Feeding behavior of the invasive bivalve *Limnoperna fortunei* (Dunker, 1857) under exposure to toxic cyanobacteria *Microcystis aeruginosa*

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(With 4 figures)

Abstract

The aim of this study was to test the effects of cyanobacteria toxicity on feeding behavior of the golden mussel *Limnoperna fortunei*. First, it was tested the hypothesis that *L. fortunei* preferentially graze on non-toxic phytoplankton and reject toxic cyanobacteria. Second, it was tested the hypothesis that toxic cyanobacteria negatively affect feeding and survival of *L. fortunei*. The present study is the first to evaluate the effects of toxic cyanobacteria on *L. fortunei* feeding and survival. In the short-term grazing, golden mussel filtration rates were evaluated in the presence of toxic and non-toxic strains of cyanobacteria *Microcystis aeruginosa*, and non-toxic phytoplankton *Nitzschia palea*. Highest filtration rates were registered when mussels fed on *Nitzschia*. Despite that, golden mussel expelled *Nitzschia* cells in large quantities and preferentially ingested *Microcystis* cells, both toxic and non-toxic strains. In the long-term grazing, mussels were exposed to toxic and non-toxic strains of *Microcystis* during 5 days. Filtration rates were not significantly different for toxic and non-toxic *Microcystis* throughout exposure period. The results have demonstrated cyanobacteria toxicity is not the main factor influencing *L. fortunei* feeding behavior. Survival of *L. fortunei* feeding on toxic cyanobacteria shows the potential of this invasive bivalve as a vector to the transference of cyanotoxins to higher trophic levels.

Keywords: golden mussel, filtration rates, exotic species, microcystin.

Comportamento alimentar do bivalve invasor *Limnoperna fortunei* (Dunker, 1857) em exposição à cianobactéria tóxica *Microcystis aeruginosa*

Resumo

O objetivo deste estudo foi testar os efeitos da toxicidade de cianobactérias sobre o comportamento alimentar do mexilhão dourado *Limnoperna fortunei*. Primeiramente, foi testada a hipótese de que *L. fortunei* ingere preferencialmente o fitoplâncton não tóxico e rejeita as cianobactérias tóxicas. Em segundo lugar, foi testada a hipótese de que as cianobactérias tóxicas afetam negativamente a alimentação e a sobrevivência de *L. fortunei*. O presente estudo é o primeiro a avaliar os efeitos de cianobactérias tóxicas na alimentação e na sobrevivência de *L. fortunei*. Na filtração de curto prazo, as taxas de filtração do mexilhão dourado foram avaliadas na presença de cepas tóxicas e não tóxicas da cianobactéria *Microcystis aeruginosa* e do fitoplâncton não tóxico *Nitzschia palea*. As maiores taxas de filtração foram registradas quando os mexilhões foram alimentados com *Nitzschia*. Apesar disso, o mexilhão dourado expeliu as células de *Nitzschia* em grandes quantidades e ingeriu, preferencialmente, as células de *Microcystis*, tanto cepas tóxicas quanto não tóxicas. Na filtração de longo prazo, os mexilhões foram expostos a cepas tóxicas e não tóxicas de *Microcystis* durante cinco dias. As taxas de filtração não foram significativamente diferentes para cepas tóxicas e não tóxicas de *Microcystis* durante todo o período de exposição. Os resultados demonstraram que a toxicidade da cianobactéria não é o principal fator que influencia o comportamento alimentar de *L. fortunei*. A sobrevivência de *L. fortunei* alimentando-se de cianobactérias tóxicas mostra o potencial desse bivalve invasor como um vetor para a transferência de cianotoxinas para os níveis tróficos superiores.

Palavras-chave: mexilhão dourado, taxas de filtração, espécies exóticas, microcistina.

1. Introduction

Limnoperna fortunei (Dunker, 1857), known as golden mussel, is an invasive bivalve native from Southeast Asia. In 1991, it was first recorded in South America in the Río de la Plata estuary, Argentina, probably introduced by ballast water from ships (Pastorino et al., 1993). In 1998, *L. fortunei* was first recorded in Brazil, in Guaíba Lake (Mansur et al., 2004). Nowadays, the distribution of this invasive species in South America includes Argentina, Uruguay, Paraguay, Bolivia, and Brazil (Darrigran, 2002, Sylvester et al., 2005). *Dreissena polymorpha* (Pallas, 1771) (Bivalvia, Dreissenidae), so called zebra mussel, is an invasive bivalve in Europe and North America, which behavior is similar to golden mussel. Both species have common characteristics such as short life cycle, rapid growth, planktonic larval stage and the presence of byssus that explain their success in colonizing new habitats (Morton, 1973, Ricciardi, 1998). Lamellibranch bivalves, such as golden mussel and zebra mussel, are extremely efficient in filter-feeding (Sylvester et al., 2006). Their presence in aquatic ecosystems, especially when they occur in high densities, may lead to strong changes in the food chain structure by filtering particulate material (mainly phytoplankton and zooplankton) and depositing them as feces and pseudofeces on sediment (Lei et al., 1996).

