Feeding selectivity of the invasive mussel Limnoperna fortunei (Dunker, 1857) on a natural phytoplankton assemblage: what really matters?

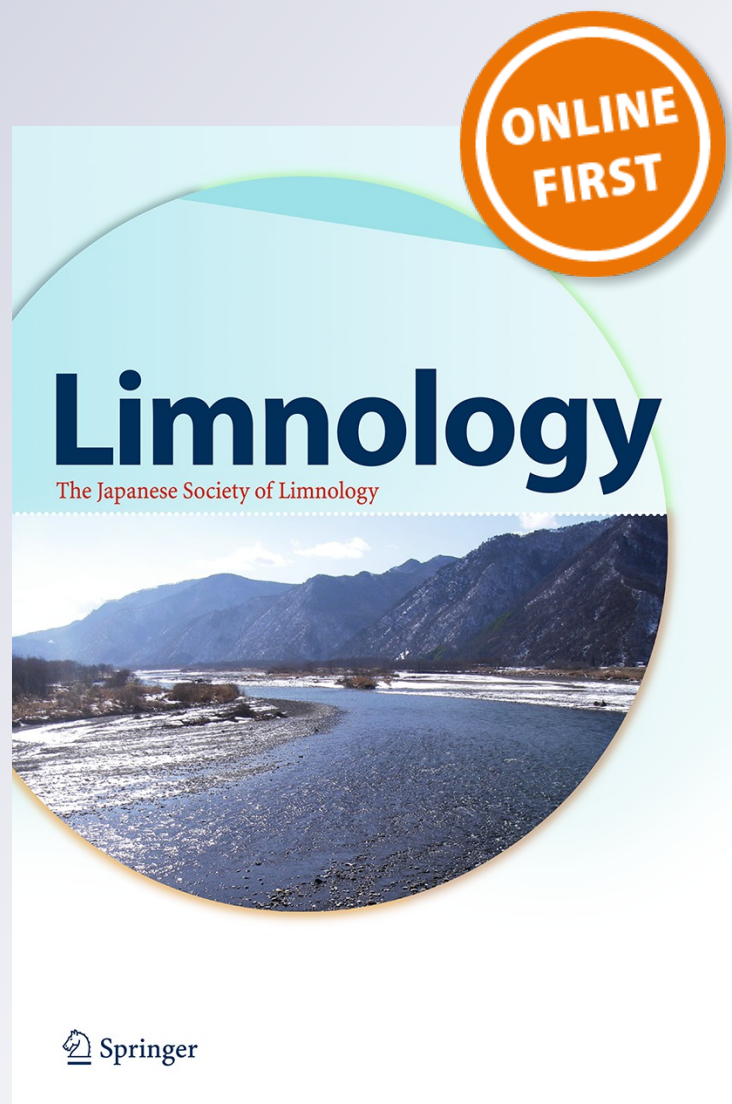**Diego Frau, Florencia Rojas Molina & Gisela Mayora**

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RESEARCH PAPER

Feeding selectivity of the invasive mussel *Limnoperna fortunei* (Dunker, 1857) on a natural phytoplankton assemblage: what really matters?

Diego Frau[1] · Florencia Rojas Molina[1,2] · Gisela Mayora[1]

Abstract The aims of this work were to analyse the feeding selectivity of *L. fortunei* in a natural assemblage of phytoplankton in a short-term microcosm experiment and to assess whether this selectivity is affected by the presence of Rotifera as a secondary, palatable feeding resource. This bivalve preferred Desmidiales, Chlorococcales, Euglenophyceae and Chrysophyceae algae with a maximum linear dimension from 20 to 100 μm. Organisms between 500 and 40×10^3 μm³ belonging to Desmidiales, Chrysophyceae and Euglenophyceae were also positively selected. Volvocales, Cryptophyceae and one group of medium-size Euglenophyceae (*Trachelomonas* sp.) had a high, negative selectivity index independent of their cell shape or size (Ivlev's index of feeding selectivity <-0.7). The mussel positively selected Rotifera, and this only had a measurable effect on large Euglenophyceae, which increase their selectivity value in the absence of Rotifera. The non-parametric multiplicative regression showed that selectivity is largely explained by a combination of cell shape, biovolume and the phytoplankton taxa offered ($R^2 > 0.8$). We concluded that the impact on phytoplankton community structure could be severe, considering that the presence of zooplankton does not have an effect on the majority of phytoplankton groups and that the mussel tends to feed on both items to improve its diet. The negative selection of some phytoplankton taxa is possibly related to the morpho-physiological characteristics of their cell shells.

Keywords Phytoplankton · Selective feeding · Invasive mussel species

Introduction

Limnoperna fortunei (Dunker 1857) is a small, mytilid, invasive bivalve thought to have originally inhabited China, Thailand, Laos, Cambodia, Vietnam, Indonesia and Korea (Ricciardi 1998), which was accidentally introduced to South America in 1991 in ballast water (Darrigran and Pastorino 1995). This mussel has an epifaunal habit and displays an aggregate behaviour during the adult phase. *Limnoperna fortunei* is a dioecious species with external fecundity and a planktonic larval phase (Darrigran and Pastorino 1993; Cataldo and Boltovskoy 2000) distinguishing it from the native fresh-water bivalves of the Neotropic region (Brugnoli et al. 2005). This bivalve has invaded not only South American water bodies from Argentina, Bolivia, Paraguay, Uruguay and Brazil, but also has invaded water bodies from Japan and Taiwan (Ricciardi 1998; Darrigran and Damborenea 2011); its larval stage has a high capability of using lotic environments to disperse throughout different kinds of continental water bodies.

In South America, the abundance and distribution of *L. fortunei* have changed the habitat complexity with relevant effects on benthos composition (Darrigran et al. 1998) as well as on water column properties (suspended matter, transparency and nutrients) (Cataldo et al. 2011). Additionally, this bivalve interacts at several levels in the food web, being consumed by fishes and macro-crustaceans (e.g.

