diet of North American fish species. According to Mills et al. (1995), only two North American fish species feed on *D. polymorpha* larvae (as opposed to at least 11 species recorded to feed on *L. fortune* larvae in South America; Paolucci et al. 2007).

The effects of these shifts in the feeding behavior of larval fishes are conceivably not restricted to the organisms directly involved in the interactions, but may have cascading effects both up and down the trophic webs (MacIssac et al. 1999; Yan et al. 2001; Clarke et al. 2004). Insofar as the new interactions modify established grazing pressures, they can strongly affect the specific composition and size structure of the zooplankton (Dumitru et al. 2001; Strecker and Arnott 2008), which in turn may change phytoplankton abundance and composition (Strecker and Arnott 2008). Indirect impacts of other fishes can derive from these rearrangements in the plankton, from direct consumption of veligers (Paolucci et al. 2007), or from changes in the availability of other food items. Because the deposit feeding adults of *P. lineatus* represent over 60% of the overall fish biomass in the Río de la Plata basin (Sverlij et al. 1993), it constitutes the main food item of larger ichthyophagous species. Thus, improved feeding conditions for *P. lineatus* may strongly affect abundances of many other fish species, an impact that is suggested by historical fish landing statistics (Boltovskoy et al. 2006).

It should be stressed that increased availability of prey items does not necessarily imply a positive effect on the predators. Previous work on the effects of *D. polymorpha* suggested that it hinders fish growth because of the negative balance in dietary change involved in the energy provided by the new resource versus its capture and manipulation (French and Bur 1996; Nagelkerke and Sibbing 1996; Pothoven and Madenjian 2008). This possibility has not yet been investigated in the case of *L. fortunei*. However, similarities with the zebra mussel may be misleading when attempting to extrapolate *D. polymorpha*'s known impacts on European and North American waterbodies to *L. fortunei* in South America (Karatayev et al. 2007). Both physically and ecologically, the South American water bodies invaded by *Limnoperna* are very different from those in Europe and North America where *Dreissena* has established in recent decades. Overall impacts depend as much on the characteristics of the invading species as on environmental traits, which underscore the need to assess these influences under an ecosystem perspective (Boltovskoy et al. 2006).

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