Zebra mussel has the ability to change the composition and abundance of planktonic communities (Holland, 1993; Nicholls and Hopkins, 1993; Fahnenstiel, 1995; Roditi et al., 1996; Caraco et al., 1997). It was suggested that this invasive bivalve has the potential to promote toxic blooms of cyanobacteria by selective feeding on plankton (Makarewicz et al., 1999; Vanderploeg et al., 2001). Cyanobacteria dominance is a common problem in eutrophic freshwaters due to bloom formation and toxin production. Cyanotoxins can poison human and animals by ingestion of contaminated water or aquatic organisms which bioaccumulate these toxins previously (Carmichael et al., 2001). Microcystins are the most studied and widespread cyanotoxins, which can cause death from liver hemorrhage or liver failure, and can be considered a tumor promoter in chronic exposure to low doses (Sivonen and Jones, 1999).

The effects of zebra mussel on cyanobacteria densities and occurrence of toxic blooms remain contradictory. Some studies have shown that *D. polymorpha* can decrease cyanobacteria densities (Bastviken et al., 1998; Baker et al., 1998; Smith et al., 1998; Dionisio-Pires and van Donk, 2002; Dionisio Pires et al., 2005). Other studies have shown opposite effects, in which *D. polymorpha* can promote the increasing of cyanobacteria densities (Lavrentyev et al., 1995; Makarewicz et al., 1999; Vanderploeg et al., 2001; Nicholls et al., 2002; Juhel et al., 2006).

However, studies about the effects of golden mussel on trophic structure of planktonic communities are still scarce. Since *L. fortunei* invasion in South America, studies were focused on spatial and temporal distribution (Darrigran and Pastorino, 1995; Mansur et al., 2004), reproductive cycle (Darrigran et al., 1999), and larval development (Santos et al., 2005; Cataldo et al., 2005). *L. fortunei* filtration rates were estimated for the first time recently in laboratory experiments using a green algae *Chlorella vulgaris* as food (Sylvester et al., 2005). The results showed that golden mussel filtration rates are among the highest recorded for filtering bivalves, including the invasive species *D. polymorpha*, *D. bugensis* and *Corbicula fluminea* (Sylvester et al., 2006). High filtration rates of golden mussel plus its occurrence in massive densities exceeding 140.000 $ind.m^{-2}$ (Darrigran and Mansur, 2006) for over fifteen years already point out to the powerful potential of this invasive bivalve to promote changes in aquatic trophic chains.

The aim of the present study was to evaluate feeding behavior of golden mussel under exposure to toxic cyanobacteria. First hypothesis accounts for the fact that golden mussel preferentially graze on non-toxic phytoplankton and reject toxic cyanobacteria, leading to a decrease of non-toxic species and an increase of toxic cyanobacteria and, indirectly, toxic blooms (short and long term grazing experiments). Second hypothesis sustains that toxic cyanobacteria affect negatively feeding and survival of *L. fortunei* (long term grazing experiment). The present study is the first to evaluate the effects of toxic cyanobacteria on *L. fortunei* feeding and survival.

2. Material and Methods

2.1. L. fortunei sampling and acclimation

L. fortunei individuals used in these experiments were collected from Guaíba Lake, Southern Brazil. In the laboratory, mussels were kept in flasks filled with water from the sampling site at controlled temperature of 24 °C at constant aeration during 24 hours for acclimation. Those mussels selected for the experiments were apparently healthy as indicated by their filtration activity. The mussels were of similar sizes (approximately 30 mm) as to avoid possible differences in filtration rates related to their sizes. The individuals were washed and brushed to remove microorganisms attached to their shells. Then, they were placed in flasks containing mineral water for a 4-hour period without food in order to have their guts cleared.