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Montalto et al. 1999; Collins et al. 2007) and establishing itself as a plankton filter-feeder that can severely impact the whole community. Several studies have shown that *L. fortunei* can ingest zooplankton (mainly Rotifera) and phytoplankton, affecting their abundance (e.g. Rojas Molina et al. 2010; Cataldo et al. 2011; Frau et al. 2013).

Despite the fact that several studies have addressed the issue of selectivity in *L. fortunei* grazing using natural, mixed plankton and various combinations of cultured algae, the results available to date are still scarce and contradictory (Boltovskoy et al. 2015), demonstrating the complexity of the feeding behaviour of this mussel. Cataldo et al. (2011) and Rückert et al. (2004) did not describe selective feeding by *L. fortunei* with regards to cell biovolume or phytoplankton taxa, whereas Gazulha et al. (2012a, b) found that this bivalve preferred small cell-size organisms, expelling filamentous, colonial Cyanobacteria and Bacillariophyceae (diatoms) as pseudofaeces. Contrarily, Rojas Molina et al. (2010) found that *L. fortunei* selected larger phytoplankton and that Euglenophyceae was the most selected food item.

Relevant aspects such as cell shape, biovolume or even the influence of different phytoplankton taxa have not been considered in an integrated way. Moreover, none of these studies have addressed whether phytoplankton selection could be affected by the presence of Rotifera, which is highly selected by *L. fortunei* (Rojas Molina et al. 2010; Fachini et al. 2012) and provides higher biomass per individual as compared to phytoplankton.

We are dealing with an invasive mussel that feeds on a complex community of planktonic organisms and whose presence could have consequences for the productivity and diversity of the invaded water systems (Higgins and Vander Zanden 2010). For all these reasons, this study aims to analyse, in a short-term microcosm experiment, the feeding selectivity of *L. fortunei* in a natural assemblage of phytoplankton and determine whether this selectivity is affected by the presence of Rotifera as an additional, palatable feeding resource. We predict that: (1) phytoplankton selection will be highly dependent on a combination of cell shape, size and taxa composition; (2) the presence of Rotifera will favour phytoplankton assemblage though a reduction of the predation pressure on it.

Methods

Experimental organisms

The adult specimens of *L. fortunei* were manually removed from macrophyte roots located in a shallow lake of the Middle Paraná River floodplain (31°40′40″S–60°42′44″W, Argentina). The mussels were maintained in laboratory conditions (20 $\pm$ 2 °C, 16 h light: 8 h dark photoperiod) in 30-l aquaria filled with continuously aerated water for 7 days. Mussels were fed daily with commercial fish food and *Chlorella vulgaris* algae. The mean size ($\pm$ standard deviation, SD) of experimental mussels was 17.11 $\pm$ 1.87 mm in maximum valve length (individuals were measured to the nearest 0.01 mm with a digital caliper).

Plankton was collected in a second shallow lake in the Middle Paraná River system (31°37′S–60°41′W, Argentina). Forty litres of water was taken to the laboratory and filtered through a 1500 μm mesh to remove detritus and vegetation. This lake is characterised by the absence of *L. fortunei* and by a continuous plankton species composition throughout the year. Chlorophyceae dominate in density and Euglenophyceae in biovolume. There is no filamentous algae development, and there is a high abundance of Rotifera, with a mean maximum linear dimension (MLD) >55 μm (Frau 2012; José de Paggi et al. 2012).

Experimental design

The experiment was carried out during 12 diurnal hours, at 20 °C, in 12 transparent cylindrical glass containers (13 cm in diameter by 22 cm deep) with a capacity of 3 l. Four different treatments were developed: the first with phytoplankton + zooplankton (PZ), the second with phytoplankton + zooplankton + mussels (PZM), the third one with phytoplankton + mussels (PM) and the last one with only phytoplankton (P) as a control to corroborate algae reproduction in the absence of predation during the study period. Each treatment was replicated three times ($n = 12$). Containers were randomly assigned to the treatments, and PZ and PZM containers were filled up to 3 l with water from the lake. The water used in PM and P treatments was also filtered through a 55 μm mesh to remove almost all zooplankton and was poured in containers to the same volume as the other treatments.

The experiment began (0 h) by taking an initial plankton sample and measuring the environmental variables described below in each treatment. Immediately afterwards, five *L. fortunei* individuals ($\sim$377 ind m^{-2}) were added to the PZM and PM treatments. Mussel density was in accordance with observations of mussels attached to macrophytes in natural environments (between 90 and 2000 ind m^{-2}, Marcal and Callil 2008). After 12 h, plankton samples were collected, environmental variables were measured again and the experiment was finished.

Water temperature (°C), conductivity (μS cm^{-1}), pH and dissolved oxygen (mg l^{-1}) were measured using portable meters. Nitrate + nitrite (N–NO$_3^-$+N–NO$_2^-$), soluble reactive phosphorus (SRP) and ammonium (N–NH$_4^+$) concentration (μg l^{-1}) were measured following the

methods proposed in APHA (1992) with the intention of securing an equal phytoplankton nutritional state among treatments during the experience and then attributing changes in the phytoplankton concentration to the mussel feeding activity.

