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Veligers of an introduced bivalve, *Limnoperna fortunei*, are a new food resource that enhances growth of larval fish in the Paraná River (South America)

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SUMMARY

1. Larvae of 'sábalo', *Prochilodus lineatus*, whose adults represent over 60% of overall fish biomass in the Río de la Plata Catchment, have been observed to feed intensively on veligers of the exotic bivalve *Limnoperna fortunei*.
2. To assess the effects of this dietary shift on the growth of *P. lineatus*, 28-day laboratory experiments were carried out feeding newly hatched *P. lineatus* larvae with three diets: zooplankton artificially enriched with *L. fortunei* veligers; natural zooplankton; and zooplankton artificially enriched with cladocerans and copepods. The average length, weight and gut contents of the fish larvae were assessed weekly and metabolic rates of fish larvae were measured.
3. Proportions of veligers in gut contents were always higher than those in the experimental diet: 100, 76 and 21% for veliger-enriched, natural and low-veliger diets, respectively. Larvae fed a veliger-enriched diet grew to a significantly larger size than larvae fed the other two diets. In energetic balance comparisons using metabolic rates and prey energy content, all three diets were sufficient to support metabolism and growth. The greatest values of excess energy at the end of each week were in the veliger-enriched experiments.
4. Feeding on veligers of *L. fortunei* significantly enhances the growth of *P. lineatus* larvae and supports the idea that this new and abundant resource is selectively preyed upon by *P. lineatus* during its larval stage. Higher growth rates may stem from the higher energy contents of veligers compared to crustaceans and/or from the lower energy costs of capturing slower prey.

Keywords: exotic bivalve, growth, larval fish, *Limnoperna fortunei*, veligers

Introduction

Populations of introduced bivalves have increased dramatically worldwide, and both negative and positive effects have been documented on the native ecosystems. One of the most notable effects of exotic bivalves on ecosystems is the impact on trophic interactions (Karatayev, Burlakova & Padilla, 2002; Karatayev *et al.*, 2007; Noonburg, Shute & Abrams,

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2003; Bulté & Blouin-Demers, 2008). This impact is generally the result of high rates of filter feeding combined with the secondary production these new organisms represent themselves. There can be positive impacts on the bivalve's predators because of increased biomass (Molloy *et al.*, 1997; Karatayev *et al.*, 2002; Boltovskoy *et al.*, 2006), but declines in the biomass of fish stocks have also been noted. These have been chiefly attributed to the indirect effects of exotic mussels, such as the competition for food between bivalves and zooplanktivorous fishes (Bartsch, Richardson & Sandheinrich, 2003; Feyrer *et al.*, 2003; Strayer, Hattala & Kahnle, 2004). Even in fish that are important predators of bivalves, some negative effects have been reported. These have been explained by the low caloric content of the bivalve compared to traditional food items, mainly because of the presence of shells which are not assimilated, as well as the increased energetic costs of handling prey (French & Bur, 1996; Nagelkerke & Sibbing, 1996; Magoulick & Lewis, 2002).

Since its introduction into the Río de la Plata from Asia in 1990 (Pastorino *et al.*, 1993), the exotic bivalve *Limnoperna fortunei* (Dunker, 1857) has colonised practically the entire Río de la Plata Catchment, including parts of Bolivia, Paraguay, Uruguay and Brazil, at densities reaching over 200 000 ind. m^{-2} (Boltovskoy *et al.*, 2006). This invasive mussel has become a significant prey item in the diet of at least 17 adult native fish (Montalto *et al.*, 1999; Garcia & Protogino, 2005; Boltovskoy *et al.*, 2006). A recent study has also shown that the larvae of at least 11 fish species of the Paraná-Río de la Plata watershed feed on the veligers of *L. fortunei* (Paolucci *et al.*, 2007). Notably, zooplanktivorous larvae of 'sábalo' (*Prochilodus lineatus*, Valenciennes 1836), whose adults represent over 60% of fish biomass in these rivers (Iwaszkiw, 2001), have sometimes been found to feed exclusively on *L. fortunei* veligers. Moreover, while a decline in the numbers of zooplankton has been associated with the arrival of *L. fortunei* (Rojas Molina & José de Paggi, 2007), these bivalve larvae have become a new and abundant item in the water column, reaching concentrations of over 30 000 veligers m^{-3} (Boltovskoy *et al.*, 2009). The shift from crustaceans to veligers in this ecosystem's zooplankton assemblage and in the diet of larval fish could have a positive impact on fish populations. Potential positive effects, such as higher growth rates or increased survival of fish larvae, will probably depend on the biochemical composition, energetic content and energetic costs associated with prey capture of veligers in comparison with indigenous zooplankton prey.

To assess the effects of this dietary shift on the growth of *P. lineatus*, we conducted laboratory experiments feeding newly hatched *P. lineatus* larvae with (i) plankton artificially enriched with *L. fortunei* veligers; (ii) natural zooplankton and (iii) plankton artificially enriched with cladocerans and copepods. The caloric content of food items and metabolic rates of larval fish were measured to assess the importance of veligers on the energy balance of sábalo.

Methods

Larvae of *P. lineatus* with yolk-sac absorption completed were collected from the Lower Paraná River near the city of Zárate (34°6.190'S–59°0.418'W), where the water temperature was 21–22 °C. For growth experiments, fish larvae were collected in December, 2006, and for respiration experiments, fish larvae were collected in December, 2007. Larvae were collected with a 1-m-long conical plankton net with a 0.35 m mouth diameter and 300-μm mesh. Larvae were transported within 3 h to the laboratory, placed in a 50-L tank and acclimated for 3 days on a diet of *Artemia persimilis*. A subsample of freshly collected larval fish was frozen for later biochemical analyses. Zooplankton for the different diet treatments were collected every other day, during December–January, in the Río de la Plata estuary (34°32.838'S–58°25.809'O W) using the same size plankton net but with a 25-μm mesh, transported within half an hour to the laboratory and used over 2 days.