2.2. Cyanobacteria and phytoplankton

Species used in the experiments were toxic and non-toxic strains of cyanobacteria *Microcystis aeruginosa*, and non-toxic diatom *Nitzschia palea*. Toxic (NPLJ-4) and non-toxic (NPCD-1) strains of *M. aeruginosa* were provided by the Laboratory of Ecophysiology and Toxicology of Cyanobacteria from Federal University of Rio de Janeiro, Brazil and cultivated in ASM-1 growth medium (Gorham, 1964). Non-toxic *Nitzschia* (N) was isolated from Guaíba Lake and cultivated in D growth medium (Jebram, 1993). These species were batch-cultured in 250 mL Erlenmeyer flasks in a 25 °C incubator with a 14:10 h light:dark cycle and light intensity of 2000 lux. Analyses of microcystins (MC-LR) from *M. aeruginosa* were performed using an ELISA assay test kit (Beacon®).

2.3. Filtration, ingestion and pseudofeces production rates

Filtration rates (FR) or clearance rates (CR) were assessed by considering the amount of particles captured by the mussels. Ingestion rate (IR) equaled filtration rate (FR) less pseudofeces production rate (PPR). Pseudofeces are the filtered particles agglomerated with mucus which are expelled periodically by inhalant opening, i.e. particles filtered but not ingested. Therefore, filtration rate equaled ingestion rate only if no pseudofeces were produced.

Golden mussel filtration rates were estimated in short and long term grazing experiments using the following equation based on Coughlan (1969) (Equation 1):

$$FR = V(\ln(C_o/C_t) - \ln(C'_o/C'_t))/NT \quad (1)$$

where FR is the filtration rate (mL.mussel^{-1}.h^{-1} or mL.mgDW^{-1}.h^{-1}), V is the volume of water in the experimental chamber (mL), N is the number of mussels per chamber or their dry weight (mgDW), T is the total filtration time (h), C0 is the food concentration (mm^3.L^{-1}) at T = 0, Ct is the food concentration at time T in flasks with mussel, C'0 is the concentration of food in the control flask (without mussel) at T = 0 and the C'0 concentration of food in the control flask at time T.

Mussel tissue was removed from shells and oven-dried at 60 °C for 48 hours to assess the dry weight (mgDW). Food concentration (mm^3.L^{-1}) before and after filtration was estimated by Sedgewick-Rafter chamber counting. Samples were preserved in 1% Lugol solution. Food biovolume (mm^3) was calculated according to Hillebrand et al., (1999).

2.4. Short term grazing of L. fortunei

Short term grazing experiment was carried out to evaluate *L. fortunei* feeding behavior in the presence of toxic and non-toxic cyanobacteria, and non-toxic phytoplankton. The experiment was carried out in flasks containing 400 mL of mineral water, food suspension, and one mussel per flask. Different food strategies were used (Table 1) with 8 replicates each at an initial biovolume of 2 mm^3.L^{-1}. Flasks were kept in an acclimatized room (24 °C) and gently stirred each 15 minutes to keep food particles in suspension during filtration time (1 hour). Flasks were prepared under the same conditions, but without mussels, to assess possible phytoplanktonic growth during filtration time.

During the course of experiment, each specimen of *L. fortunei* was monitored under a stereomicroscope coupled to a digital camera. The number of pseudofeces and feces expelled was registered (events.h^{-1}). A method was developed to estimate accurately pseudofaeces production by mussels, as follows (Gazulha 2010). Pseudofeces and feces were captured in the moment of expelling using a micropipette and preserved in 1% Lugol solution. Pseudofeces were disintegrated for 10 minutes using ultrasound Bandelin Sonorex RK100H to separate cells from the mucus and then enable the counting of food particles. The application of ultrasound was efficient to separate cells from the mucus and did not damage the cells. Food particles (mm^3.L^{-1}) were estimated by Sedgewick-Rafter chamber counting to assess PPRs. Mussels used in this experiment kept their filtering ability, with the valves opened and the inhalant siphon exposed during the total filtration time (1 hour). FRs, IRs, and PPRs were determined in the present experiment.

2.5. Long term grazing of L. fortunei

Long term grazing experiment was carried out to evaluate the effects of toxic cyanobacteria on *L. fortunei* feeding and survival. This experiment was conducted in aquaria containing 35 L of mineral water, food suspension, and 70 mussels at controlled temperature of 24 °C and continuous aeration. Two treatments were used: a toxic strain of *M. aeruginosa* (NPLJ-4), and a non-toxic strain of the same species (NPCD-1) as a control with 3 replicates each. Mussels were daily fed with a food biovolume of 2 mm^3.L^{-1} during 5 days (120 hours). Food suspension and water were replaced every 24 hours. Control aquaria with no mussels under the same conditions were used to assess cyanobacteria growth. The water was stirred to get the pseudofeces suspended prior sampling for final cyanobacteria concentration. Therefore, FR equaled IR due to pseudofeces resuspension. IRs were estimated every 24 hours. Microcystin concentration in the water was analyzed by ELISA assay to compare toxins assimilated by mussels and toxins remaining in experimental aquaria.