Phytoplankton samples were taken in 70-ml bottles from the sub-superficial region and fixed with 1 % acidified Lugol solution. The organism count followed the Utermöhl method (Utermöhl 1958) and density was expressed as ind ml^{-1}. Zooplankton samples were obtained using the method proposed by Szlauer (Szlauer 1964); samples were stained with erythrosine and fixed with 10 % formalin solution. The quali-quantitative analyses were performed under a binocular microscope (rotifers and *Copepoda nauplii* were counted in a 1 ml Sedgwick-Rafter chamber and microcrustaceans in a 5 ml Bogorov chamber) (Wetzel and Likens 1979). Zooplankton density was recorded as ind l^{-1}. In both cases (phytoplankton and zooplankton) organism volume was estimated by approximation to regular geometric shapes (Dumont et al. 1975; Ruttner-Kolisko 1977; Hillebrand et al. 1999) and biovolume was expressed as μm^3. Maximum linear dimension (MLD) was used as a measure of organism shape (Weithoff 2003) for phytoplankton and zooplankton. At least 20 individuals were measured per taxa (Lewis 1976). With the exception of Copepoda, the taxonomic identification of plankton was performed up to the most specific level possible (species level when was possible). Adult and juvenile Copepoda individuals were grouped into Calanoida, Cyclopoida and Harpacticoida.

At the end of the experiment *L. fortunei* specimens from each treatment (PZM and PM) were dissected under a stereo binocular microscope (4×) and their stomach and gut contents were analysed (5 stomach and gut contents per replica, 15 individuals per treatment). Phytoplankton was examined under an inverted binocular microscope, whereas zooplankton was studied in a Sedgwick Rafter chamber under a conventional optical microscope. Feeding selectivity was calculated using the formula proposed by Ivlev (1961): $E_i = (r_i - p_i)/(r_i + p_i)$ [where E_i = feeding selectivity index; r_i = relative abundance (density or biovolume) of the food item i in the diet and p_i = relative abundance (density or biovolume) of item i in the environment]. This selectivity index ranges from -1 to $+1$. When $E_i = 0$ selective feeding does not occur, when $E_i < 0$ food item i occurs less in the diet than expected from random feeding, indicating negative selection (avoidance or inaccessibility). When $E_i > 0$, food item i occurs more frequently in the diet than expected by chance, indicating positive selection (preference). The index was estimated for different categories of food items, considering combinations of phytoplankton taxa with cell shape expressed as MLD (μm) or with biovolume (μm^3) (Table 1). The

Table 1 Categories of maximum linear dimension (MLD) and biovolume defining phytoplankton groups

MLD (μm)	Category
2–20	S
21–40	M
41–70	L
71–110	XL
Biovolume (μm^3)	
<500	S
500–5 × 10^3	M
5 × 10^3–40 × 10^3	L

S small, *M* medium, *L* large, *XL* extra large

phytoplankton taxa registered were: Chlorococcales (Chlo), Volvocales (Volvo), Desmidiales (Desmi), Cryptophyceae (Crypto), Chrysophyceae (Chryso), Bacillariophyceae (Bacill), Chroococcales (Chro), Euglenophyceae (Eugle), centric diatoms (Cen), pennate diatoms (Pen) and Oscillatoriales (Oscill). Zooplankton was also grouped by shape [Rotifera: Roti < 150 μm (S), Roti > 150 μm (M); Cladocera: Clad < 410 μm (M), Clad > 410 μm (L); Copepoda: adults and nauplii]. Each zooplankton group was included in a single biovolume category with the exception of Rotifera, which exhibited a wider size range: smaller Rotifera (<530 × 10^3 μm^3, S category), larger Rotifera (>530 × 10^3 μm^3, M category), Cladocera (15 × 10^6 μm^3), adult Copepoda (12 × 10^6 μm^3) and Copepoda nauplii (7 × 10^6 μm^3).

Statistical analyses

All treatments (PZ, PZM and PM) were compared by a one-way ANOVA test with Tukey post hoc comparison at the beginning of the experiment (0 h) to verify that they had similar plankton concentrations. At the end of the experiment (12 h), the same statistical analysis was applied to evaluate the feeding impact of the mussel on the different phytoplankton groups in the presence and absence of zooplankton as a complementary feeding resource. The PZ treatment was used as a control to evaluate the possible influence of predation by zooplankton on phytoplankton density and also to statistically prove the effect of mussel feeding on zooplankton. The mean E_i obtained for each food item considered was compared to zero (null hypothesis) using a two-tailed, Student's one sample t test. The E_i value obtained for every phytoplankton category was compared between PZM and PM treatments using a Student's test for independent samples. Additionally, Student's test was used to compare the total biovolume registered in the stomach and gut content in each treatment (PM and PZM) to evaluate changes in mussel feeding when zooplankton was present. The predictive power of MLD,

biovolume (quantitative variables) and taxa (qualitative variable) to describe E_i (dependant variable) was estimated for PZM and PM treatments using a non-parametric multiplicative regression (NPMR). This regression model is used when data cannot be adjusted to a predictable curve shape and there is high correlation among explicative variables (Mcune and Mefford 2004). In this analysis, the Epanechnicov smoothing method was used with a polynomial grade 1 and a constant band size. These calibration parameters were chosen after trying different models and comparing them in accordance with their determination coefficient (R^2) and the sum of squared errors of the model (SSE).

Results

During the experiment, all treatments showed similar mean (±SD) environmental conditions of temperature (20.97 ± 1.14 °C), pH (6.30 ± 0.50), dissolved oxygen (6.07 ± 0.80 mg l$^{-1)}$ and conductivity (142.66 ± 5.15 µS cm^{-1}). Nutrient concentration varied little between 0 and 12 h in all treatments (at 0 h: N–NO$_3^-$+N–NO$_2^-$: 1039 ± 158 µg l^{-1}, SRP: 9.04 ± 3.11 µg l^{-1}, N–NH$_4^+$: 106 ± 38 µg l^{-1}; at 12 h: N–NO$_3^-$+N–NO$_2^-$: 1192 ± 71 µg l^{-1}, SRP: 10.15 ± 4.42 µg l^{-1}, N–NH$_4^+$: 115 ± 24 µg l^{-1}). No statistical significance was found for any nutrient measured among treatments or hours (repeated measure two-way ANOVA $p > 0.05$ for all cases).

