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O MEXILHÃO DOURADO *LIMNOPERNA FORTUNEI* (DUNKER, 1857) NA PRESENÇA DE CIANOBACTÉRIAS: TAXAS DE FILTRAÇÃO, COMPORTAMENTO ALIMENTAR E SOBREVIVÊNCIA

VANESSA GAZULHA

PORTO ALEGRE, ABRIL DE 2010.

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VANESSA GAZULHA

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ORIENTADOR: Prof. Dr. Luiz Fernando de Abreu Cybis

CO-ORIENTADORA: Dra. Maria Cristina Dreher Mansur

BANCA EXAMINADORA:

Prof. Dr. Antonio Domingues Benetti (UFRGS)

Profa. Dra. Maria Teresa Raya-Rodriguez (UFRGS)

Profa. Dra. Sandra Maria Feliciano de Oliveira e Azevedo (UFRJ)

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RESUMO

O objetivo deste estudo foi avaliar o comportamento alimentar e a sobrevivência do bivalve invasor *Limnoperna fortunei*, conhecido como mexilhão dourado, na presença de cianobactérias tóxicas e não-tóxicas. O presente estudo é o primeiro a avaliar os efeitos de cianobactérias tóxicas na alimentação e sobrevivência de *L. fortunei*, e o primeiro a estimar as taxas de filtração das larvas de *L. fortunei*. Primeiro, foi testada a hipótese de que *L. fortunei* ingere preferencialmente fitoplâncton não-tóxico e rejeita cianobactérias tóxicas, e que as toxinas de cianobactérias têm um efeito negativo na sobrevivência do mexilhão. Em segundo lugar, foi testada a hipótese de que *L. fortunei* filtra com mais eficiência as partículas menores, como as células solitárias, do que as partículas maiores, como as cianobactérias coloniais e filamentosas. Em terceiro lugar, foi testada a hipótese de que as toxinas de cianobactérias afetam negativamente a alimentação e sobrevivência das larvas de *L. fortunei*. As taxas de filtração mais elevadas foram registradas quando os mexilhões foram alimentados com o fitoplâncton não-tóxico *Nitzschia*. Apesar disso, o mexilhão dourado expeliu células de *Nitzschia* em grandes quantidades e ingeriu, preferencialmente, células de *Microcystis*, tanto tóxicas, quanto não-tóxicas. Os mexilhões foram expostos a cepas tóxicas e não-tóxicas de *Microcystis* durante 5 dias, e não foram registrados efeitos tóxicos na sua alimentação e sobrevivência. Os resultados demonstraram que a toxicidade das cianobactérias não é o principal factor que influencia o comportamento alimentar de *L. fortunei*. As taxas de filtração do mexilhão dourado na presença de cianobactérias solitárias, coloniais e filamentosas mostraram que as células solitárias foram preferencialmente aceitas como alimento, enquanto os filamentos e colônias foram massivamente expelidos como pseudofeces. A sobrevivência das larvas de *L. fortunei* foi elevada na presença de algas verdes e seston natural durante todo o experimento. Após quatro dias de exposição, a sobrevivência das larvas diminuiu na presença das cianobactérias. A baixa sobrevivência das larvas observada em todos os tratamentos contendo cianobactérias, até mesmo as cepas não tóxicas, pode ter sido influenciada pela toxicidade e também pela qualidade das cianobactérias. A baixa concentração de lipídeos nas cianobactérias pode ter causado uma deficiência nutricional nas larvas. As larvas ingeriram as algas verdes *Monoraphidium*, assim como cepas tóxicas e não-tóxicas de *Microcystis* a taxas de filtração similares. Estes resultados indicam que as toxinas de cianobactérias não tiveram nenhum efeito sobre a atividade de filtração de *L. fortunei*, possivelmente relacionado com a incapacidade das larvas de detectar a toxicidade do alimento. A sobrevivência dos adultos de *L. fortunei* na presença de cianobactérias tóxicas indica o potencial deste bivalve invasor como um vetor para a transferência de cianotoxinas para os níveis tróficos superiores. As densidades massivas de *L. fortunei* em associação com sua elevada capacidade de filtrar evidenciam o potencial desta espécie invasora para promover grandes alterações na estrutura das cadeias tróficas dos ecossistemas invadidos.

ABEXTRACT

The aim of this study was to evaluate feeding behavior and survival of the invasive bivalve *Limnoperna fortunei*, socalled golden mussel, in the presence of toxic and non-toxic cyanobacteria. The present study is the first to evaluate the effects of toxic cyanobacteria on feeding and survival of *L. fortunei*, and the first to estimate filtration rates of *L. fortunei* larvae. First, it was tested the hypothesis that *L. fortunei* preferentially graze on non-toxic phytoplankton and reject toxic cyanobacteria, and that cyanobacteria toxins have a negative effect on mussel survival. Second, it was tested the hypothesis that *L. fortunei* filter more efficiently smaller particles, such as single-celled, than larger particles, such as colonial and filamentous cyanobacteria. Third, it was tested the hypothesis that cyanobacteria toxins negatively affect feeding and survival of *L. fortunei* larvae. Highest filtration rates were registered when mussels fed on non-toxic phytoplankton *Nitzschia*. Despite that, golden mussel expelled *Nitzschia* cells in large quantities and preferentially ingested *Microcystis* cells, both toxic and non-toxic strains. Mussels were exposed to toxic and non-toxic strains of *Microcystis* during 5 days and no toxic effects were registered on their feeding and survival. Results have demonstrated cyanobacteria toxicity is not the main factor influencing *L. fortunei* feeding behavior. Filtration rates of golden mussel in the presence of single-celled, colonial, and filamentous cyanobacteria have demonstrated that single cells were widely accepted as food, while filaments and colonies were massively expelled as pseudofeces. *L. fortunei* larvae survival was high in the presence of green algae and natural seston during all experiment. After four days of exposure, larvae survival decreased in the presence of cyanobacteria. Low larvae survival observed in all cyanobacteria treatments, including the non-toxic, might have been influenced by cyanobacteria toxicity and also by quality. Low lipid concentration of cyanobacteria may have caused a nutritional deficiency to larvae. Golden mussel larvae ingested *Monoraphidium* as well as non-toxic and toxic *Microcystis* at similar filtration rates. It indicates cyanobacteria toxins had no effect on filtration activity of *L. fortunei* possibly relating to larvae incapability to detect food toxicity. Survival of *L. fortunei* adults in the presence of toxic cyanobacteria shows the potential of this invasive bivalve as a vector for the transference of cyanotoxins to higher trophic levels. Massive densities of *L. fortunei* in association to its powerful filtering capability point out to the potential of this invasive species to promote great changes in the structure of trophic chains from invaded ecosystems.

APRESENTAÇÃO

A presente tese de doutorado foi produzida no formato de artigos. Está composta por uma parte introdutória, que inclui o objetivo geral, os objetivos específicos, as hipóteses testadas, a revisão bibliográfica e o material e métodos, seguida pelos artigos (**Artigo I**, submetido e **Artigos II** e **III,** manuscritos) e pelas considerações finais.

Os seguintes artigos compõem a presente tese de doutorado:

ARTIGO I.