2.6. Statistical analysis

Analysis of variance (One-way ANOVA) with Tukey's test for multiple comparison were carried out to detect significant differences in filtration, ingestion, and pseudofeces production rates among food combinations ($\alpha = 0.05$) in the short and long term grazing experiments. Tukey's test has been applied after confirming the normality of data using Kolmogorov-Smirnov (KS) test ($\alpha = 0.05$).

Table 1. Food strategies (species, strain, MC-LR, GLD and cell volume) used in *Limnoperna fortunei* short term grazing experiment. Microcystin-LR (MC-LR), Greatest Linear Dimension (GLD).

Species	Strain	MC-LR	GLD	Cell volume
Toxic *Microcystis aeruginosa*	NPLJ-4	7 μg.MC-LR L^{-1}	3.7 μm	29.2 μm^3
Non-toxic *Microcystis aeruginosa*	NPCD-1	-	3.7 μm	29.2 μm^3
Nitzschia palea	N	-	22.5 μm	355.3μm^3
Toxic *M. aeruginosa* + *N. palea* (50:50 mixture)	NPLJ-4 + N	3.5 μg.MC-LR L^{-1}		

3. Results

3.1. Short term grazing of *L. fortunei*

Golden mussel FRs varied from 2.4 to 24.5 mL.mgDW^{-1}.h^{-1}, and the mean value was 10.6 mL.mgDW^{-1}.h^{-1}. Mean values varied from 14.8 mL.mgDW^{-1}.h^{-1} feeding on *Nitzschia* to 8.8 mL.mgDW^{-1}.h^{-1} feeding on non-toxic *Microcystis* (Figure 1). *L. fortunei* FRs were significantly higher on non-toxic *Nitzschia* than other food combinations ($p < 0.05$, ANOVA). Despite higher FRs on non-toxic phytoplankton, golden mussel expelled more *Nitzschia* cells ($p < 0.05$, ANOVA) and ingested more *Microcystis* cells ($p < 0.05$, ANOVA; Figure 1).

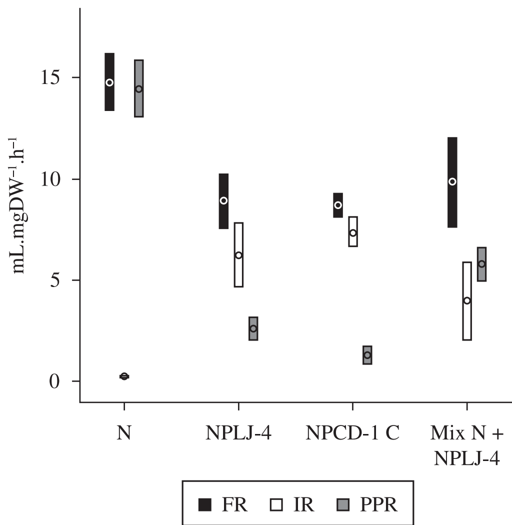

Figure 1. Filtration Rates (FR), Ingestion Rates (IR) and Pseudofeces Production Rates (PPR) of *Limnoperna fortunei* (mL.mgDW^{-1}.h^{-1}) on non-toxic *Nitzschia* (N), toxic (NPLJ-4) and non-toxic (NPCD-1) *Microcystis*, and mixture of *Nitzschia* + toxic *Microcystis* (Mix N+NPLJ-4) (symbol = mean, and bar = SE).

IRs of golden mussel ranged from 0.1 to 15.7 mL.mgDW^{-1}.h^{-1}, and the mean value was 4.5 mL.mgDW^{-1}.h^{-1}. Mean IRs varied from 7.4 mL.mgDW^{-1}.h^{-1} on non-toxic cyanobacteria to 0.3 mL.mgDW^{-1}.h^{-1} on *Nitzschia* (Figure 1). Golden mussel ingested significantly more cyanobacteria cells, both toxic and non-toxic, than diatom cells ($p < 0.05$, ANOVA).

PPRs of golden mussel ranged from 0.3 to 20.2 mL.mgDW^{-1}.h^{-1}, with a mean value of 6.1 mL.mgDW^{-1}.h^{-1}. The highest pseudofeces production was registered in the presence of diatom *Nitzschia*, and the lowest in the presence of non-toxic cyanobacteria (Figure 1). *L. fortunei* expelled significantly more pseudofeces in the presence of diatom *Nitzschia* than of toxic and non-toxic cyanobacteria ($p < 0.05$, ANOVA).