Growth experiment

After the 3 day acclimation period, 150 larval fish specimens were weighed and measured to estimate the initial size of the experimental larval fish population. Thirty-five specimens were transferred to each of nine chambers. Chambers were divided into three groups, and one of three zooplankton feeding experiments was assigned randomly to each chamber. Growth experiments lasted 28 days and were carried

out in 3.5-L plastic chambers containing freshwater with streptomycin sulphate and penicillin (Rontag S.A., Buenos Aires, Argentina; 50 ppm, each; Struhsaker *et al.*, 1973). The temperature was maintained at 22 ± 1 °C, with a natural photoperiod (14 h light: 10 h dark) and constant slight aeration. Oxygen content was checked daily and maintained at 80–90% saturation. Before being fed zooplankton, 100% of the holding water was replaced with freshly aerated incubation water containing antibiotics at the experimental temperature.

Each treatment consisted of feeding the fish with natural zooplankton at one of three different concentrations of *L. fortunei* veligers: 'Veliger-enriched', where proportions of veligers were artificially enhanced and represented 90% of zooplankton biomass > 100 μm (see below); 'Natural', with unmodified proportions of veligers (average biomass about 43%), as found in the zooplankton samples during field sampling; and 'Low-veliger', with proportions of veligers artificially reduced, where *L. fortunei* accounted for about 1% of the zooplankton biomass. Enrichment and dilution of veligers in the zooplankton samples were accomplished by pouring the net-concentrated field-collected plankton in dark 50-cm long, 3 cm in diameter glass cylinders illuminated from the top. Because of the different swimming capabilities and photo- and geotropic behaviour patterns of the veligers and the crustaceans, most veligers tended to settle on the bottom, whereas crustaceans migrated *en masse* 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 *L. fortunei* veligers (see Table 1 and Fig. 1).

Each day, the zooplankton to be used for feeding was split into four fractions with a Folsom plankton splitter. Three fractions were utilised for growth experiments as described above and one was further split into two sub-samples. One of these sub-samples was fixed in 4% buffered formaldehyde and utilised for biomass measurements and diet composition analysis (Table 1). Prey items in these sub-samples were classified as cladocerans, copepods (including all nauplii and copepodites) or *L. fortunei* veligers, counted and measured using a Leica stereomicroscope. For cladocerans and copepods, dry weight values were calculated applying the equations proposed by Dumont, Van de Velde & Dumont (1975) and Bottrell *et al.* (1976) to all the individuals present in the sub-samples. These dry weight values were subsequently corrected for ash contents to achieve ash-free dry weight (AFDW) using three sets of 350–450 individuals which were dried to constant weight at 60 °C and then ashed at 500 °C for 4 h. For veligers, mean AFDW values were derived from three sets of 500 individuals each, processed similarly to cladocerans and copepods. The other sub-sample was stored in a freezer for later biochemical analyses. Food was supplied once per day *ad libitum*; surplus food was always observed 24 h after each feeding. Additionally, no significant differences between the daily mean dry weights of the sub-samples representing feeding treatments were found (one way ANOVA, $P = 0.629$; Table 1).

The total lengths of ten fish larvae from each experimental chamber were measured weekly. These larvae were anaesthetized with 0.5 μM benzocaine (Cepacol®: Gilderhus, Lemm & Woods, 1991), and total length was measured under a microscope to the closest 0.01 mm. Each week, two or three larvae were sampled from each experimental chamber and utilised for wet weight measurements and gut content analyses. Numeric abundance and biomass of the prey items in the guts of fish larvae were classified and calculated as described above for diet composition analysis.

Table 1 Biomass composition and average dry weight of zooplankton prey provided daily to larval *Prochilodus lineatus* during experiments with three diets: veliger-enriched, natural and low-veliger

Diet (veliger concentrations)	*n*	Mean dry weight biomass ± SD (mg day^{-1}; %) *Limnoperna fortunei*	Cladocerans	Copepods	Total
Enriched	28	4.26 ± 0.26 (87.05)	0.24 ± 0.01 (4.99)	0.38 ± 0.03 (7.94)	4.9 ± 0.31
Natural	28	1.46 ± 0.14 (33.99)	1.24 ± 0.04 (28.89)	1.59 ± 0.06 (37.1)	4.29 ± 0.25
Low	27	0.03 ± 0.01 (0.8)	1.44 ± 0.04 (31.93)	3.04 ± 0.13 (67.25)	4.52 ± 0.18

n is the number of samples utilised for biomass measurements and diet composition.