Feeding resource characterisation and *L. fortunei* effects

In total, 90 phytoplankton species were recorded across treatments, including Chlorophyceae (Chlorococcales: 28 species; Desmidiales: 9 species; Volvocales: 5 species), Cryptophyceae (3 species), Chrysophyceae (2 species), Bacillariophyceae (25 species), Euglenophyceae (6 species) and Cyanobacteria (12 species). Ninety per cent of the individuals were from just four genera: *Chlamydomonas* and *Dictyosphaerium* (Chlorophyceae), followed by *Synura* (Chrysophyceae) and *Cryptomonas* (Cryptophyceae). At the beginning of the experiment, the average initial phytoplankton density was 1341 ± 144 ind ml^{-1} and the densities of each phytoplankton group were similar among treatments (Chlorococcales: $F = 0.704$, $p = 0.576$; Desmidiales: $F = 0.897$, $p = 0.484$; Volvocales: $F = 0.821$, $p = 0.305$; Cryptophyceae $F = 2.779$, $p = 0.110$; Euglenophyceae: $F = 0.749$, $p = 0.434$; Chrysophyceae: $F = 1.555$, $p = 0.274$; Bacillariophyceae: $F = 0.572$, $p = 0.649$ and Cyanobacteria: $F = 1.553$, $p = 0.275$).

Forty-four zooplankton taxa were recorded across all treatments, and the average initial density was 2801 ± 844 ind l^{-1}. The assemblage was dominated in richness and abundance by Rotifera (over 80 % of zooplankton abundance), with *Colurella*, *Lecane*, *Lepadella* and *Thrichocerca* as dominant genera. Cladocera and Copepoda were not well represented and made up only 2 and 18 % of total zooplankton abundance, respectively. Cladocera species were primarily *Ceriodaphnia dubia*, *Diaphanosoma birgeii* and chidorids. Most juveniles and adults of Copepoda were Cyclopoida, and more than 65 % of the Copepoda were nauplii. Likewise for phytoplankton, there was little variation in the density of zooplankton groups between treatments at the beginning of the experiment: Rotifera ($t = 0.824$, $p = 0.456$), Cladocera ($t = -0707$, $p = 0.519$) and Copepoda ($t = -1.342$, $p = 0.251$). It is worth pointing out that some Rotifera would be able to pass through the 50 µm-mesh net used to filter those treatments without zooplankton (PM and P treatment). We found some individuals in the PM and P treatments at the beginning of the experiment, but this density was lower than those in the microcosms with zooplankton (PZM) (less than 25 % in both treatments).

At the end of the experiment (12 h) the density of all of the phytoplankton groups declined noticeably in mussel treatments with respect to PZ (more than 90 % and up to 100 %) with the exception of Cryptophyceae in PM and PZM (Fig. 1). The density of Cryptophyceae increased more than 20 % in PZM and 50 % in PM in comparison with PZ. The differences in density between the treatments without *L. fortunei* (PZ) and mussel treatments (PZM and PM) were statistically significant for Chlorococcales, Desmidiales, Volvocales, Bacillariophyceae and Cyanobacteria (Table 2). In the phytoplankton control (P) a reduction in density was observed after 12 h of the experiment. However, these reductions in density were notoriously lower than the reductions observed in the presence of the mollusc (less than 50 % in all groups) and very similar to the reduction observed in the PZ treatment (Student test, PZ vs. P $p > 0.05$, in nearly all cases). The exceptions were the Volvocales and Cryptophyceae groups, which presented an increase of their initial density in a 56 and 46 %, respectively. This increment was statistically significant for Volvocales (Student's test PZ vs. P $t = 0.504$, $p = 0.045$), but not for Cryptophyceae (PZ vs. P $t = 0.408$, $p = 0.704$).