FEEDING BEHAVIOR OF THE INVASIVE BIVALVE *LIMNOPERNA FORTUNEI* (DUNKER, 1857) UNDER EXPOSURE OF TOXIC CYANOBACTERIA *MICROCYSTIS AERUGINOSA*. Submetido para a revista Hydrobiologia em 19 de março de 2010. Status: em revisão.

ARTIGO II.

GRAZING IMPACTS OF THE INVASIVE BIVALVE *LIMNOPERNA FORTUNEI* (DUNKER, 1857) ON SINGLE-CELLED, COLONIAL, AND FILAMENTOUS CYANOBACTERIA. Manuscrito.

ARTIGO III.

EFFECTS OF TOXIC CYANOBACTERIA *MICROCYSTIS AERUGINOSA* ON FILTRATION AND SURVIVAL OF GOLDEN MUSSEL LARVAE. Manuscrito.

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1. INTRODUÇÃO

Os impactos provocados por espécies invasoras são temas de preocupação no mundo inteiro, por trazerem sérias conseqüências ambientais e econômicas. Estes impactos estão associados, principalmente, ao crescimento descontrolado de suas populações. O bivalve invasor *Limnoperna fortunei* (Dunker, 1857) (Bivalvia, Mytilidae), conhecido como mexilhão dourado, é originário do sudeste asiático (Coréia e China). Em 1991, foi registrado na América do Sul pela primeira vez no Rio da Prata (Argentina), provavelmente introduzido via água de lastro de navios (Pastorino et al., 1993). Em 1998, foi descoberto nas proximidades do porto da cidade de Porto Alegre, lago Guaíba, sul do Brasil (Mansur et al., 2003). Esta espécie invasora distribui-se além de 3.000 km do seu ponto original de entrada na América do Sul, sendo encontrada na Argentina, na Bolívia, no Uruguai, no Paraguai e no Brasil (Sylvester et al., 2005).

Os impactos econômicos do mexilhão dourado desde sua invasão na América do Sul têm sido bem documentados (Darrigran & Drago, 2000, Mansur et al., 2004, Brugnoli et al., 2005). Diversas indústrias (indústrias nucleares, várias centrais hidrelétricas, estações de tratamento de água, refinarias, etc.) localizadas no estuário do Rio da Prata e nos rios Paraná, Paraguai e Uruguai e seus afluentes, começaram a apresentar problemas de obstruções associados às incrustações do mexilhão dourado (Boltovskoy et al., 2006). Estas incrustações obstruem os filtros de captação de água, as tubulações, os trocadores de calor e os condensadores de indústrias e companhias de energia que utilizam água bruta.

Os custos das indústrias para a limpeza e remoção destas incrustações são extremamente elevados, pois também implicam na parada de atividades, como a geração de energia e o abastecimento de água potável.

A respeito dos impactos ecológicos que o mexilhão dourado *L. fortunei* pode causar na América do Sul, as pesquisas ainda são escassas. Desde sua invasão, foram enfocados aspectos relacionados à sua distribuição espacial e temporal (Darrigran & Pastorino, 1995, Mansur et al., 2004), ao ciclo reprodutivo (Darrigran et al., 1999), ao desenvolvimento larval (Santos et al., 2005, Cataldo et al., 2005) e às taxas de filtração (Sylvester et al., 2005). Os efeitos da filtração do mexilhão dourado na disponibilidade de nutrientes e na estrutura das comunidades planctônicas ainda são pouco conhecidos.

Os problemas econômicos que o mexilhão dourado *L. fortunei* vem causando na América do Sul assemelham-se muito com os problemas provocados na Europa e América do Norte pelo bivalve invasor *Dreissena polymorpha* (Pallas, 1771), conhecido como mexilhão zebra. Estas espécies apresentam características em comum que explicam seu sucesso na invasão e colonização de novos habitats, como ciclo de vida curto, crescimento rápido, estágio larval planctônico e presença de bisso, estrutura protéica que permite a fixação dos bivalves em qualquer substrato duro (Morton, 1973, Ricciardi, 1998).

É provável que os impactos ecológicos que o mexilhão zebra promoveu na Europa e América do Norte também ocorram na América do Sul com o mexilhão dourado, devido à semelhança entre estas espécies. Os impactos ecológicos do mexilhão zebra na Europa e América do Norte estão relacionados à sua capacidade de alterar a estrutura da cadeia trófica via filtração de partículas. O mexilhão zebra tem potencial para alterar a composição e a abundância das comunidades planctônicas através da filtração seletiva das partículas em suspensão (Nicholls & Hopkins, 1993, Fahnenstiel, 1995, Roditi et al., 1996, Caraco et al., 1997, Bastviken et al., 1998).

Alguns estudos sugerem que a filtração seletiva do mexilhão zebra em ecossistemas com a presença de cianobactérias poderia promover a ocorrência de florações tóxicas (Baker et al., 1998, Vanderploeg et al., 2001). A dominância de cianobactérias em ecossistemas de água doce é um problema mundial, especialmente naqueles sujeitos à eutrofização, devido à formação de florações e produção de toxinas, conhecidas como cianotoxinas. Estas toxinas podem provocar a intoxicação e morte de animais silvestres e domésticos, e até mesmo a morte de seres humanos pela ingestão de água contaminada (Carmichael et al., 2001).

Os estudos a respeito dos efeitos do mexilhão zebra sobre as densidades de cianobactérias e a ocorrência de florações tóxicas são contraditórios. Alguns estudos observaram o declínio das densidades de cianobactérias como efeito da filtração seletiva do mexilhão zebra (Bastviken et al., 1998, Dionisio-Pires Pires & Van Donk, 2002). Outros estudos demonstraram efeito contrário, onde a filtração do mexilhão zebra promoveu o aumento das densidades de cianobactérias (Makarewicz et al., 1999, Vanderploeg et al., 2001, Nicholls et al., 2002).

O mexilhão dourado é invasor na América do Sul há mais de quinze anos, ocorrendo sempre em densidades massivas que podem superar 140.000 ind m^{-2} (Darrigran & Mansur, 2006). As taxas de filtração registradas para o mexilhão dourado são mais elevadas do que aquelas registradas para outros bivalves invasores de água doce, como *D. polymorpha, D. bugensis* e *Corbicula fluminea* (Sylvester et al., 2005). As elevadas taxas de filtração indicam o grande potencial do mexilhão dourado para alterar a estrutura das comunidades planctônicas dos ecossistemas invadidos.

O lago Guaíba, onde o mexilhão dourado é invasor desde 1998, apresenta florações freqüentes de cianobactérias tóxicas (Cybis et al., 2006, Bendati et al., 2007, Werner et al., 2007). A coexistência do mexilhão dourado e das cianobactérias indica que o bivalve

invasor apresenta mecanismos que permitem sua sobrevivência em exposição a florações tóxicas. Além disso, pode indicar que o bivalve invasor está contribuindo para a predominância das cianobactérias, via ingestão preferencial das partículas não toxicas e rejeição das tóxicas.

Desta forma, pretende-se entender estes mecanismos através do estudo das taxas de filtração e do comportamento alimentar do mexilhão dourado na presença de cianobactérias tóxicas. O presente estudo é o primeiro a avaliar os efeitos de cianobactérias tóxicas na alimentação e sobrevivência do mexilhão dourado *L. fortunei*, assim como a estimar as taxas de filtração das larvas deste bivalve invasor.