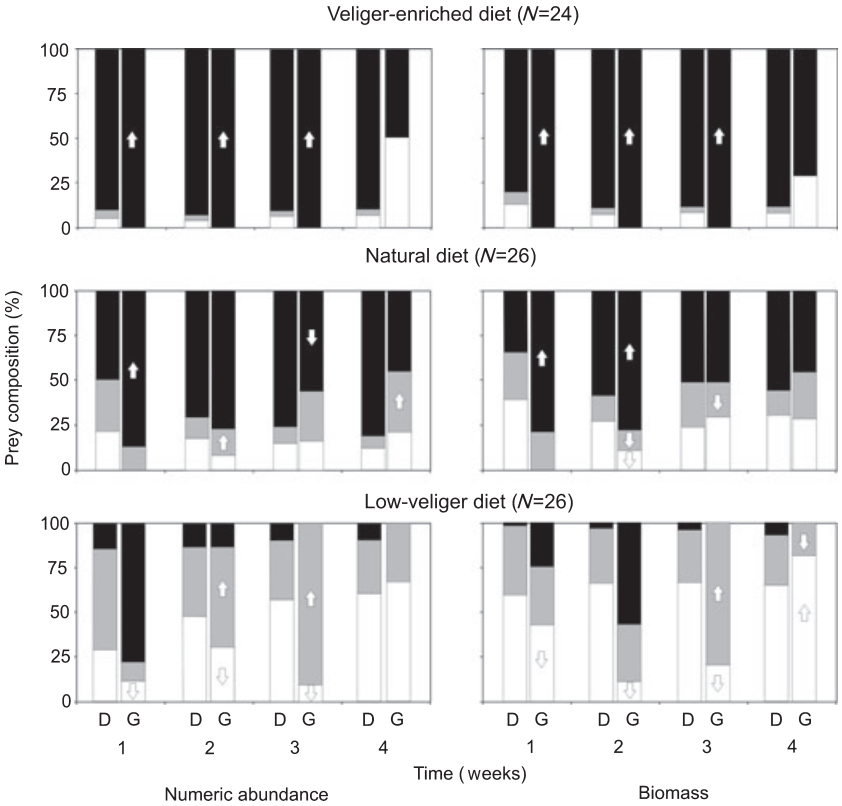

Fig. 1 Mean numeric abundance (%) and biomass (%) of prey items in the diet treatment (D) and larval fish gut contents (G) each week during the 4-week growth study with three different diets: veliger-enriched, natural and low-veliger. Solid black, solid grey and open bars indicate veligers, cladocerans and copepods, respectively. Up and down arrows indicate a significantly higher or lower proportion of a prey in the gut contents than in the diet treatment (Kruskal–Wallis test). *N* denotes the total number of larval fish utilised for gut content analyses.

Biochemical analyses

Biochemical compositions of the fish larvae at the beginning and end of each growth experiment and of the different prey items were analysed by standard biochemical methods. Soluble protein was measured using the Lowry method (Lowry *et al.*, 1951) against a bovine albumin standard (Sigma No A-4503). Total lipids were determined by a microcolorimetric assay developed by Van Handel (1985) and modified for small samples (Inouye & Lotufo, 2006). Ash contents of the different items and larval fish were obtained by reweighing dried samples after combustion at 500 °C for 6 h. All assays were carried out on triplicate samples of 200–500 individuals (copepods, cladocerans or veligers) or 3–5 individuals (larval fish), and components were expressed in terms of % AFDW. Carbohydrate contents of larval fish and food items were calculated as AFDW minus lipids and protein. Energetic contents were calculated using the conversion values of Paine (1971): 5.65, 9.45 and 4.10 kcal g^{-1} AFDW of protein, lipids and carbohydrates, respectively, and a conversion value of 4.18 kJ $kcal^{-1}$.

Oxygen measurements

Respirometry measurements were carried out on 32 larval fish specimens. These larval fish were kept in the growth experiment conditions described above but fed exclusively with the field-collected zooplankton. Rates of oxygen consumption were measured using PreSens-type B2-NTH fibre optic oxygen optodes connected to a PreSens Microx TX3 temperature-compensated oxygen meter (Precision Sensing, Regensburg, Germany). Optodes were calibrated at two points using an aqueous 5% sodium sulphite solution for oxygen-free water and gently stirred filtered water (22 °C) for oxygen-saturated water. Data were recorded on a personal computer through a serial connector. Each day, 4–5 specimens were chosen at random, transferred into a separate holding chamber at 22 °C with filtered water (0.22 μm) containing antibiotics (100 mg L^{-1} each of streptomycin and ampicillin) and starved for 24 h. Small glass syringes (2 mL) were used as respiration chambers for small larval fish, and large specimens were placed in 30-mL glass syringes. Syringes were sealed with Luer-type fittings and Teflon septa for insertion of oxygen

optodes similar to methods described by Rutherford & Thuesen (2005). Respiration experiments lasted *c*. 2 h. The total lengths of fish were measured afterwards and then returned to the maintenance chambers. Control experiments were performed in an identical fashion to respirometry experiments, except no specimens were used in the syringes. Controls showed no significant bacterial respiration over the time in which experimental trials were conducted.

To derive weight–length relationships, 10 larvae were sampled weekly to measure total length, wet and dry weight. The dry weight to wet weight relationship was calculated and utilised to convert the wet weight measurements of the growth experiment specimens. The growth rates of specimens used in respiration experiments were calculated as in the growth experiments described above and used to estimate metabolic energetic costs of growth using the values of energetic density of fish larvae feeding on natural veliger concentrations.

Energetic analysis

We utilised a model of energy partitioning represented as $C = R + P + E$, where the available energy (C) equals the energy used to support life (R) and to elaborate new tissue (P), plus that lost from the system (E) (Rombough, 1994). The variables C, R and P, in association with the gut content analyses of larvae fed different diets (this study) and the feeding rate values reported by Paolucci, Cataldo & Boltovskoy (2009), were used to determine whether the energy contents of these diets are sufficient to support larval metabolism and growth. Using a daily feeding period corresponding to the local photoperiod of *c*. 14 h, the same standard metabolic rate (SMR) for all diets, diet-specific growth rates and the average energy contents of each prey item, energy consumed each week was compared with the energy needed for metabolism and growth.

Data and statistical analyses

Respirometry data were analysed using Graphical Analysis (Vernier Software, Sarasota, FL, USA) to perform linear regressions of the oxygen concentrations against time. Oxygen consumption rates were converted to rates of energy transformation using an oxycaloric equivalent of 450 kJ mol O_2^{-1} (Elliott & Davison, 1975; Wieser *et al*., 1988). A polynomial regression was fitted to individual oxygen consumption data as a function of dry weight (Parra & Yúfera, 2001). Differences of total length and wet weight of the larval fish between the three diets and with those used in respiration experiments were analysed with repeated measures ANOVA adjusted using a Greenhouse–Geisser correction and orthogonal contrasts for each pair-wise comparison in each week. Differences in biochemical compositions of the food items and larval fish were assessed with one-way ANOVA following arc-sin transformation of the percentage data (Sokal & Rohlf, 1995). The biochemical data met statistical assumptions of normality and homogeneity of variance. Duncan's multiple contrast was used as a *post hoc* test for all pair-wise comparisons. Comparisons of prey composition and biomass of each prey item in stomach contents and diets were also assessed with one-way ANOVA following arc-sin transformation of the percentage data. However, assumptions for parametric tests were not fulfilled, and differences were assessed with nonparametric Kruskal–Wallis techniques (Daniel, 1978).