Pseudofeces releasing by *L. fortunei* varied from 9 to 115 events.h^{-1}. The mean value was of 39.1 events.h^{-1}. Mean values of pseudofeces expelled in each food combination varied from 69 events.h^{-1} in the presence of diatom *Nitzschia* to 23.9 events.h^{-1} in the mixture *Nitzschia* + toxic *Microcystis* (Figure 2). Golden mussel released considerably more pseudofeces when fed with *Nitzschia* than toxic and non-toxic cyanobacteria ($p < 0.05$, ANOVA), which was observed as well in terms of PPRs.

Feces releasing by *L. fortunei* ranged from 0 to 6 events.h^{-1}, and the mean value was 2.4 events.h^{-1}. Mean values varied from 2.6 events.h^{-1} on *Nitzschia* and toxic *Microcystis* to 2 events.h^{-1} in the mixture *Nitzschia* + toxic *Microcystis* (Figura 4). There were no significant differences of feces expelled among food combinations ($p > 0.05$, ANOVA).

3.2. Long term grazing of *L. fortunei*

Golden mussel IRs on toxic *Microcystis* ranged from 31.8 to 54.6 mL.mussel^{-1}.h^{-1} (Figure 3) and on non-toxic *Microcystis* ranged from 36.3 to 62.5 mL.mussel^{-1}.h^{-1} (Figure 4), with mean values of 40.9 and 48 mL.mussel^{-1}.h^{-1}, respectively. In terms of body mass, IRs on toxic *Microcystis*

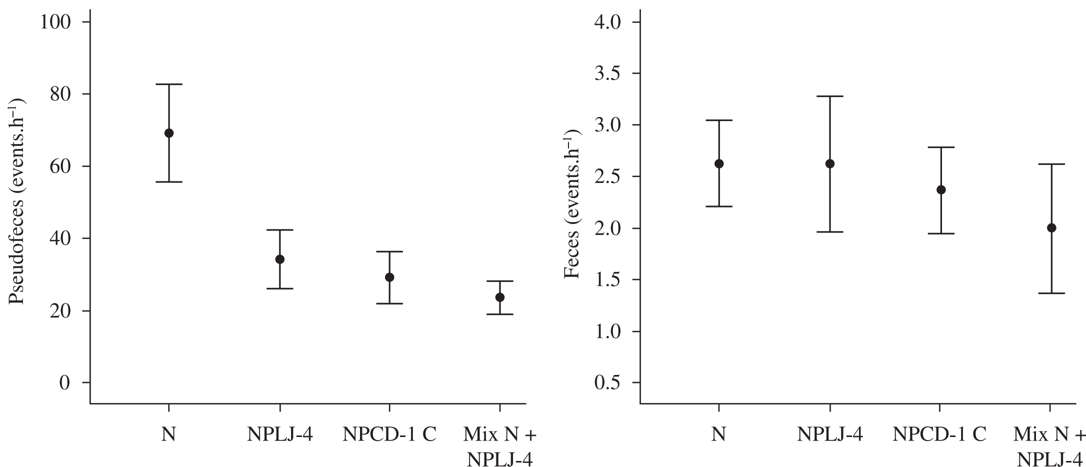

Figure 2. Pseudofeces and feces of *Limnoperna fortunei* (events.h^{-1}) on non-toxic *Nitzschia* (N), toxic (NPLJ-4) and non-toxic (NPCD-1) *Microcystis*, and mixture of *Nitzschia* + *Microcystis* (Mix N + NPLJ-4) (symbol = mean, and bar = SE).