1.1. Objetivo geral

Avaliar as taxas de filtração, o comportamento alimentar e a sobrevivência do bivalve invasor mexilhão dourado (*L. fortunei*) na presença de cianobactérias tóxicas e não-tóxicas.

1.2. Objetivos específicos

- Analisar o comportamento alimentar do mexilhão dourado (*L. fortunei*) na presença da cianobactéria tóxica (*M. aeruginosa*) e do fitoplâncton não-tóxico (diatomácea *N. palea*) **(Artigo I)**.

- Avaliar o efeito da cianobactéria tóxica (*M. aeruginosa*) nas taxas de filtração e na sobrevivência do mexilhão dourado (*L. fortunei*) **(Artigo I)**.

- Comparar as taxas de filtração e o comportamento alimentar do mexilhão dourado (*L. fortunei*) na presença de cianobactérias solitárias (*M. aeruginosa*), coloniais (*M. aeruginosa*) e filamentosas (floração natural de *Planktothrix* sp.) **(Artigo II)**.

- Avaliar as taxas de filtração e a sobrevivência das larvas do mexilhão dourado (*L. fortunei*) na presença de cianobactérias tóxicas e não-tóxicas (*M. aeruginosa*), e fitoplâncton não-tóxico (clorofícea *Monoraphidium* sp. e seston natural) **(Artigo III)**.

1.3. Hipóteses

- O mexilhão dourado ingere preferencialmente o fitoplâncton não-tóxico e rejeita a cianobactéria tóxica, levando ao decréscimo das espécies não-tóxicas e ao aumento das espécies tóxicas e assim, a ocorrência de florações tóxicas **(Artigo I)**;

- A cianobactéria tóxica afeta negativamente a filtração e a sobrevivência do mexilhão dourado **(Artigo I)**;

- O mexilhão dourado filtra com mais eficiência as partículas pequenas, que passarão com maior facilidade pelo seu aparato filtrador **(Artigo II)**;

- O mexilhão dourado *L. fortunei* filtra com maior eficiência as partículas arredondadas, como as colônias de *Microcystis*, ingeridas mais facilmente do que as cianobactérias filamentosas longas **(Artigo II)**;

- A cianobactéria tóxica (*M. aeruginosa*) afeta negativamente a sobrevivência das larvas do mexilhão dourado (*L. fortunei*) **(Artigo III)**;

- A cianobactéria tóxica (*M. aeruginosa*) tem efeito negativo nas taxas de filtração das larvas do mexilhão dourado (*L. fortunei*) levando-as a filtrar com menor eficiência **(Artigo III)**;

1.4. Revisão Bibliográfica

1.4.1. A invasão do mexilhão dourado L. fortunei na América do Sul

O mexilhão dourado, *Limnoperna fortunei* (Dunker, 1857), é um bivalve de água doce da família Mytilidae, nativo da Coréia e do sudeste da China (Iwasaki & Uryu, 1998). No entanto, em 1965 foi registrado como invasor em Hong Kong (Morton, 1973), e nos anos 90, no Japão (Kimura, 1994) e na Ilha de Formosa (Taiwan) (Ricciardi, 1998). Em 1991, foi registrado pela primeira vez na América do Sul, no Balneário Bagliardi, estuário do Rio da Prata, Argentina (Pastorino et al., 1993).

No ano de 1998, o mexilhão dourado foi registrado pela primeira vez no Brasil, em duas localidades: no rio Paraguai, nas proximidades de Corumbá, Mato Grosso do Sul, e no lago Guaíba, próximo ao porto de Porto Alegre, Rio Grande do Sul. No entanto, estas introduções foram independentes, visto que o rio Paraguai não tem comunicação com o lago Guaíba (Figura 1.1). Porém, provavelmente tiveram uma origem comum: a população de *L. fortunei* que chegou ao Rio da Prata através da água de lastro de navios mercantes, originários do sudeste asiático (Mansur et al., 2004, Darrigran & Mansur, 2006).

A introdução do mexilhão dourado nas proximidades de Corumbá teria ocorrido através da proliferação da população do Rio da Prata em direção à montante dos rios Paraná e Paraguai, auxiliada pela intensa navegação fluvial. A introdução no lago Guaíba teria ocorrido via Laguna dos Patos através de deslastre de água contaminada, provavelmente de navios argentinos (Mansur et al., 2004). A Laguna dos Patos comunica-se com o Atlântico através da barra de Rio Grande, no sul do Rio Grande do Sul, e ao norte, conecta-se ao lago Guaíba, através do estreito de Itapuã. Atualmente, esta espécie invasora distribui-se além de 3.000 km do seu ponto original de entrada na América do Sul,

sendo encontrada na Argentina, Bolívia, no Uruguai, Paraguai e Brasil (Sylvester et al., 2005).

Figura 1.1. Vias de ingresso do mexilhão dourado *Limnoperna fortunei* na América do Sul. Fonte: modificado de Mansur (2008).

1.4.1.1. Impactos econômicos da invasão do mexilhão dourado na América do Sul

Os impactos do mexilhão dourado nas atividades humanas foram observados pouco tempo após sua invasão na América do Sul. No Brasil, Uruguai, Paraguai, na Argentina e Bolívia, muitas atividades econômicas (duas indústrias nucleares, várias centrais

hidrelétricas, estações de tratamento da água, refinarias, etc.) localizadas no estuário do Rio da Prata e nos rios Paraná, Paraguai e Uruguai e seus afluentes, começaram a experimentar problemas de obstruções associados às incrustações de *L. fortunei* (Darrigran & Drago, 2000, Brugnoli et al., 2002, Boltovskoy et al., 2006). O bivalve invasor provocou o entupimento dos filtros de captação de água, das tubulações, dos trocadores de calor, dos condensadores, etc., tornando-se um grande incômodo para indústrias e companhias de energia que utilizam água bruta, principalmente, para fins de resfriamento (Boltovskoy et al., 2006).

O maior prejuízo financeiro da invasão do mexilhão dourado vem se constatando junto às hidrelétricas. Problemas nas unidades hidrelétricas de Jaciretá (rio Paraná) e Salto Grande (rio Uruguai) na Argentina, e Itaipu (Usina Hidrelétrica Binacional de Itaipu) no Brasil, já ocorreram diversas vezes (Mansur, comunicação pessoal). Desde 2001, Itaipu luta com uma rotina de limpeza que se torna cada vez mais intensa para impedir perdas na produção energética. Conforme cálculos fornecidos por Itaipu, a empresa já gastou 12 milhões de reais, desde o ano 2001, na remoção do mexilhão de suas turbinas, adaptações de filtros, etc. A UHE de Porto Primavera, desde abril de 2004, vem enfrentando obstruções em quase 16 filtros e mais de 3600 metros de tubulações. Relatos de barrageiros anunciam os primeiros problemas nas represas mais a montante do rio Paraná e do Tietê, como em Ilha Solteira, São Simão e Barra Bonita.