Results

Experimental protocols were successful as demonstrated by the general trends in growth and feeding experiments. Although significant differences in growth rates were observed between the three diets (Table 2), even those larvae with the lowest growth rates displayed high survival. The overall survival rate throughout all experiments was > 90.0%. Feeding experiments showed that 95.0% of the larvae used for stomach content analysis ($n = 76$) had at least one food item in the gut and on average, there were 6.2 prey items per stomach.

Feeding

The relative number of individual prey items and biomass proportions of *L. fortunei* in fish gut contents mirrored the concentrations of veligers in each diet, yet the proportions of veligers in fish gut contents were invariably higher than the proportions of veligers available in the experimental diet (Fig. 1). Larval fish fed with the three experimental diets displayed different gut content compositions during the 4 weeks of the experiment (Fig. 1). For larvae fed a

Table 2 Chemical composition of food items and *Prochilodus lineatus* larvae at time 0 and after 28 days consuming three different diets: veliger-enriched, natural and low-veliger. Means and SDs are for three replicates of 340–500 individuals each for prey item, and for 3–5 larval *P. lineatus*

Item	Dry weight Mean ± SD (μg)	Length Mean ± SD (mm)	Chemical composition of the ash-free dry weight Mean ± SD (%) Protein	Lipid	Carbohydrate	Ash	Caloric content Mean ± SD (Kcalgr^{-1}) Protein	Lipid	Carbohydrate	Total	Energy content Mean ± SD (%) (J ind.$^{-1}$)	(kJ g^{-1} dry wet)
Cladocerans	0.39 ± 0.30	0.51 ± 0.16	54.2 ± 3.0*	5.3 ± 0.3	40.4 ± 2.8	6.1 ± 3.1	3.06 ± 0.17	0.5 ± 0.03	1.65 ± 0.11	5.22 ± 0.10	0.009	21.84 ± 0.45
Copepods	2.80 ± 2.96	0.63 ± 0.39	66.0 ± 5.9*	3.2 ± 0.2	30.6 ± 6.1	2.5 ± 1.6	3.73 ± 0.33	0.31 ± 0.02	1.25 ± 0.25	5.30 ± 0.20	0.056	22.15 ± 0.85
Veligers	0.47 ± 0.09	0.16 ± 0.04	58.4 ± 11.1*	17.6 ± 2.3**	23.8 ± 11	28.6 ± 1.3	3.30 ± 0.62	1.67 ± 0.22	0.97 ± 0.45	5.95 ± 0.43**	0.012	24.88 ± 1.81**
P. lineatus (mg)												
Initial	0.14 ± 0.01	6.57 ± 0.82	67.6 ± 2.4**	11.2 ± 1.2	21.2 ± 3.2	9.5 ± 0.8	3.82 ± 0.13	1.05 ± 0.11	0.87 ± 0.13	5.74 ± 0.13	–	24.00 ± 0.54
Final (28 days)												
Enriched	0.68 ± 0.05**	15.08 ± 1.49**	88.5 ± 3.3	9.7 ± 0.6*	1.8 ± 2.4	12 ± 1.0	4.99 ± 0.19	0.91 ± 0.06	0.07 ± 0.09	5.99 ± 0.11	–	25.04 ± 0.49
Natural	0.29 ± 0.09	11.20 ± 0.96	83.3 ± 4.5	10.4 ± 1.6	6.3 ± 5.5	12.1 ± 2.6	4.7 ± 0.25	0.98 ± 0.15	0.25 ± 0.22	5.95 ± 0.21	–	24.87 ± 0.89
Low	0.23 ± 0.02	9.53 ± 1.01	79.5 ± 4.7	11.8 ± 0.5	8.6 ± 4.9	10.1 ± 2.2	4.49 ± 0.27	1.11 ± 0.05	0.35 ± 0.20	5.96 ± 0.17	–	24.93 ± 0.73

* and ** denote ANOVA significant differences at $P < 0.05$ and $P < 0.01$, respectively.

veliger-enriched diet, veligers were the main prey item found in the stomach contents, in terms of both biomass and numeric abundance. Notably, all the fish that were fed the veliger-enriched diet consumed only veligers during the first 3 weeks; proportions of veligers in these guts were significantly higher than those in the experimental diets offered each week in both numeric abundance (Kruskal–Wallis test; $H_{1,14} = 5.42$, $H_{15} = 5.71$, $H_{13} = 4.23$, $P < 0.05$ for all comparisons; Fig. 1) and biomass (Kruskal–Wallis test; $H_{14} = 5.42$, $H_{15} = 5.71$, $H_{13} = 9.96$, $P < 0.05$ for all comparisons; Fig. 1). In the last week, when fish were 15.1 ± 1.1 mm in size, copepods became part of the diet. For specimens fed a natural diet, the consumption of the three prey items was more balanced than under the veliger-enriched diet. Although veligers reached a significantly higher numeric abundance in the gut contents than in the available diet for week 1 (Kruskal–Wallis test $H_{12} = 4.87$, $P = 0.027$), on average, the importance of veligers, cladocerans and copepods was roughly similar in terms of biomass over all weeks (Fig. 1). In spite of this similarity in the mean biomass of the prey items, veligers were more important than crustaceans in the gut contents and significantly higher than the available prey during the first 2 weeks (Kruskal–Wallis test $H_{12} = 6.42$, $P = 0.011$ and $H_{14} = 5.0$, $P = 0.025$ for weeks 1 and 2, respectively). In weeks 3 and 4, the percentage of veligers dropped to less than 50%. On the other hand, in larval fish that were fed a low-veliger diet, veligers accounted on average for 18 and 30% of the biomass and numeric abundance, respectively (Fig. 1), and the weekly percentages of veligers in the guts were never significantly higher than the percentages in the diet. In terms of prey numeric abundance, cladocerans were the first item in fish stomachs under the low-veliger diet, but copepods replaced cladocerans when evaluating feeding in terms of prey biomass. Veligers were an important prey item during the first 2 weeks of the low-veliger diet experiments, and then, unlike the natural diet experiments, veligers were completely replaced by crustaceans in the gut contents (Fig. 1).