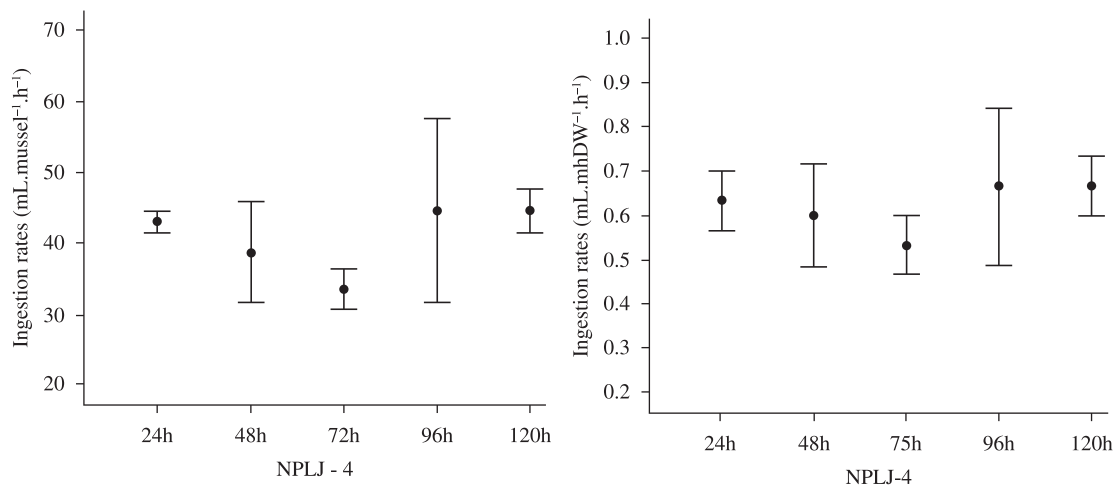

Figure 3. Ingestion rates (IR) of *Limnoperna fortunei* ($mL.mussel^{-1}.h^{-1}$ and $mL.mgDW^{-1}.h^{-1}$) on toxic *Microcystis* (NPLJ-4) (symbol = mean, and bar = SE).

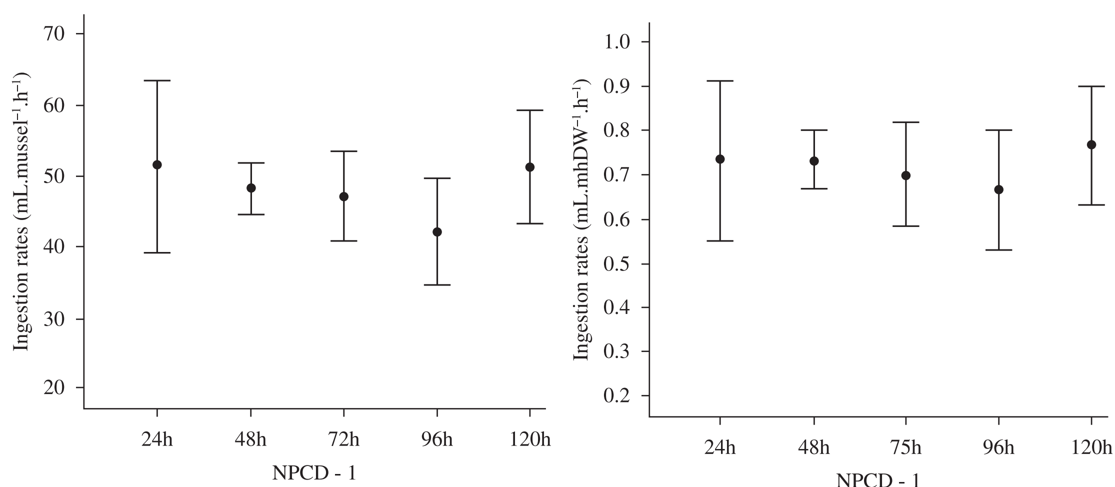

Figure 4. Ingestion rates (IR) of *Limnoperna fortunei* ($mL.mussel^{-1}.h^{-1}$ and $mL.mgDW^{-1}.h^{-1}$) on non-toxic *Microcystis* (NPCD-1) (symbol = mean, and bar = SE).

varied from 0.5 to 0.8 $mL.mgDW^{-1}.h^{-1}$, with a mean value of 0.62 $mL.mgDW^{-1}.h^{-1}$, and on non-toxic *Microcystis* ranged from 0.6 to 0.9 $mL.mgDW^{-1}.h^{-1}$, with a mean value of 0.72 $mL.mgDW^{-1}.h^{-1}$. No mussel mortality was registered on both toxic and non-toxic treatments.

A slightly decrease of IRs was observed at 72 hours in the presence of toxic *Microcystis* (Figure 3). However, *L. fortunei* IRs throughout the 5-day exposure to toxic *Microcystis* did not decrease significantly ($p > 0.05$, ANOVA), indicating there is no negative effects of cyanobacteria toxicity on golden mussel grazing. IRs of golden mussel did not vary significantly in the presence of toxic and non-toxic *Microcystis* ($p > 0.05$, ANOVA).

Microcystis toxins in the water varied from 1.8 to 2.6 µg. $MC\text{-}LR.L^{-1}$ (Table 2). There were no significant differences between initial and final microcystin concentrations every 24 hours of grazing during the time of exposure ($p > 0.05$, ANOVA). It suggests there was a constant excretion of cells containing microcystins returning to the water.