A Companhia Riograndense de Saneamento (CORSAN) teve gastos em torno de 20 mil reais na remoção de material incrustado em bomba de captação de água para tratamento, sem contabilizar o prejuízo decorrente do tempo de ócio do equipamento durante a limpeza (Comunicação Pessoal da direção da CORSAN ao Grupo Limnoperna da PUCRS, em 19 de abril de 2006). A rede de captação e distribuição da CORSAN deixou de atender 26 bairros, ou seja, 419 mil pessoas, durante 4 horas utilizadas para a

remoção de incrustações de mexilhão dourado nas tubulações (ZERO HORA, caderno geral, 15 de junho de 2003, página 33).

1.4.1.2. Impactos ecológicos da invasão do mexilhão dourado

Quanto aos impactos ecológicos que o mexilhão dourado *L. fortunei* pode causar na América do Sul, pouco se conhece. Desde sua invasão, foram estudados aspectos relacionados à sua distribuição espacial e temporal (Darrigran & Pastorino, 1995, Mansur et al., 2004), ao ciclo reprodutivo (Darrigran et al., 1999), ao desenvolvimento larval (Santos et al., 2005, Cataldo et al., 2005) e às taxas de filtração (Sylvester et al., 2005).

Efeitos positivos do mexilhão dourado sobre a fauna bentônica e a ictiofauna foram observados na América do Sul. Darrigran et al. (1998) observaram que a presença de *L. fortunei* levou ao aumento da diversidade e abundância da fauna bentônica no Rio da Prata. Penchaszadeh et al. (2000) e Cataldo et al. (2002) verificaram o grande consumo do mexilhão por várias espécies de peixes do rio Paraná e Rio da Prata, uma vez que o bivalve invasor representa um novo recurso alimentar no ecossistema.

Os impactos negativos do mexilhão dourado vêm sendo observados sobre a fauna de moluscos nativos e a vegetação aquática do lago Guaíba (Mansur et al., 2003). Os indivíduos de *L. fortunei* aglomeram-se e fixam-se nas aberturas das conchas de moluscos nativos, dificultando sua atividade de filtração da água para obtenção de alimento, causando o seu sufocamento e, consequentemente, a morte. A redução das áreas de juncais nas margens do lago Guaíba também foi relacionada com a presença do mexilhão dourado, que estaria formando aglomerados na base dos rizomas dos juncos, levando ao apodrecimento da vegetação aquática.

Os efeitos da filtração do mexilhão dourado na disponibilidade de nutrientes e na estrutura das comunidades planctônicas ainda são pouco conhecidos. Os bivalves filtradores podem exercer forte controle sobre as comunidades planctônicas (*top-down control*) por serem grandes consumidores de plâncton (Caraco et al., 1997). O mexilhão dourado apresenta as taxas de filtração mais elevadas em comparação com os demais bivalves invasores de água doce como, *Dreissena polymorpha, D. bugensis* e *Corbicula fluminea* (Sylvester et al., 2005). Portanto, fica claro o potencial do mexilhão dourado de alterar a estrutura das comunidades planctônicas, assim como a importância de se entender os efeitos de *L. fortunei* nos ecossistemas invadidos.

1.4.1.3. Mecanismo de seleção de partículas do mexilhão dourado

Para o estudo do potencial de impacto do mexilhão dourado sobre o ecossistema, é essencial o entendimendo dos seus mecanismos de filtração e seleção de partículas. O estudo da anatomia e morfologia desta espécie invasora foi realizado por Morton (1973), com descrições detalhadas das correntes ciliares e do mecanismo de seleção de partículas (Figura 1.2).

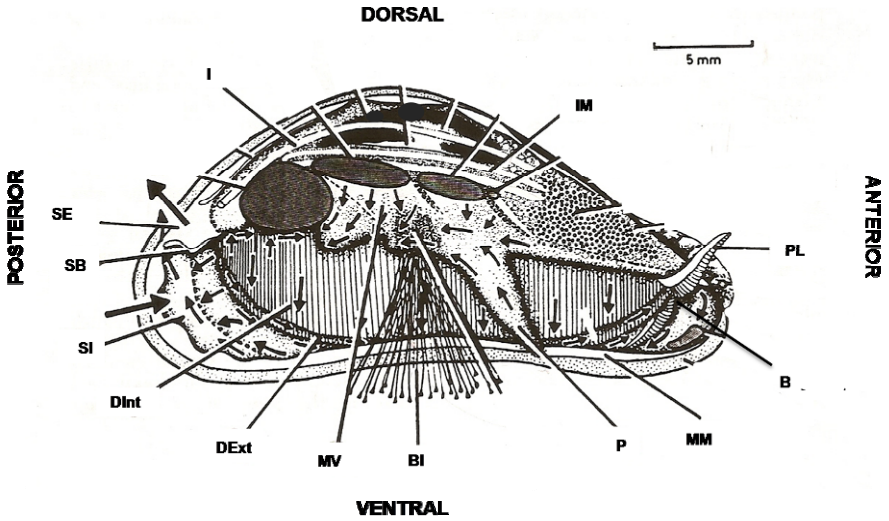

Figura 1.2. Anatomia e correntes ciliares de *Limnoperna fortunei*. Sifão exalante (SE), septo branquial (SB), sifão inalante (SI), demibrânquia interna (DInt), demibrânquia externa (DExt), massa visceral (MV), bisso (BI), pé (P), margem do manto (MM), boca (B), palpo labial (PL), intestino médio (IM), intestino (I). Modificada de Morton (1973).

Nos bivalves filtradores (lamelibrânquios), como o mexilhão dourado, a entrada da água contendo as partículas alimentares ocorre pelo sifão inalante, e a saída desta juntamente com as fezes ocorre pelo sifão exalante. A seleção das partículas ocorre no manto, na massa visceral, no pé e principalmente, nas brânquias e nos palpos labiais.

O mexilhão dourado direciona as partículas filtradas em correntes de aceitação, que vão para a boca do bivalve para serem ingeridas, e correntes de rejeição, que vão para a região do sifão exalante para serem expelidas. As correntes ciliares no manto (incluindo os sifões), na massa visceral e no pé são de rejeição e movem as partículas para serem concentradas na região posterior da massa visceral, servindo para manter a cavidade do manto livre de partículas muito grandes ou indesejáveis. A partir daí, as partículas são direcionadas nas demibrânquias internas, ventral ou dorsalmente, em direção aos sulcos alimentares ventral ou dorsal (Figura 1.3).