Growth experiments

Growth experiments were carried out successfully utilising the three diets. The different veliger concentrations had significant effects on the growth of

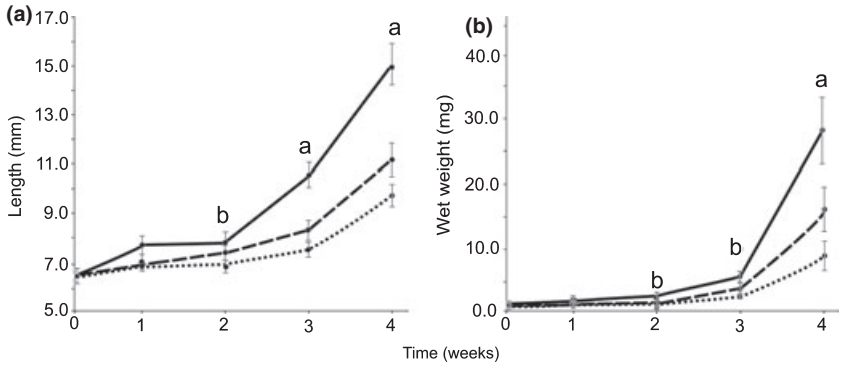

Fig. 2 Growth as (a) total length (mm) and (b) wet weight (mg) of larval fish fed three different diets: veliger-enriched (solid line), natural (dashed line), and low-veliger (dotted line). Vertical bars denote 95% confidence intervals. Letters denote significant differences (ANOVA, $P < 0.01$) as (a) between all diets; or (b) between at least one pair diets.

P. lineatus larvae: those fed a veliger-enriched diet had the highest growth performance, followed by those fed natural and low-veliger diets. This trend was evident in the total length and wet weight of larval fish where significant differences were found between larvae during the growth experiment (ANOVA, $F_{6, 111} = 119.91$, $P < 0.01$ and ANOVA, $F_{6, 111} = 30.54$, $P < 0.01$ for length and wet weight, respectively; Fig. 2). The orthogonal contrasts showed that the length of larval fish fed with veliger-enriched and natural diets were significantly higher than those fed a low-veliger diet starting as early as day 14 (ANOVA, $F_{1, 37} = 15.9$, $P < 0.01$ and $F_{1, 37} = 6.95$, $P = 0.01$, respectively). Beginning day 21, the total lengths of larvae fed veliger-enriched and natural diets were significantly different (ANOVA, $F_{1, 37} = 50.35$, $P < 0.001$). Similarly, the mean weight of larval fish fed a veliger-enriched diet was significantly higher than those fed natural (ANOVA, $F_{1, 37} = 19.9$, $P < 0.01$) and low-veliger (ANOVA, $F_{1, 37} = 7.5$, $P = 0.01$) diets starting day 14. On the other hand, wet weights of fish fed natural and low-veliger diets never differed significantly. Therefore, the final mean lengths and weights were lower when larval fish ate diets with lower proportions of veligers (total lengths: 15.1 ± 1.1, 11.2 ± 0.6 and 9.5 ± 0.4 mm, and average wet weights: 28 ± 5.3, 18.7 ± 2.3 and 8.5 ± 3.1 mg, for veliger-enriched, natural and low-veliger diets, respectively).

Biochemical composition and energetic content

All three prey items had high protein contents (over 50% AFDW), but the chemical compositions were significantly different (Table 2; ANOVA, $F_{2, 6} = 21.7$, $P < 0.002$). Copepods had the highest proportions of proteins, followed by veligers and then cladocerans. After proteins, carbohydrates were the second body component for all prey items, ranging from 23.0 to 40.0% of the AFDW. Veligers had the highest proportion of lipids. These were significantly higher than those in the other two prey items, whereby lipid content was up to 3 and 5 times more than that found in cladocerans and copepods, respectively (Table 2; ANOVA, $F_{2, 6} = 94.6$, $P < 0.001$). Because of these differences in the chemical compositions, the energy contents showed significant differences, too.

While the specific caloric contents of cladocerans and copepods were similar, veligers had a significantly higher specific caloric content (Table 2; ANOVA, $F_{2, 6} = 26.8$, $P < 0.01$). However, the average energy content per individual was highest for copepods because of their larger mass (Table 2). Similarly, the average dry weight for veligers was higher than for cladocerans, and consequently, veliger energy contents were higher too. Protein contents of fish larvae increased significantly during the experiment (Table 2; ANOVA, $F_{3, 8} = 12.8$, $P = 0.002$). Although the larval fish fed a veliger-enriched diet had the highest protein percentage, there was no significant difference in final protein content between these larvae and those fed natural and low-veligers diets (Duncan test, $P = 0.08$ and 0.36, respectively). On the other hand, larvae fed a veliger-enriched diet had significantly lower lipid contents than those fed the other diets (ANOVA, $F_{3, 8} = 12.8$, $P = 0.002$). Lipid contents in fish fed veliger-enriched diets were also lower when compared to the initial composition of the larval fish (Table 2). The specific caloric content of fish larvae showed a slight increase during development, but this was not significantly different between treatments (ANOVA, $F_{3, 8} = 3.19$, $P = 0.08$; Table 2).

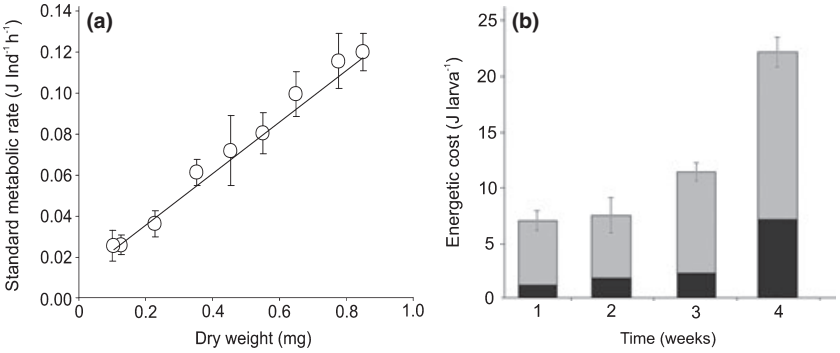

Fig. 3 (a) Average standard metabolic rate (SMR) as a function of the body dry weight in *Prochilodus lineatus* larvae ($y = -0.046x^2 + 0.181x + 0.001$; $R^2 = 0.837$, $n = 91$). (b) Weekly energy costs of growth (black bars) and SMR (grey bars) according to the mean weight of the larval fish used in respiration experiments each week. Vertical bars denote 95% confidence intervals of the combined energetic costs.