At 12 h Rotifera abundance was decreased by 75 % in PZM compared to PZ treatment, Cladocera 68 % and Copepoda 20 % (Fig. 2). These differences between treatments were statistically significant for Rotifera ($t = 3.914$, $p = 0.017$), but not for Cladocera ($t = 2.033$, $p = 0.122$) or Copepoda ($t = 0.322$, $p = 0.764$).
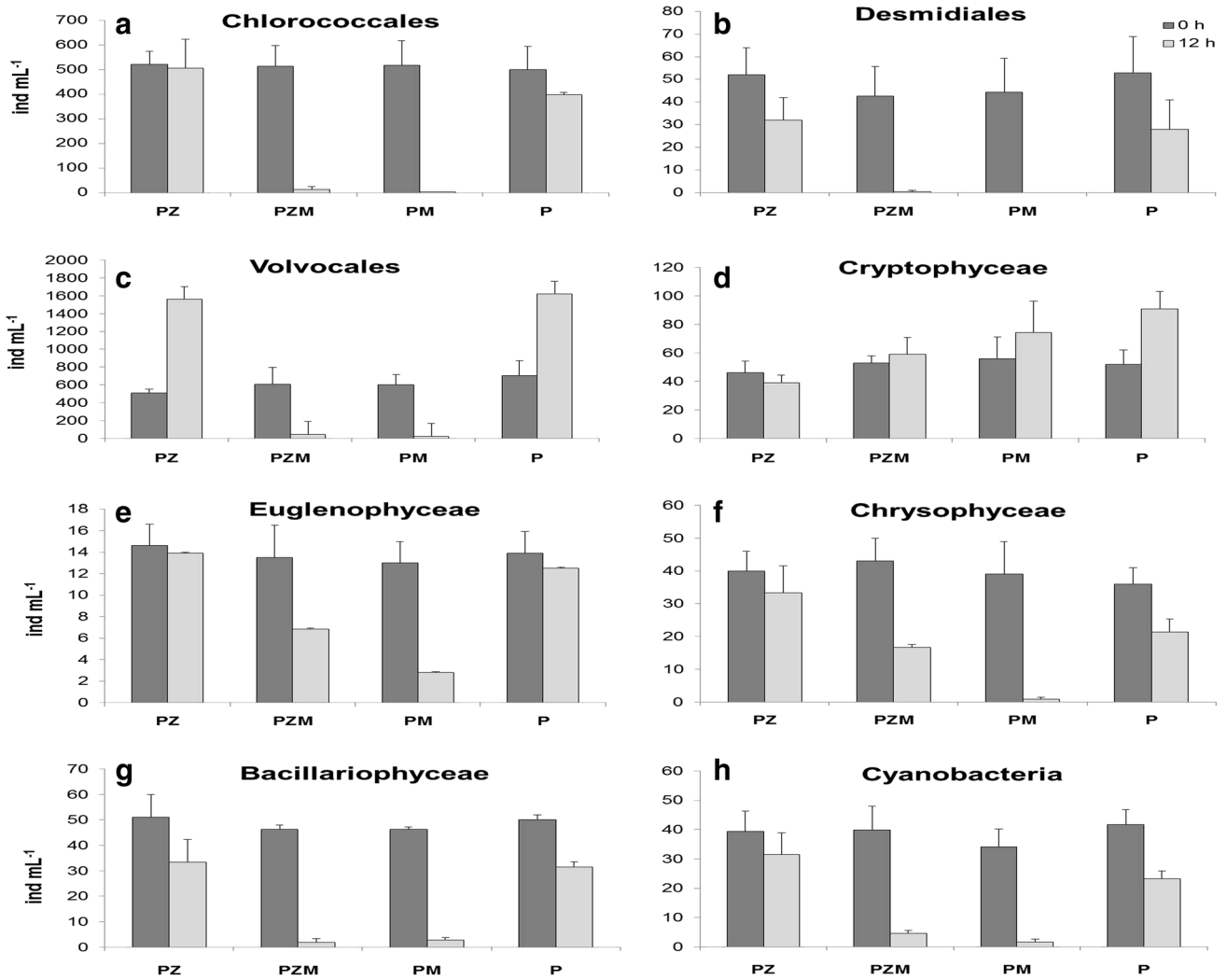
Fig. 1 Mean density and standard deviation (*vertical tiny bars*) of each phytoplankton group at 0 and 12 h of the experiment in each of the four treatments used. PZ (phytoplankton + zooplankton), PZM (phytoplankton + zooplankton + mussels), PM (phytoplankton + mussels), P (just phytoplankton)

Mussel feeding selectivity

A total of 46 phytoplankton taxa, representing only three genera (71 % of all the individuals recorded), *Dyctiosphaerium*, *Cosmarium* and *Euglena*, were found in the course of stomach and gut content processing. Zooplankton was represented by 26 taxa, with Rotifera as the most abundant group (97 % of the organisms) with *Colurella, Lecane* and *Lepadella* making a large contribution to the total zooplankton.

The E_i index showed that the majority of food items provided during the experiment were positively selected by the mussel (considering taxa in combination with MLD or biovolume) in both PZM and PM treatments. The exceptions were Volvocales (by MLD and biovolume), Cryptophyceae (by MLD and biovolume) and Eugle (M) just by

MLD, since each of these groups demonstrated a high and statistically significant negative selection (Fig. 3). In PZM, the most highly selected items by MLD were Chlo (L), Chlo (XL), Desmi (L), Eugle (L) and Chryso (L) ($p < 0.05$). When zooplankton was unavailable (PM treatment), *L. fortunei* also demonstrated a preference for Chlo (M) and Eugle (XL) (Fig. 3a), indicating a preference for items ranging between 20 and 110 μm in both treatments. With respect to biovolume, a significant ($p < 0.05$) positive E_i was found for Desmi (L) and Chryso (M) in PZM treatment. In the absence of zooplankton, Eugle (M) was also positively selected (Fig. 3b).

There were no statistically significant differences in the E_i values of almost any phytoplankton MLD or biovolume categories between PZM and PM ($p > 0.05$) with the lonely exception of the Eugle (XL) group, which statistically

Table 2 ANOVA test comparison between treatments at 12 h

Algal group	ANOVA		Tukey's test
Chlorococcales	$F = 27.50$	**$p < 0.001$**	**PZ vs. PZM $p < 0.0001$**
			PZ vs. PM $p < 0.0001$
			PZM vs. M $p = 0.999$
Desmidiales	$F = 5.53$	**$p = 0.024$**	PZ vs. PZM $p = 0.054$
			PZ vs. PM $p = 0.049$
			PZM vs. PM $p = 0.999$
Volvocales	$F = 147.3$	**$p < 0.001$**	**PZ vs. PZM $p < 0.0001$**
			PZ vs. PM $p < 0.0001$
			PZM vs. PM $p = 0.497$
Euglenophyceae	$F = 2.45$	$p = 0.138$	
Cryptophyceae	$F = 4.23$	$p = 0.103$	
Chrysophyceae	$F = 1.450$	$p = 0.306$	
Bacillariophyceae	$F = 15.86$	**$p = 0.001$**	**PZ vs. PZM $p = 0.004$**
			PZ vs. PM $p = 0.005$
			PZ vs. PM $p = 0.999$
Cyanobacteria	$F = 12.46$	**$p = 0.02$**	**PZ vs. PZM $p = 0.008$**
			PZ vs. PM $p = 0.004$
			PM vs. PZM $p = 0.957$

All phytoplankton groups were considered in each treatment: phytoplankton + zooplankton (PZ), phytoplankton + zooplankton + mussels (PZM) and phytoplankton + mussels (PM)