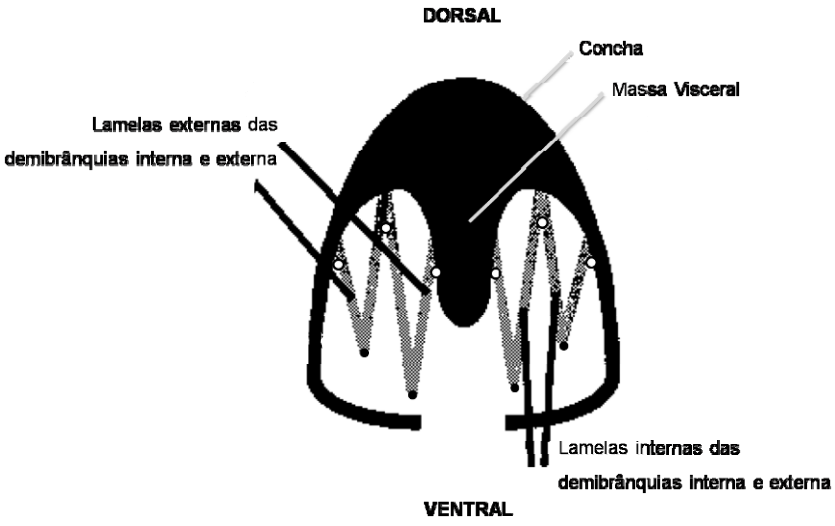

Figura 1.3. Esquema das brânquias lamelibrânquias (bivalves filtradores). Sulcos ventrais= ●, sulcos dorsais= ○. Modificada de Baker et al. (2000).

As áreas de aceitação estão situadas na margem ventral das demibrânquias, no eixo das brânquias e na junção das lamelas externas das demibrânquias interna e externa com a massa visceral e o manto, respectivamente. A superfície das brânquias e dos palpos labiais é ciliada, e desta forma possibilita a seleção das partículas desejáveis e indesejáveis, direcionando-as para correntes de aceitação ou rejeição.

As partículas que chegam à região dos palpos labiais (Figura 1.4), vindas do interior do sulco alimentar ventral das demibrânquias internas, passam do sulco oral proximal diretamente para a boca. As demais partículas estão sujeitas à seleção nas correntes ciliares dos palpos labiais, antes de sua ingestão. As partículas selecionadas nos palpos labiais como desejáveis são movidas diretamente para a boca, e as indesejáveis são movidas lateralmente para a margem oposta do palpo, para posterior rejeição. As correntes ciliares das margens da boca realizam a última seleção das partículas antes da ingestão, onde somente as desejáveis passam diretamente para a boca, e as indesejáveis retornam aos palpos labiais.

As partículas que são muito grandes caem no manto e são direcionadas para a região posterior, para serem expelidas. O material rejeitado, denominado pseudofezes, vai acumulando-se nesta região, próximo ao sifão inalante. Os lobos do sifão inalante são altamente móveis e com cílios fortes na sua superfície interna, que direcionam o material rejeitado dorsalmente na direção do sifão exalante para sua expulsão (Figura 1.5).

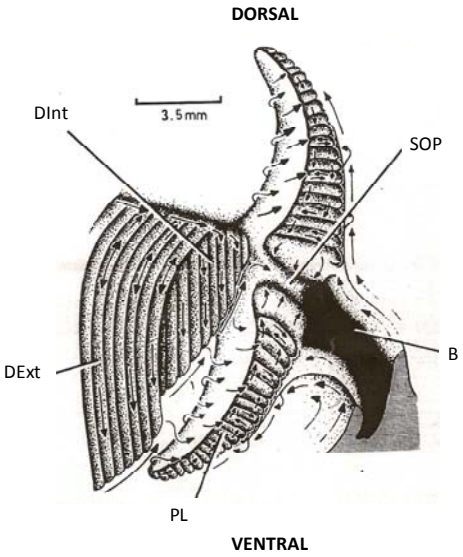

Figura 1.4. Correntes ciliares das brânquias e dos palpos labiais de *Limnoperna fortunei*. Demibrânquia interna (DInt), demibrânquia externa (DExt), palpo labial (PL), boca (B), sulco oral proximal (SOP). Modificada de Morton (1973).

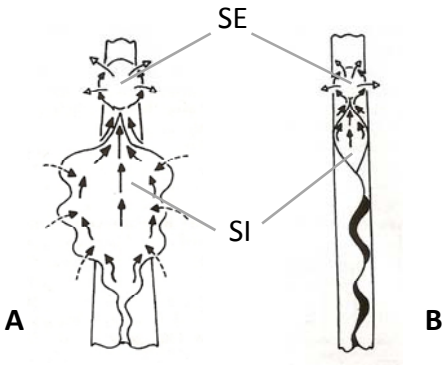

Figura 1.5. Correntes ciliares inalantes, exalantes e de rejeição de *Limnoperna fortunei* (A) filtrando ativamente e (B) filtrando pouco. Sifão exalante (SE), sifão inalante (SI). Modificada de Morton (1973).

1.4.1.4. Taxas de filtração do mexilhão dourado

O primeiro trabalho sobre as taxas de filtração de *L. fortunei* foi realizado por Sylvester et al. (2005), utilizando mexilhões coletados no Rio Paraná, Argentina. Em experimentos laboratoriais, estes mexilhões foram alimentados com a clorofícea *Chlorella vulgaris*. As taxas de filtração obtidas variaram de 125 a 350 mL ind^{-1} h^{-1}. Estes valores

estão entre os mais elevados já registrados para bivalves filtradores, incluindo as espécies invasoras *D. polymorpha*, *D. bugensis* e *Corbicula fluminea*.

Em estudo posterior, Sylvester et al. (2006) compararam graficamente as taxas de filtração de *L. fortunei*, com as de outros bivalves de água doce e marinhos. O mexilhão dourado demonstrou taxas de filtração superiores em relação às demais espécies, evidenciando seu potencial de impacto sobre o ecossistema (Figura 1.6).

A comparação entre as taxas dos bivalves tem certas limitações devido à grande variabilidade das condições ambientais e experimentais entre os trabalhos publicados, p.ex. oxigênio dissolvido, velocidade da corrente, etc. Apesar destas limitações, é possível fazer uma comparação geral entre os bivalves filtradores, e observar que as taxas de filtração de *L. fortunei*, nitidamente, estão entre as superiores.

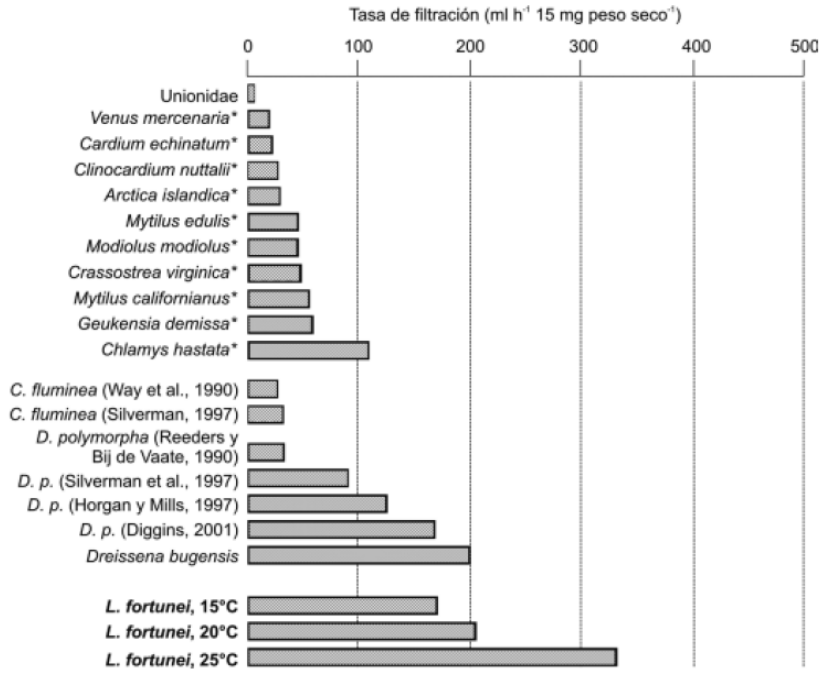

Figura 1.6. Taxas de filtração (mL h^{-1} 15 mg PS^{-1}; 15 mg PS= aprox. 1 mexilhão jovem) de *Limnoperna fortunei* em comparação com as de outros bivalves de água doce e marinhos (*Jørgensen, 1990) (Sylvester et al., 2006).