Oxygen consumption

A total of 91 respirometry measurements were carried out on 32 larval fish specimens; these larvae grew well throughout the experiment, from 7 ± 0.46 to 14 ± 1.01 mm total length and from 0.1 ± 0.02 to 0.58 ± 0.18 mg dry weight at 22 ± 1 °C. During this period, the mean specific oxygen consumption of *P. lineatus* larvae was 0.73 ± 0.26 mg O_2 g wet weight^{-1} h^{-1} at an average wet weight of 6.45 ± 4.15 mg. The total length and wet weight of these larvae were not significantly different from those fed a natural veliger diet in the growth experiments (ANOVA, $F_{4, 22} = 1.32$, $P = 0.29$ and ANOVA, $F_{4, 22} = 2.52$, $P = 0.09$ for length and wet weight, respectively). The SMR at this temperature and range of larval sizes varied between 0.025 and 0.120 J h^{-1} per larva (Fig. 3a). Changes in the SMR with respect to larval dry mass fitted a polynomial function ($y = -0.046x^2 + 0.181x + 0.001$) with $n = 91$ and $r^2 = 0.83$, $P = 0.005$. The total energy consumption per larva and per week, including the energy costs of growth and SMR, increased during the experimental period (Fig. 3b). This total consumption ranged from 7 to 21 J ind.$^{-1}$, with the energetic increase due growth per week ranging between 1 and 6 J ind.$^{-1}$ (Fig. 3b).

Energetic analysis

The energy used by the fish larvae during this experiment was lower than the energy available to them during the first 3 weeks of the study, as indicated by the energy available in their prey in relation to the energy allocated to respiration and growth (Table 3). The differences between these values were highest during the second week of the treatments and decreased towards the end of the experiment. Only once, during week 4 under a natural diet, did the energy balance become negative (Table 3).

Discussion

This is the first study on the growth rates and SMR of *P. lineatus* larvae. This study demonstrates that veligers of *L. fortunei* can significantly enhance the growth of *P. lineatus* larvae and supports the idea that this new and abundant resource is selectively preyed upon by this larval fish. Before the arrival of *L. fortunei*, cladocerans were the main prey item for larvae less than 14.0 mm (Rossi, 1992), whereas after the bivalve's arrival, average proportions of veligers in the diet ranged between 20 and 90%, depending on veliger concentrations in the water (Paolucci *et al.*, 2007). This suggests that our laboratory results are representative of the effects on growth that are happening in the natural environment since early larval stages of *P. lineatus* have incorporated veligers into their diet. Enhanced growth rates will be dependent on the biochemical composition and caloric content of prey, as well as the energy costs involved in prey capture.

Chemical composition analyses showed that veligers have high protein contents, and that lipid contents and energy densities are higher than those of cladocerans and copepods. In general, the biochemical compositions of the food items were consistent with those seen in other crustacean and mollusc studies which have found high protein levels (over 50%) and variable proportions of lipids and carbohydrates (Williams & McMahon, 1989; Sprung, 1993; Riccardi & Mangoni, 1999; Chaparro, Navarrete & Thompson, 2006). Specifically, the cladocerans and

Table 3 Growth, energy consumption rate and energy balance of *Prochilodus lineatus* larvae under veliger-enriched, natural and low-veliger diets. The number of food items in the diet was derived using the total prey consumption rate of Paolucci *et al*, (2009) and each item's percentage in the respective diet. The available energy of each prey type was calculated with the prey consumption rate, average energy content of 0.009, 0.056 and 0.012 J Ind^{-1} for cladocerans, copepods and veligers, respectively, and a feeding period of 14 h

Treatment	Week	Average wet weight (mg)	Growth (% per day)	Prey consumption rate (ind.h^{-1})	Energy consumed (J $week^{-1}ind^{-1}$)				Energy allocation (J $week^{-1}ind^{-1}$)			Excess energ
					Cladocerans	Copepods	Veligers	Total	SMR	Growth	Total	
Veliger-enriched diet	1	2.01 ± 1.33	4.81 ± 1.33	8.4 ± 3.28	0.00	0.00	9.88	9.88	5.79 ± 0.85	0.69 ± 0.12	6.49 ± 0.97	3.40
	2	2.54 ± 0.92	3.76 ± 0.92	15.0 ± 11.11	0.00	0.00	17.64	17.64	5.57 ± 1.55	0.58 ± 0.11	6.15 ± 1.66	11.49
	3	5.7 ± 1.61	17.77 ± 1.61	15.0 ± 11.11	0.00	0.00	17.64	17.64	9.04 ± 0.82	4.44 ± 0.12	13.49 ± 0.94	4.16
	4	28 ± 15.46	55.88 ± 15.46	15.0 ± 12.55	0.00	41.16	8.82	49.98	14.97 ± 1.34	29.18 ± 0.76	44.15 ± 2.10	5.83
Natural diet	1	1.69 ± 0.76	1.77 ± 0.76	6.0 ± 3.84	0.48	0.00	6.41	6.90	5.79 ± 0.85	0.20 ± 0.72	5.99 ± 1.57	0.91
	2	2.3 ± 0.71	5.15 ± 0.71	9.8 ± 5.54	1.04	4.30	9.22	14.56	5.57 ± 1.55	0.72 ± 0.07	6.30 ± 1.62	8.25
	3	4.8 ± 1.88	15.52 ± 1.88	9.8 ± 5.54	1.85	5.76	7.82	15.44	9.04 ± 0.82	3.50 ± 0.12	12.55 ± 0.94	2.89
	4	17.5 ± 3.33	37.79 ± 3.33	12.0 ± 4.76	2.12	15.15	8.04	25.31	14.97 ± 1.34	16.47 ± 0.23	31.44 ± 1.57	-6.15
Low-veliger diet	1	1.66 ± 1.61	1.48 ± 1.46	3.8 ± 3.56	0.37	2.32	3.48	6.17	5.79 ± 0.85	0.15 ± 0.23	5.95 ± 1.08	0.21
	2	1.8 ± 1.36	1.2 ± 0.25	6.2 ± 5.06	0.55	10.21	4.37	15.13	5.57 ± 1.55	0.04 ± 0.14	5.62 ± 1.69	9.50
	3	3.01 ± 1.57	9.6 ± 0.2	6.2 ± 5.06	4.10	8.51	0.00	12.61	9.04 ± 0.82	1.68 ± 0.14	10.72 ± 0.96	1.88
	4	8.5 ± 4.52	26.05 ± 2.95	5.8 ± 1.51	1.71	21.22	0.00	22.93	14.97 ± 1.34	7.03 ± 0.27	22.00 ± 1.61	0.92