It is interesting to mention that the remaining mussels were kept in a 24 hours starvation period after the end of long term experiment. High mussel mortality was registered in both food types (mean = 65%), indicating that *L. fortunei* survived better feeding on toxic cyanobacteria than without food.

4. Discussion

4.1. Short term grazing of L. fortunei

Sylvester et al., (2005) have registered *L. fortunei* FRs ranging from 9.9 to 29.5 $mL.mgDW^{-1}.h^{-1}mussel^{-1}.h^{-1}$, which are amongst the highest reported for filter-feeding bivalves, including the invasive species *D. polymorpha*, *D. bugensis* and *Corbicula fluminea*. FRs in the present

Table 2. Microcystins (μg.MC-LR.L^{-1}) from *Microcystis aeruginosa* (NPLJ-4) in the water in *Limnoperna fortunei* long term grazing experiment. Microcystin-LR (MC-LR).

μg.MC-LR.L^{-1}	24 hours	48 hours	72 hours	96 hours	120 hours
Initial concentration	2.0	2.1	1.9	2.2	2.6
Final concentration	1.8	2.0	2.0	2.4	2.5

short term experiment (from 9 to 14.8 mL.$mgDW^{-1}.h^{-1}$) were comparable to that observed by Sylvester et al., (2005).

The effects of zebra mussel *D. polymorpha* on cyanobacteria have been widely researched in laboratory and field experiments since its invasion in Europe and North America (Makarewicz et al., 1999; Vanderploeg et al., 2001; Dionisio Pires et al., 2004; Naddafi et al., 2007). Several studies have shown that zebra mussel can promote the decrease of cyanobacteria densities by selective feeding (Baker et al., 1998; Dionisio Pires and van Donk, 2002; Dionisio-Pires et al., 2005; Sarnelle et al., 2005).

Bastviken et al., (1998) conducted laboratory experiments to verify the impact of zebra mussel on natural phytoplankton communities from Hudson River, under the addition of cultured cyanobacteria (*M. aeruginosa* and *Anabaena* sp.). They found that single cells of *Microcystis* were removed more efficiently by zebra mussel, whereas colonies of *Microcystis* and diatoms were removed less efficiently. Smith et al. (1998) observed a shift of the dominant phytoplankton from diatoms to cyanobacteria in Hudson River. In laboratory studies with zebra mussel populations from the same river, Baker et al. (1998) found *Microcystis* were largely ingested, while diatoms were commonly rejected as pseudofeces.

In laboratory experiments, where zebra mussel were fed with a mixture of green algae (*Scenedesmus*) and cyanobacteria (*Microcystis*) it was observed that cyanobacteria were preferably ingested, while green algae were commonly incorporated in a mucus string and rejected as pseudofeces (Baker et al., 2000). Therefore, it was suggested that food selection by zebra mussel occurred mainly due to the size of particles. Smaller sizes (*Microcystis*) were preferably ingested, whereas larger ones (*Scenedesmus*) were rejected.

Dionisio-Pires and Van Donk (2002) tested *D. polymorpha* filtration rates in the presence of toxic and non-toxic strains of *Microcystis* and green algae *Chlamydomonas*. Zebra mussel grazed on cyanobacteria and green algae as well, although *Chlamydomonas* cells were more rejected than *Microcystis* cells. Differences in sizes were too small to be the reason of rejection (*Chlamydomonas*, 5.6 μm; *Microcystis*, 3.8 μm). Thus, it was attributed to the thickness of *Chlamydomonas* cell wall the difficulty to digestion and its further expelling as undesirable food.

Quality of food, besides cell size and structure, may influence feeding selection by bivalves. High concentrations of long chain PUFA (Polyunsatured Fatty Acid) in the food, particularly EPA (Eicosapentaenoic Acid), and DHA (Docosahexaenoic Acid), have a positive effect on growth and recruitment of bivalves being preferentially ingested (Vanderploeg et al., 1996; Naddafi et al., 2007). Cryptophytes, chrysophytes, and dinoflagellates are usually rich in both EPA and DHA; diatoms are rich in EPA; and cyanobacteria and green algae contain no or little EPA and DHA (Naddafi et al., 2007). However, the present study does not sustain this hypothesis since it was observed a preferential ingestion of cyanobacteria and rejection of diatom on pseudofeces.