1.4.2. Semelhanças entre o mexilhão dourado L. fortunei e o mexilhão zebra D. polymorpha, bivalve invasor na Europa e América do Norte

Os problemas econômicos que o mexilhão dourado vem causando na América do Sul assemelham-se muito com os problemas ocorridos na Europa e América do Norte com o bivalve invasor *Dreissena polymorpha* (Pallas, 1771) (Bivalvia, Dreissenidae), conhecido como mexilhão zebra. Estes bivalves apresentam características de espécies invasoras que se tornam pragas como, tempo de geração curto, comportamento gregário, abundância em seu habitat natural, ampla tolerância ambiental e associação a atividades humanas (Darrigran & Damborenea, 2005). Tais características são decorrentes de aspectos comuns às duas espécies como, ciclo de vida curto, crescimento rápido, estágio larval planctônico e presença de bisso, estrutura protéica que permite a fixação dos bivalves em qualquer substrato duro (Morton, 1973, Ricciardi, 1998).

É provável, que os impactos ecológicos que o mexilhão zebra promoveu na Europa e América do Norte, também ocorram na América do Sul com o mexilhão dourado, devido à semelhança entre estas espécies. O mexilhão zebra tem a capacidade de alterar a estrutura trófica dos ecossistemas aquáticos, principalmente a composição e a abundância das comunidades planctônicas através do mecanismo de filtração seletiva (Fahnenstiel, 1995, Caraco et al., 1997, Bastviken et al., 1998).

1.4.2.1. A invasão do mexilhão zebra na Europa e América do Norte

Os impactos ecológicos da invasão de *D. polymorpha* estão relacionados principalmente ao crescimento descontrolado de suas populações, e ao seu potencial para alterar a estrutura da cadeia trófica aquática. A sua presença nos ecossistemas aquáticos

pode resultar na alteração da disponibilidade de alimento para as espécies pelágicas e bentônicas (Fahnenstiel et al., 1995, Karatayev et al., 1997, Strayer et al., 1999), na redução da biomassa fitoplanctônica (Caraco et al., 1997, Caraco et al., 2006) e na mudança da composição de espécies do fitoplâncton (Baker et al., 1998, Smith et al., 1998, Strayer et al., 1999).

O potencial do mexilhão zebra para alterar a composição e a abundância das comunidades planctônicas foi demonstrado em diversos estudos (Holland, 1993, Nicholls & Hopkins, 1993, Heath et al., 1995, Roditi et al., 1996). O mexilhão zebra quando submetido a misturas de fitoplâncton natural e cultivado, em condições laboratoriais, rejeitou determinadas espécies do fitoplâncton nas pseudofezes. Estas pseudofezes continham células viáveis de fitoplâncton, que retornando à coluna d'água, poderiam ter sua predominância favorecida em relação àquelas espécies que foram ingeridas (Baker et al., 1998, Vanderploeg et al., 2001). Em longo prazo, a seleção de partículas do plâncton por densas populações de *D. polymorpha* pode gerar grandes mudanças nos ecossistemas.

A invasão do Rio Hudson pelo mexilhão zebra levou à drásica diminuição da biomassa fitoplanctônica (Caraco et al, 1997). Através da modelagem do ecossistema, antes (1987-1991) e depois (1993-1994) da invasão por *D. polymorpha*, foi observado que a pressão de predação do mexilhão levou a biomassa fitoplanctônica ao declínio em cerca de 85%. Além disso, a disponibilidade de luz aumentou, as concentrações de fosfato dobraram, e alguns predadores planctônicos diminuíram em densidade após a invasão. O fitoplâncton é a base da cadeia trófica aquática, logo, mudanças na sua estrutura podem causar alterações em cascata nos níveis tróficos superiores.

Os bivalves podem afetar fortemente os processos pelágicos e bentônicos pela remoção de fitoplâncton, deposição de fezes e pseudofeces e ciclagem de nutrientes dissolvidos. Portanto, a forma como os bivalves interagem com a mistura heterogênea de

partículas em suspensão pode afetar a quantidade e a qualidade do material que é trocado de volta para a coluna de água ou entregue ao bentos via pseudofeces e fezes (Ward et al., 2003).

1.4.2.2. Impactos do mexilhão zebra nas cianobactérias

A filtração seletiva do mexilhão zebra tem efeito sobre as densidades de cianobactérias, podendo promover a ocorrência de florações tóxicas (Baker et al., 1998, Vanderploeg et al., 2001, Dionisio-Pires Pires & Van Donk, 2002). Alguns estudos observaram o declínio das densidades de cianobactérias como efeito da filtração seletiva do mexilhão zebra (Bastviken et al., 1998, Dionisio-Pires Pires & Van Donk, 2002). Outros estudos demonstraram efeito contrário, em que a filtração seletiva do mexilhão zebra promoveu o aumento das densidades de cianobactérias (Makarewicz et al., 1999, Vanderploeg et al., 2001, Nicholls et al., 2002).

A preferência do mexilhão zebra a ingerir ou rejeitar espécies fitoplanctônicas foi estudada por Baker et al. (1998) para tentar explicar as mudanças observadas no Rio Hudson (Nova Iorque, EUA) desde sua invasão, como o declínio das cianobactérias e o aumento das diatomáceas. Os autores observaram que o mexilhão zebra ingeriu preferencialmente as cianobactérias (*Microcystis*) dentre as demais partículas testadas. Entretanto, Bastviken et al. (1998) observaram que a presença do mexilhão zebra promoveu o aumento significativo das densidades de *Microcystis* spp. coloniais em experimentos de microcosmos com água natural. Dionisio-Pires Pires & Van Donk (2002), em experimentos laboratoriais, alimentaram o mexilhão zebra com a clorofícea *Chlamydomonas reinhardtii* e a cianobactéria *Microcystis aeruginosa*, e observaram maior ingestão da cianobactéria e rejeição preferencial da clorofícea. Deste modo, a presença do

mexilhão zebra poderia levar a uma diminuição das densidades de cianobactérias e da ocorrência de florações.

Entretanto, a capacidade do mexilhão zebra de promover o aumento das densidades de cianobactérias também foi observada (Lavrentyev et al., 1995, Makarewicz et al., 1999, Vanderploeg et al., 2001). Desde o estabelecimento do mexilhão zebra na região dos grandes lagos (*Great Lakes*), foi observada uma forte mudança no fitoplâncton, onde as cianobactérias começaram a predominar. Vanderploeg et al. (2001) realizaram experimentos com culturas laboratoriais puras de *M. aeruginosa* e com água natural destes lagos contendo a cianobactéria. As colônias maiores foram rejeitadas com maior eficiência, enquanto que as menores foram ingeridas, indicando o potencial do mexilhão zebra de promover florações através da rejeição seletiva das colônias de *M. aeruginosa* nas pseudofezes.