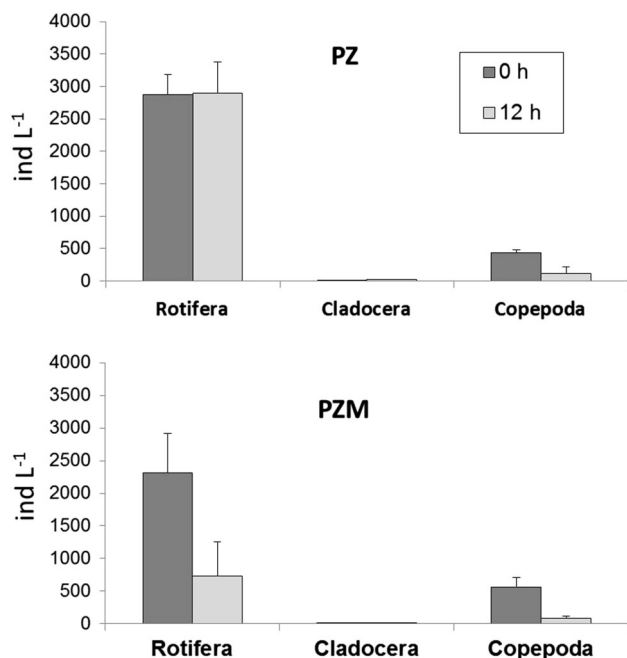Statistically significant values ($p < 0.05$) are indicated in bold

Fig. 2 Mean density and standard deviation (*vertical tiny bars*) of each zooplankton group in PZ (phytoplankton + zooplankton) and PZM (phytoplankton + zooplankton + mussels) treatments

differed between PZM vs. PM treatments ($t = -3.23$, $p = 0.032$), demonstrating a higher and statistically significant selectivity in the absence of zooplankton.

With respect to the zooplankton items offered, small Rotifera (S) showed a low but statistically significant positive selectivity index ($E_i = 0.3$). In contrast, Cladocera was not selected in any MLD category ($E_i = 0$), and Copepoda (including nauplli) presented a high and negative selectivity in both treatments ($E_i = -1$) (Fig. 3c). Biovolume selectivity followed a pattern similar to MLD for all zooplankton categories, although the positive selection of Rotifera biovolume categories ($E_i > 0.8$) was higher than Rotifera MLD categories (Fig. 3d). Rotifera represented 70 % of the total biovolume registered in the gut and stomach content (phytoplankton + zooplankton items) of PZM, but there were no statistically significant differences between treatments when the biovolume of the different phytoplankton groups was considered separately ($p > 0.05$ in all phytoplankton groups) or as total phytoplankton biovolume ($t = 0.627$, $p = 0.541$) (Fig. 4).

The NPMR model using the biovolume of each species found in the gut and stomach content ($n = 46$), their MLD and taxa as predictive variables for E_i demonstrated a high predictive power in both PZM (R^2: 0.851, SSE = 3.280) and PM (R^2: 0.812, SSE = 3.116) treatments.

Discussion

In these microcosm experiments, phytoplankton density was clearly affected by the presence of *L. fortunei*. The density of every group, with the exception of Cryptophyceae, was
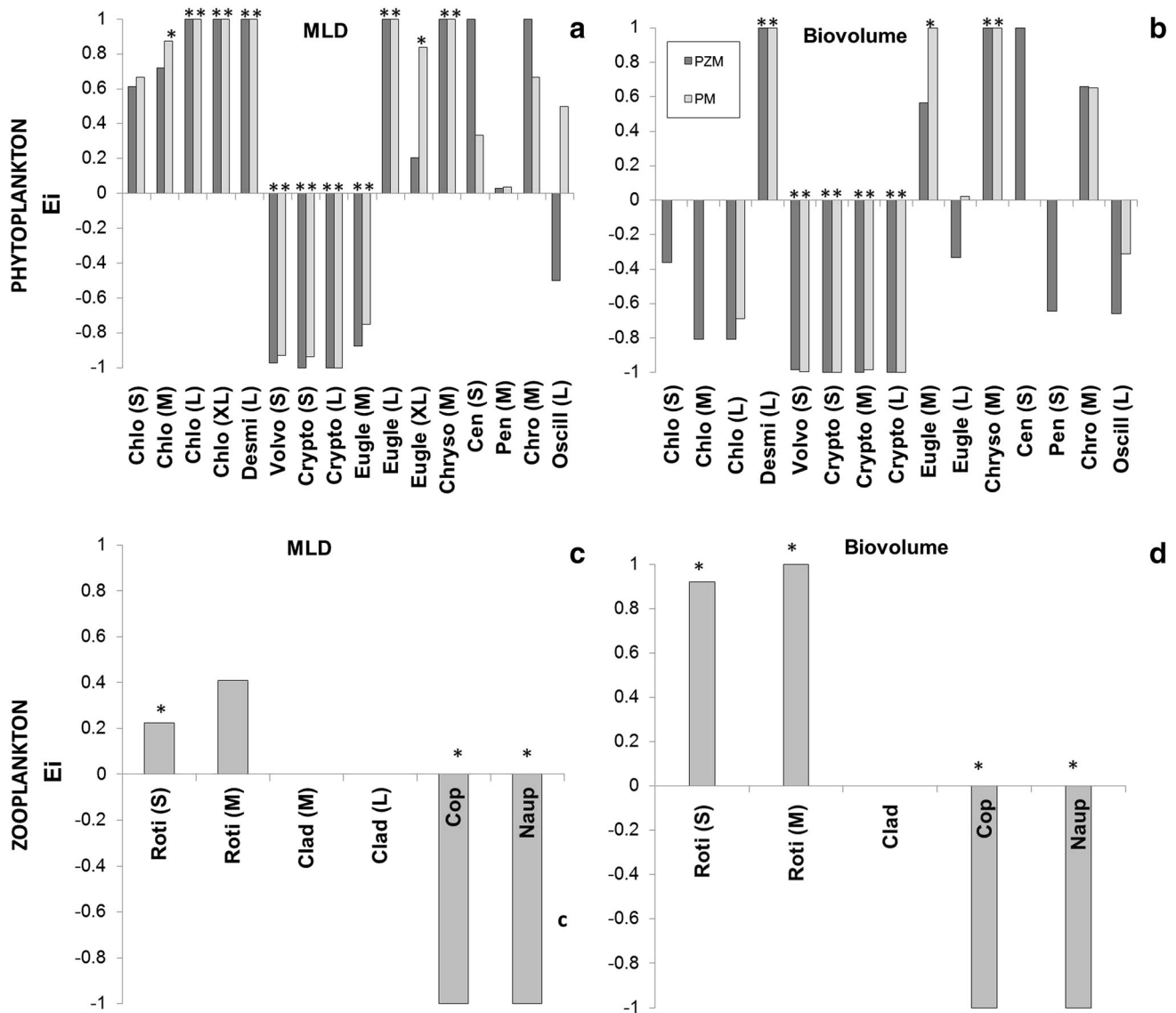
Fig. 3 Mean selectivity index (E_i) obtained for each group and category (MLD and biovolume) of phytoplankton (**a**, **b**) and zooplankton (**c**, **d**) in PZM (phytoplankton + zooplankton + mussel), and PM (phytoplankton + mussel) treatments. *Asterisks* indicate that the E_i value is statistically different from 0 ($p < 0.05$). *Chlo* Chlorococcales, *Desmi* Desmidiales, *Volvo* Volvocales, *Crypto* Cryptophyceae, *Eugle* Euglenophyceae, *Chryso* Chrysophyceae, *Cen* Centric diatoms, *Pen* Pennate diatoms, *Chro* Chroococcales, *Oscill* Oscillatoriales, *Roti* Rotifera, *Clad* Cladocera, *Cop* Copepoda, *Naup* Nauplii. Code: *S*, *M*, *L*, *XL* refers to the categories expressed in Table 1 for MLD and biovolume, respectively