The rejection of diatom *Nitzschia* observed in the present study, by all appearances, can be attributed both to size and structure of the food particle. *Nitzschia* cell volume is larger than *Microcystis* cell volume (Table 1). In addition, *Nitzschia* stiff silicate frustules are likely to make it an undesirable food for golden mussel. Rejection of diatoms has also been observed in bivalves such as *Mytilu edulis* (Cucci et al., 1985) and *Ostrea edulis* (Shumway et al., 1985).

On the other hand, several studies with *D. polymorpha* have shown zebra mussel promoted the increasing of cyanobacteria densities by low filtration and high rejection in pseudofeces (Lavrentyev et al., 1995; Vanderploeg et al., 2001; Nicholls et al., 2002; Juhel et al., 2006). Those studies were performed with natural seston containing natural populations of *Microcystis* predominantly in large colonies, and most likely non-preferentially ingested by bivalves. Laboratory strains are usually single-celled, and colonies eventually formed are usually small and without mucilage (Dionisio-Pires and Van Donk, 2002). Vanderploeg et al. (2001) observed that zebra mussel when fed with *Microcystis* preferentially ingested single cells and small colonies, whereas natural large colonies were rejected.

Those studies corroborate with the present results, in which small particles are preferably ingested regardless of its toxicity. Therefore, golden mussel selective feeding seems to be more related to the size of particles than to the toxicity. The present experiment was conducted with single cells of *Microcystis* simply aiming to test the effect of toxicity on *L. fortunei* grazing.

4.2. Long term grazing of L. fortunei

Long term grazing experiment showed there was not a decrease in *L. fortunei* IRs under exposure to toxic *Microcystis*. It indicated that golden mussel ingested cyanobacteria cells during the 5-day experiment and any toxic effect could be observed. Besides that, no mussel mortality was registered. The ingestion of toxic *Microcystis* by golden mussel suggests this invasive bivalve presents survival mechanisms in face of toxins. Therefore, the

hypothesis that cyanobacteria toxicity has an effect on golden mussel grazing and survival was rejected.

An experiment with the marine mussel *Mytilus galloprovincialis* feeding on a toxic strain of *Microcystis* showed there was no mussel mortality during 4 days of exposure (MC-LR concentration = 1.5 $\mu g.L^{-1}$) (Amorim and Vasconcelos, 1999). A similar experiment with zebra mussel showed higher filtration rates on toxic *Microcystis* than on non-toxic food (*Nannochloropsis*) with no mussels mortality in a 3-week assimilation period (MC-LR concentration = 11.7 $\mu g.L^{-1}$) (Dionisio Pires et al., 2004), which endorse our results.

The ability of bivalves to accumulate and store toxins has been demonstrated in some studies (Vasconcelos, 1995; Amorim and Vasconcelos, 1999; Yokoyama and Park, 2002). A possibility for that ability is that microcystins can be detoxified through the conjugation of the toxin with the enzyme glutathione via soluble GST (glutathione-S-transferases), which was shown in several aquatic organisms, including zebra mussel (Pflugmacher et al., 1998). Another explanation is that the ingestion of intact cells could be less toxic to mussels. Vasconcelos et al. (2007) showed that intact *Microcystis* cells did not induce any major response (GST activity) from mussel *Mytilus*, indicating mussels are quite resistant to cyanobacteria when those cells are intact. However, it was registered a large effect in different organs of mussels when they tested cell extracts, mimicking the decay of bloom in natural systems.

Golden mussel IRs were higher in the short term (1 hour) than on long term (120 hours) grazing experiment. These differences could be related to: 1) intraspecific variations, since it was observed a great difference on filtration rates between specimens in the same experimental conditions (replicates), which seems to be common for other bivalve species, including *L. fortunei* (Sylvester et al., 2005); 2) filtration rates on the short term grazing were closer to optimum rates (overestimated rates), in which mussels kept actively filtering (with the valves opened) during total experiment time (1 hour); 3) filtration rates on long term grazing were closer to natural conditions, including periods of lower activity (e.g. low filtration rates, closing of valves) (underestimated rates).

5. Conclusion

Golden mussel was able to survive feeding on toxic cyanobacteria. This fact points out to the risk of this invasive bivalve as a possible vector for the transference of cyanobacteria toxins to higher trophic levels. Massive densities of golden mussel in South American waters associated to its powerful filtering capability may lead to changes on the structure of trophic chains, mainly the planktonic communities. The presence of *L. fortunei* might promote a decrease of toxic and non-toxic *Microcystis* cells, and an increase of diatoms. In the presence of cyanobacteria blooms, however, the ability of golden mussel to remove *Microcystis* cells could be reduced. Cyanobacteria blooms are usually formed by large colonies and filaments that would probably be rejected by golden mussel.

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