1.4.3. As cianobactérias e a produção de toxinas

As cianobactérias ou cianofíceas, também conhecidas popularmente como algas azuis, são microorganismos aeróbicos fotoautotróficos. Seus processos vitais requerem somente água, dióxido de carbono, substâncias inorgânicas e luz. A fotossíntese é seu principal modo de obtenção de energia para o metabolismo, entretanto, sua organização celular demonstra que esses microorganismos são procariontes e, portanto, muito semelhantes bioquimica e estruturalmente às bactérias (Mur et al., 1999).

O processo de eutrofização dos ecossistemas aquáticos favorecido pelo enriquecimento de nutrientes, especialmente fósforo e nitrogênio, é reconhecido como a principal causa do aumento da freqüência e intensidade das florações de microalgas ou cianobactérias. As florações, conhecidas também como *blooms*, são eventos de

multiplicação e acumulação de microalgas ou cianobactérias nos corpos hídricos, que podem durar de algumas horas ao longo do dia a meses (Ceballos et al., 2006).

1.4.1.4. Cianotoxinas

Vários gêneros e espécies de cianobactérias produzem toxinas. As toxinas de cianobactérias, que são conhecidas como cianotoxinas, constituem uma grande fonte de produtos naturais tóxicos (Carmichael, 1992). As cianotoxinas são classificadas quanto a seu mecanismo de toxicidade ou estrutura química. Quanto aos seus mecanismos de toxicidade, podem ser hepatotóxicas, neurotóxicas e dermatotóxicas. Quanto à sua estrutura química, correspondem respectivamente aos peptídeos cíclicos, alcalóides e lipopolissacarídeos. As cianotoxinas estão presentes principalmente no interior das células, e são liberadas na lise celular, que ocorre principalmente por senescência natural (Sivonen & Jones, 1999).

O tipo mais comum de intoxicação envolvendo cianobactérias é ocasionado pelas hepatotoxinas (microcistina e nodularinas), que agem sobre o citoesqueleto das células provocando hemorragias. As hepatotoxinas têm a ocorrência mais ampla e são as mais estudadas (Sivonen & Jones, 1999).

Dentre as hepatotoxinas, destacam-se as microcistinas, produzidas por diversos gêneros incluindo *Microcystis*, *Anabaena*, *Planktothrix* (*Oscillatoria*), *Anabaenopsis*, *Nostoc* e *Hapalosiphon* (Carmichael, 1992). As microcistinas são potentes inibidoras das proteínas fosfatases do tipo 1 e 2A (PP-1 e PP-2A) de células eucariontes (Mackintosh et al., 1990, Williams et al., 1997). Estas toxinas são hidrossolúveis, portanto incapazes de penetrar diretamente a membrana lipídica de células de bactérias, plantas e animais. Para obter os seus efeitos tóxicos, a entrada nas células ocorre através de transportadores de

membrana que em outras circunstâncias, transportam nutrientes e bioquímicos essenciais (Sivonen & Jones, 1999). Sendo assim, as microcistinas atingem as células do fígado dos vertebrados através do transporte ativo da membrana celular por meio de transportadores dos ácidos biliares. A morte dos vertebrados é conseqüência dos severos danos no fígado, que começam com a desorganização do citoesqueleto das células hepáticas e podem incluir perda da integridade da membrana celular, danos no DNA, apoptose celular, necrose, hemorragia intra-hepática, e por fim a morte por insuficiência hepática (Wiegand & Pflugmacher, 2005). A exposição crônica a doses não letais destas hepatotoxinas pode causar tumores no fígado, e consequentemente, trazer sérios danos a seres humanos e animais (Falconer, 1991, Carmichael, 1992).

A cilindrospermopsina, produzida por gêneros como *Cylindrospermopsis*, *Umezakia* e *Aphanizomenon*, é um composto alcalóide hepatotóxico, seu mecanismos de ação ocorre pela inibição da síntese protéica. A hepatotoxina nodularina, produzida por *Nodularia spumigena*, é um pentapeptídeo semelhante à microcistina.

As neurotoxinas são as saxitoxinas, anatoxina-a e anatoxina-a (s). As saxitoxinas, produzidas por *Anabaena, Aphanizomenon, Cylindrospermopsis* e *Lyngbia*, são alcalóides carbamatos que inibem a transmissão nervosa através do bloqueio dos canais de sódio das células. A anatoxina-a, produzida por *Anabaena, Planktothrix* e *Aphanizomenon*, é um alcalóide que mimetiza a ação da acetilcolina e bloqueia os receptores de sinapses neuromusculares. A anatoxina-a (s) é um organofosforado natural, produzido por *Anabaena*, que inibe a atividade da acetilcolinesterase (Ceballos et al., 2006). Os lipopolissacarídeos, de efeito dermatotóxico, são componentes da membrana celular das cianobactérias, formados por carboidratos e lipídeos que podem causar irritações na pele e alergias, e estão presentes em todos os gêneros de cianobactérias (Sivonen & Jones, 1999).

A toxicidade das espécies de cianobactérias presentes nas florações pode apresentar variação temporal, desde intervalos de tempo curtos até diferenças sazonais, assim como espacial, provavelmente decorrentes de alterações na proporção de cepas tóxicas e não-tóxicas na população. Estas variações na toxicidade ainda não foram devidamente esclarecidas, todavia a ocorrência de florações tóxicas é cada vez mais freqüente, sendo que de 50 a 75% das florações de cianobactérias apresentam espécies produtoras de toxinas (Ceballos et al., 2006).

1.4.1.5. Florações de cianobactérias no Brasil

Os gêneros identificados no Brasil em situações de floração, que se destacam pela sua ampla distribuição, capacidade de produzir toxinas e pelo seu efeito na biota aquática, são *Microcystis, Anabaena, Aphanizomenon, Planktothrix* e *Cylindrospermopsis* (Ceballos et al., 2006). As florações de cianobactérias tóxicas foram registradas em 11 dos 26 estados brasileiros, distribuídos do norte ao sul do país, ocorrendo em rios, estuários, lagoas costeiras, e com maior freqüência, em reservatórios (Ceballos et al., 2006).

1.4.1.6. Florações de cianobactérias no lago Guaíba

As florações de cianobactérias no lago Guaíba, estado do Rio Grande do Sul, vêm sendo registradas há mais de trinta anos como ocorrências eventuais (Bendati et al., 2007). Entretanto, no ano de 2004, foi registrada uma floração intensa de *Planktothrix isothrix* que afetou a qualidade da água do lago. O Departamento Municipal de Água e Esgotos de Porto Alegre (DMAE) teve que acrescentar medidas operacionais nas Estações de Tratamento de Água para a remoção de gosto e odor, provocados pela floração de

cianobactérias. Os valores de microcistinas foram detectados em baixas concentrações no lago Guaíba, e abaixo de 1 μg L^{-1} na água tratada.