negatively impacted by the feeding activity of the mussel, demonstrating that *L. fortunei* is a filter-feeder that can consume nearly all phytoplankton groups, with few exceptions.

Is phytoplankton selectivity affected by the presence of Rotifera?

Although Rotifera represented an important proportion of total dietary biovolume and was a highly selected item by the mussel, our results indicate that the pattern of phytoplankton selection was very similar in both treatments. We initially predicted that in presence of Rotifera (PZM treatment), the mollusc would select Rotifera and this would change selectivity on phytoplankton groups as compared to the group without zooplankton (PM treatment). Rotifera is a highly desirable food item because it has a high nutritional quality (28–67 % of its dry weight is protein, Øie and Olsen 1997), has relatively low mobility, which would not be enough to avoid mussel predation (Rojas Molina et al. 2010; Fachini et al. 2012), and has greater biovolume than several phytoplankton species. Two
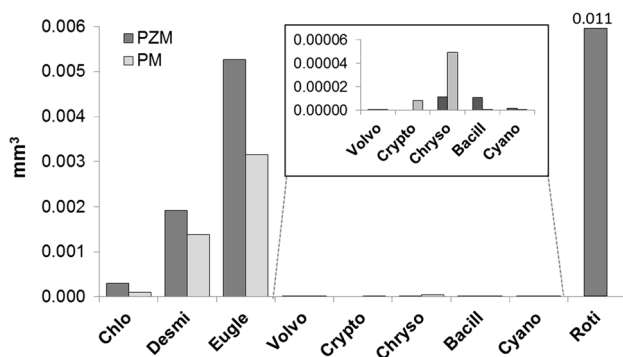
Fig. 4 Mean total biovolume of plankton groups in the stomach and gut content of *L. fortunei* for PZM (phytoplankton + zooplankton + mussel) and PM (phytoplankton + mussel) treatments. Abbreviations used here are the same as Fig. 3

important conclusions arise from our results. First, the plankton assemblage offered did not satiate *L. fortunei* during the study period, since almost all of the phytoplankton individuals offered were consumed in the presence of the mussel. Previous studies of other filter-feeding bivalves have indicated that satiation only occurs at higher phytoplankton concentrations ($>500,000$ ind ml^{-1}) and for short periods of time (Pascoe et al. 2009). Second, our results of feeding selectivity indicate that this mussel prefers an omnivorious feeding behaviour. Wong and Levinton (2004) indicate that suspension feeders benefit from omnivorous feeding as opposed to consuming only phytoplankton or zooplankton. *Limnoperna fortunei* fed on both plankton groups and Rotifera did not affect the feeding selectivity of almost all phytoplankton groups. The exception was Eugle (XL) mainly represented by *Euglena* spp. and *Phacus* sp. This group of algae with a MLD >70 μm presented a higher filtration in the absence of Rotifera as feeding resource, suggesting that a compensation of biomass in the mussel diet occurs when zooplankton is absent. With respect to Copepoda (negative selectivity), these results are consistent with those of Rojas Molina et al. (2010), who attributed the negative selectivity to Copepoda (including nauplii) to their ability to avoid predation, whereas absence of Cladocera selectivity in our study can be explained by the low density registered in the treatments.

Selectivity multi-criteria

The feeding selectivity of filter-feeding molluscs operates on different levels (Shumway et al. 1985; Baker et al. 2000). The first pre-ingestive mechanism occurs in the ctenidium, which can retain particles within a specific size spectrum (represented in our study by plankton biovolume and shape). The selected particles are transported to the labial palps where a second pre-ingestive selection occurs.

This selection is largely dependent on the morpho-physiological characteristics of the particles (represented in our study by the features of the different phytoplankton taxa offered). In both pre-ingestive steps negatively selected particles are bound in mucus and expelled as pseudofaeces through the inhalant siphon.