copepods in the present study had chemical compositions similar to those recorded previously for similar species from several environments (Vijverberg & Frank, 1976; Riccardi & Mangoni, 1999; Macedo & Pinto-Coelho, 2001). Although lipid contents displayed great variation between prey items, veligers contained up to 3 and 5 times more lipids than that found in cladocerans and copepods, respectively. Lipid contents in veligers reached 17.0% of the AFDW, and hence the energy density of the veligers was greater than either of the other prey items. The energy contents measured in this study were comparable with those reported previously for other species of bivalves, copepods and cladocerans (Williams & McMahon, 1989; Riccardi & Mangoni, 1999; Magoulick & Lewis, 2002). Although no previous data of energy density for *L. fortunei* veligers are available, the energy density for *L. fortunei* veligers (24.88 ± 1.81 kJ g^{-1} dry weight) was slightly higher than that recorded for adults of the invasive bivalve *Dreissena polymorpha* and other bivalve larvae (between 17.3 and 22.7 kJ g^{-1}; Blaber, 1979). In addition to high energy density, the veligers had a higher dry biomass than crustacean prey of the same or greater total length; and consequently, veligers had comparatively higher total energy content.

Similar to that reported in other studies with larval fish, the differences in growth rates observed in this experiment were probably influenced by the biochemical composition of prey (Lankford & Targett, 1997; Halver, 2001; Teshima *et al*., 2004). Several authors have highlighted the combination of high protein and fat contents, like that found in veligers, as important to the diet of larval fish in general (Rodríguez *et al*., 1997; Sargent *et al*., 1999; Lazo, 2000; Rønnestad *et al*., 2007). While lipids provide necessary energy during the fast-paced larval fish development period, protein is the most important body component and accounts for over 50.0% of the AFDW in these organisms. Moreover, higher rates of growth in larval fish are seen when the diet is rich in free amino acids, peptides and unsaturated essential fatty acids, as docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids. Particularly, the latter two cannot be synthesised by the larval fish themselves. These components are known to be incorporated into the diets of different species of zooplankton (Sprung, 1984; Vanderploeg, Liebig & Gluck, 1996; Barnard *et al*., 2006), and the veligers of the bivalve *D. polymorpha* can

absorb these dissolved organic compounds directly from the water (Baines, Fisher & Cole, 2007). Although there are no such studies on veligers of *L. fortunei*, it is most likely that they are able to incorporate these dissolved chemical compounds, which will then be available in the diet of larval fish.

Reduced energetic costs associated with the capture of slow prey, such as veligers, in comparison with fast prey, such as cladocerans, and especially copepods may also have had an effect on larval growth. Actually, our results showed clear evidence of selectivity on mussel veligers, particularly in the first weeks of development of *P. lineatus* larvae, when their consumption selectively targeted this prey item (cf. Fig. 1). This evidence of selectivity is also supported by field data (Paolucci *et al.*, 2007) and laboratory tests (Paolucci *et al.*, 2009), where veligers were preferred to other items such as copepods and cladocerans, apparently because of their lower ability to avoid predation. Selective feeding on slow and easy prey implies a lower energetic cost of feeding which results in a positive energetic impact that could enhance growth rates (Lazzaro, 1987; Lankford & Targett, 1997).

The mean SMR of larval *P. lineatus* in this study is exponentially proportional to those obtained for juvenile *P. lineatus* by Parma de Croux (1994), whereby the mean specific oxygen consumption of larvae (0.73 mg O_2 h^{-1} mg^{-1} at 6.5 mg wet weight) was *c.* 3 times higher than that measured for *P. lineatus* juveniles (0.24 mg O_2 h^{-1} at 10 g wet weight), achieving a mass-specific scaling coefficient of about −0.15. The measured larval SMR is also similar to those measured on larval fish of similar weight in other species (Wieser *et al.*, 1988; Wieser & Medgyesy, 1990; Parra & Yúfera, 2001).

All three diets seem sufficient to support SMR and growth in agreement with the experimental observations. The greatest values of excess energy at the end of each week were in the veliger-enriched experiments. Under all three diet regimes, the greatest amount of excess energy is apparent at the end of week 2, right before a doubling in the amount of energy used for growth. The only time when there appeared to be insufficient energy to support growth and metabolism was at the end of week 4 under a natural diet. A more detailed study is needed, but the negative value of excess energy at the end of the natural diet experiment was probably related to a transition in feeding behaviour of post-larvae and juveniles. In a field-based investigation, Rossi (1992) observed that at *c.* 14 mm, *P. lineatus* larvae begin shifting from a crustacean-based diet to a diet rich in diatoms, fungi, protozoa, rotifers, and detritus before adopting the detritivorous diet of adult fish. The estimates above do not include other energetic costs, such as specific dynamic action, coefficients of utilisation, dissimilar energetic costs of prey capture for different prey types, the energy lost from the system, etc., needed to understand the actual energetic balance. However, in spite of these gaps in our knowledge, the energetic importance of veligers compared to other items in the diets of larval *P. lineatus* is very high.

Many cases of incorporation of exotic bivalves into the diets of native adult fishes have been recorded, and these dietary shifts generally are considered to constitute positive impacts on fish populations (French & Bur, 1996; Molloy *et al.*,1997; Cataldo & Boltovskoy, 2000; Magoulick & Lewis, 2002; Garcia & Protogino, 2005; Boltovskoy *et al.*, 2006; Paolucci *et al.*, 2007; Cantanhêde *et al.*, 2008). Relatively little research has been conducted on predation of zebra mussel veligers by fry fish (reviewed by Molloy *et al.*, 1997). Despite the high numbers of veligers in the gut contents of larval fishes, the direct impact of this feeding on fish growth has not been studied. However, studies on adult *D. polymorpha* have found that this invasive mussel represents a lower-quality food item than traditional prey such as fish, clams and non-mollusc invertebrates (French & Bur, 1996; Nagelkerke & Sibbing, 1996; Magoulick & Lewis, 2002; Pothoven & Madenjian, 2008). In general, *D. polymorpha* represents a lower caloric content compared to natural prey. This can be up to an order of magnitude less when taking into account the energetic density of the whole organism and the energy cost involved in prey handling (Magoulick & Lewis, 2002). Especially, when the percentage of non-digestible material is high, between 67 and 94% of stomach contents (French & Bur, 1996), the energy value of the fresh tissue in relation to the total weight ingested decreased significantly. As a result of this lower energy, negative impacts on the growth rates of some fishes were observed (French & Bur, 1996). Moreover, Nagelkerke & Sibbing (1996) showed that energy requirements during handling time of *D. polymorpha* can consume a considerable proportion, up to 29%, of the energy

gained by feeding on this mussel. On the other hand, the non-digestible remains (shells) of *L. fortunei* veligers, represented less than 30% of dry weight, and, given the fact that veligers are much slower and clumsier swimmers than crustaceans, their capture times are most probably significantly lower than those of cladocerans and copepods. Consequently, veligers represent a higher-quality food in comparison with other prey, explaining the positive impact on the growth of fish larvae that feed on veligers.

In the Paraná River, consumption of veliger larvae is not limited to *P. lineatus*. At least 10 other species have been observed to feed on veligers (Paolucci *et al*., 2007), and their growth patterns may also have changed after the introduction of this exotic bivalve. Most of these zooplanktivorous larval fishes fed mainly on cladocerans and copepods prior to the arrival of *L. fortunei* (Rossi, 1992; Makrakis *et al*., 2005). The growth, as well as the mortality, of larval fish depends primarily on the quality and availability of food (Leiby, 1984; Fortier & Leggett, 1985; Li & Mathias, 1987). The biochemical composition of veligers and their availability because of the temporal overlap between fish and mussel reproduction periods (Paolucci *et al*., 2007) result in a stable supply of high-quality prey for the larval fishes. Because of the widespread occurrence of adult *L. fortunei* in the diet of fish in the Río de la Plata Catchment, Boltovskoy *et al*. (2006) have analysed the impact on the adult fish populations. According to these authors, positive impacts of *L. fortunei* are not limited to fishes that directly consume mussels, but there are also indirect positive effects on ichthyophagous and detritivorous fish species. Fish larvae, e.g. *Salminus maxillosus*, *Rhaphiodon vulpinus*, *Pseudoplatystoma coruscans*, *Pseudoplatystoma fasciatum*, and metalarvae of other Pimelodidae that prey on other larval fish probably benefit from the mussel-enhanced growth rates of their prey. Because of the importance of larval fish survival on the adult populations, it is highly probable that positive effects of veliger-rich diets radiate through fish food webs and have positive effects at the population level of many other species.

The effects of larval fish predation on zooplankton communities have been studied extensively (Lazzaro, 1987; Mehner & Thiel, 1999; Viitasalo, Flinkman & Viherluoto, 2001; Milstein, Valdenberg & Harpaz, 2006), and selective feeding on specific zooplankters results in dramatic declines in the targeted prey populations and significant changes in the composition of the zooplankton community. Although predation on veligers could potentially inhibit the initial colonisation or recolonisation of invasive mussels, it appears that there is no reduction in the total population of these exotic bivalves (Magoulick & Lewis, 2002; Sylvester, Boltovskoy & Cataldo, 2007). In the lower Paraná River, Sylvester *et al*. (2007) found that predators, mainly adult fish, harvest 26–79% of the individuals (20–85% of the biomass) in the *L. fortunei* population and suggested that despite being more actively consumed than other exotic bivalves elsewhere, e.g. *D. polymorpha*, suppression of mussel populations because of predation pressure does not seem to occur. As was discussed in Paolucci *et al*. (2009), it seems unlikely that the effect of larval fish predation may significantly reduce the standing stock of veligers found in the plankton at average ichthyoplankton densities of 3 larvae m^{-3} (Rossi, Cordiviola & Parma, 2007). According to that study, the mortality by predation is between 10 and 20% of the standing stock of veligers. Those mortality rates were based on laboratory measurements of consumption rates for *P. lineatus* larvae, and lab-based measurements may overestimate the actual predation rates in the field (cf. MacKenzie, Leggett & Peters, 1990). However, the energetic balance estimates in the present investigation indicate that lower consumption rates would not be able to maintain the high growth rates of these larval fish. Furthermore, the presence of other ichthyoplanktonic predators of veligers, some of which probably have higher consumption rates than *P. lineatus* because of their greater sizes, supports the idea that the predation impact is probably several times higher. In spite of this, the longer reproductive period of *L. fortunei* (6–9 months; Boltovskoy *et al*., 2009) compared to those of the fish species (2–4 months; Paolucci *et al*., 2009) must significantly decrease the long-term impact of larval fish on mussel veliger populations. To provide more insight into the overall ecological impacts of this introduced species, both on the veligers and on the larval fish, knowledge of some important variables, such as the fecundity of *L. fortunei* and the specific feeding ecology of other larval fishes, is needed. Such knowledge would be enhanced by implementing techniques such as stable isotopes analysis to better elucidate the ecological impacts (Lopes, Benedito-Cecilio & Martinelli, 2007; Rennie, Sprules & Johnson, 2009).

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