The NPMR model demonstrated a high predictive power of feeding selectivity (E_i) when shape (MLD), biovolume and taxa were combined as explanatory variables (in both treatments $R^2 > 0.8$), indicating that phytoplankton selectivity depends on several factors, which simultaneously influence prey selection. We demonstrated that the mollusc can feed on a wide spectrum of phytoplankton particles (between 20 and 110 μm in MLD). By comparison, *Dreissena polymorpha* (Pallas), which has invaded several water bodies in Europe and North America and is considered to be the ecological equivalent to *L. fortunei* (Karatayev et al. 2007), can feed on a wide range of phytoplankton items (≥ 7–100 μm in MLD) and selects larger items (between 71 and 95 μm) (Dionisio Pires et al. 2004; Naddafi et al. 2007). A very similar range is described in this study for *L. fortunei*.

Different patterns of selectivity were also observed for each category of algal biovolume. Our results indicate that this mussel primarily fed on particles between 500 and 40×10^3 μm^3, which is consistent with the observation made by Rojas Molina et al. (2010). In view of the importance of high biovolume algae as a food item, we expect that the feeding activity of the mussel in its natural environment will lead to an over-dominance of small biovolume algae or even encourage the development of algae that aggregate in mucilaginous colonies as a strategy to avoid mussel predation (Cataldo et al. 2012).

Limnoperna fortunei selected phytoplankton by taxa. Our results indicate that at least three groups of algae were not ingested by the mollusc, independent of their shape or biovolume. The Volvocales group was composed of unicellular flagellate species, mainly *Chlamydomonas*, whose density was severely affected after 12 h (a reduction of 97 % in PZM and 99 % in PM treatments compared to a density increase in PZ treatment). Considering that very few *Chlamydomonas* (Volvocales) individuals were found in the stomach (see Fig. 4) relative to the other 45 species registered, we assumed that the majority were expelled as pseudofaeces. Dionisio Pires and Van Donk (2002) showed that *D. Polymorpha* prefered small, non-toxic colonies of *Mycrocistis* to *Chlamydomonas*, a pattern that they attributed to the thickness of the *Chlamydomonas* cell wall.

Cryptophyceae were mainly represented by the genus *Cryptomonas*, did not experiment reduction in abundance in the presence of *L. fortunei* (Fig. 1) and had a negative selection index in both PZM and PM treatments. Moreover, very few individuals were found in the stomach contents

(see Fig. 4), suggesting that this group was not available for the mollusc filtration, or they were rapidly digested, a possibility that does not seem to be very probable considering the high number of other species with similar cell wall characteristics found in the stomach and gut content. Our results corroborate previous natural diet analyses and experimental studies of *L. fortunei*. Rojas Molina et al. (2010) did not find *Cryptomonas* individuals in the stomach content despite its presence in the environment, and in Frau et al. (2013) we did not find changes in *Cryptomonas* density in a mesocosm experiment with mussel presence. Similar conclusions have been drawn about the invasive bivalve *Corbicula fluminea* (Müller), which invaded similar water bodies to those now inhabited by *L. fortunei* in South America (see Table 2 and Fig. 6 in Boltovskoy et al. 1995). On the other hand, these results contrast with studies of *D. polymorpha* that observed high, positive selection of *Cryptomonas*, this being attributed to its high palatability and nutritional content (e.g. Bastviken et al. 1998; Naddafi et al. 2007). Specific studies related to the feeding selectivity of *L. fortunei* on *Cryptomonas* are necessary in order to clarify this pattern.

Finally, the phytoplankton group Eugle (M) in MLD was also negatively selected by the mussel. The lone representative of this group was the genus *Trachelomonas*, which is notable for its shell-like covering, known as a lorica. This structure is made of minerals and mucilage, and it can be spherical, subspherical, ellipsoidal, ovoid or ovate, variably punctate, with spines or smooth, and with or without a collar (Wolowski and Walne 2014). Epiparticulate chemical compounds produced by phytoplankton and the electrostatic charge of particles can influence the efficiency of food selection by *Mytilus edulis* (Ward and Targett 1989; Hernroth et al. 2000) and have also been suggested as a factor for *D. polymorpha* food selection (Naddafi et al. 2007). The interaction between *L. fortunei's* selectivity organs and the surface of phytoplankton cells is a key issue that has yet to be evaluated and certainly could play an important role in the negative selectivity of *Trachelomonas* or other loricated phytoplankton groups.

As for selectivity patterns by MLD and biovolume, selectivity was positive and high for Chlorococcales, Desmidiales, Euglenophyceae [except Eugle (M) in MLD] and Chrysophyceae. High concentrations of long-chain polyunsaturated fatty acids (PUFAs), particularly EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), have a positive effect on several aspects of bivalve development, such as growth, mortality, egg quality and recruitment success (Naddafi et al. 2007). According to recent studies of total lipid extracts, Chrysophycean species may be rich sources of EPA, and Euglenophyceae can be a significant source of DHA (Lang et al. 2011).

Chlorophyceae contain little to no EPA or DHA (Naddafi et al. 2007) with the exception of Desmidiales, which can have high concentrations of PUFA (Nailwal et al. 2013). Chlorococcales have little PUFA content, but some species have a high protein content that varies between 15 and 88 % of dry matter (Toyub et al. 2008). Chlorococcales was the most abundant group in the assemblage of phytoplankton offered to the mussel (Fig. 1), and this dominance is consistent with the phytoplankton composition found in water bodies in the Paraná system during the low water period (Zalocar de Domitrovic 1998, 2005). Results indicate that the mollusc can benefit from capturing and ingesting both phytoplankton with high contents of long-chained PUFA, as in the case of Desmidiales, Euglenophyceae and Chrysophyceae, and phytoplankton with less PUFA, but a high protein content such as Chlorococcales.

Conclusions

Despite the fact that complementary field experiments are necessary to confirm our findings, we can expect that *L. fortunei* would select phytoplankton in response to a combination of cell shape (represented in this study by the MLD), size and quality and that the presence of zooplankton as an additional food resource will not affect this selection on the majority of groups. Moreover, the negative selection of some phytoplankton groups and *L. fortunei's* preference for large algae suggest that *L. fortunei* feeding could dramatically affect the composition of phytoplankton species.

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