A partir de 2004, as florações de cianobactérias tornaram-se freqüentes no lago Guaíba, sendo registradas também nos verões de 2005, 2006 e 2007 (Bendati et al., 2007). As espécies predominantemente encontradas nestas florações foram *Planktothrix isothrix, Planktothrichoides raciborskii* e *Microcystis* spp. (Cybis et al., 2006; Bendati et al., 2007; Werner et al., 2007). Bioensaios em camundongos demonstraram a toxicidade das florações, com efeitos hepatotóxicos e neurotóxicos (Werner et al., 2007).

1.5. Material e Métodos

1.5.1. Coleta e aclimatação de L. fortunei

Os exemplares de *L. fortunei* utilizados nos experimentos **(Artigos I e II)** foram coletados no lago Guaíba, sul do Brasil. Em laboratório, foram mantidos em aquários com água do local de coleta sob temperatura controlada de 24ºC e aeração constante durante 24h para aclimatação. Os mexilhões foram medidos com paquímetro digital Mytutoyo e selecionados indivíduos adultos com comprimentos de aproximadamente 30 mm para evitar possíveis diferenças nas taxas de filtração em função de variações de tamanho. Os espécimes selecionados estavam aparentemente sadios, como indicado por sua atividade de filtração. Os mexilhões foram lavados e escovados para a retirada dos microorganismos aderidos às suas conchas. Posteriormente, foram colocados em água mineral (Fonte Floresta; pH= 7,0; condutividade elétrica= 2,9 x 10^{-4} mhos cm^{-1}; temperatura na fonte= 24ºC) sem adição de alimento por um período de 4 h para evacuação.

As larvas de *L. fortunei* utilizadas no experimento **(Artigo III)** foram coletadas no Lago Guaíba, sul do Brasil. Foi filtrado aproximadamente 1 m³ de água em rede de plâncton de 30 μm de abertura de malha. No laboratório, as larvas foram selecionadas sob microscópio estereoscópico e separadas para serem utilizadas no experimento. Foram utilizadas larvas na fase véliger, em que o véu ciliado, responsável pela locomoção e alimentação, está formado e desenvolvido.

1.5.2. Cianobactérias e fitoplâncton utilizados nos experimentos

As cepas tóxica (NPLJ-4) e não-tóxica (NPCD-1) da cianobactéria *M. aeruginosa* **(Artigos I, II e III)**, e a cepa de *Monoraphidium* sp. (MONO) **(Artigo III)** foram fornecidas pelo Laboratório de Ecofisiologia e Toxicologia de Cianobactérias/UFRJ e cultivadas em meio ASM-1 (Gorham, 1964). A espécie *N. palea* (N) **(Artigo I)** foi isolada de amostras de água coletadas no lago Guaíba e cultivada em meio D (Jebram, 1993). As espécies foram cultivadas em frascos Erlenmeyer de 250 mL. Os frascos foram mantidos em incubadora (25º C) num ciclo dia : noite de 14 : 10 h e intensidade luminosa de 2000 lux. A espécie *Planktothrix* sp. **(Artigo II)** foi amostrada da floração natural do verão de 2008 no lago Guaíba.

1.5.3. Taxas de filtração, ingestão e produção de pseudofezes

As taxas de filtração (FR) ou taxas de clareamento (CR) foram estimadas considerando as partículas capturadas pelo mexilhão. A taxa de ingestão (IR) é igual à taxa de filtração menos a taxa de produção de pseudofezes (PPR). As pseudofezes são as partículas filtradas aglomeradas com muco que são expelidas periodicamente pelo sifão

inalante, i.e. partículas filtradas, mas não ingeridas. A taxa de filtração é igual à de ingestão somente se não forem produzidas pseudofezes.

As taxas de filtração do mexilhão dourado foram estimadas através da seguinte equação (Coughlan, 1969) **(Artigos I, II e III)**:

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

onde FR é a taxa de clareamento (mL mexilhão^{-1} h^{-1} ou mL mgPS^{-1} h^{-1}), V é o volume de água no frasco experimental (mL), N é o número de mexilhões por frasco ou seu peso seco (mgPS), T é o tempo total de filtração (h), C_0 é a concentração de partículas (mm³ L^{-1}) em T= 0, C_t é a concentração de partículas em T nos frascos com mexilhão, C'_0 é a concentração de partículas no frasco controle (sem mexilhão) em T= 0 e C'_t é a concentração de partículas no frasco controle em T. O tecido dos mexilhões foi removido das conchas e mantido em estufa a 60ºC por 48h para estimar o peso seco **(Artigos I e II)**.

As amostras para estimativa da concentração de partículas foram preservadas em solução Lugol 1% e quantificadas em câmara de Sedgewick-Rafter (APHA, 2000). O biovolume (mm³ L^{-1}) foi estimado através do cálculo do volume das espécies (mm³), baseado em fórmulas geométricas aproximadas à forma destas, multiplicado pela concentração celular (L) de acordo com Hillebrand et al. (1999) **(Artigos I, II e III)**.

1.5.4. Análise das pseudofezes e fezes

Durante o curso dos experimentos (**Artigos I e II**), o comportamento de cada exemplar de *L. fortunei* foi monitorado sob microscópio estereoscópico acoplado a câmera digital. O número de eventos de pseudofezes e fezes foram registrados (eventos h^{-1}). As

pseudofezes e fezes foram capturadas no momento da liberação com a utilização de pipetas capilares, e fixadas com Lugol a 1% para posterior análise quantitativa. Para possibilitar a contagem das células contidas nas pseudofezes, estas foram desagregadas com a utilização de equipamento de utra-som Bandelin Sonorex RK100H durante 10 min e a análise quantitativa foi realizada em câmara de contagem de Sedgewick-Rafter para acessar a taxa de produção de pseudofezes (PPR). Este método foi desenvolvido no presente estudo e sua aplicação possibilitou a desagregação das pseudofezes e a liberação das células sob forma íntegra.

1.5.4.1. Padrão de liberação das pseudofezes e fezes registrado durante a realização dos experimentos (Artigo I)

Durante o monitoramento dos experimentos foi possível observar que a ingestão das partículas alimentares sempre ocorreu pelo sifão inalante, assim como a expulsão das pseudofezes. A liberação das fezes sempre ocorreu pelo sifão exalante. Em relação a abertura e fechamento das valvas, todos os mexilhões utilizados nos diferentes experimentos apresentaram o mesmo comportamento, as valvas mantiveram-se sempre abertas por toda a duração do experimento. Em todos os experimentos, os mexilhões mantiveram-se filtrando ativamente, com o sifão inalante exposto.

O padrão de liberação das pseudofezes foi diferenciado entre os tratamentos. A maior diferença observada ocorreu entre os tratamentos fitoplâncton não-tóxico e cianobactéria (tóxica/não-tóxica). As pseudofezes no tratamento fitoplâncton não-tóxico foram liberadas constantemente, durante toda a duração do experimento, e sempre sob a forma de grandes aglomerados globulares (Figuras 1.7 e 1.8), ou sob a forma de cordões (Figura 1.9). No tratamento cianobactéria (tóxica/não-tóxica), as pseudofezes foram

liberadas com menor freqüência, sempre sob a forma de pequenos aglomerados globulares (Figuras 1.10 e 1.11).

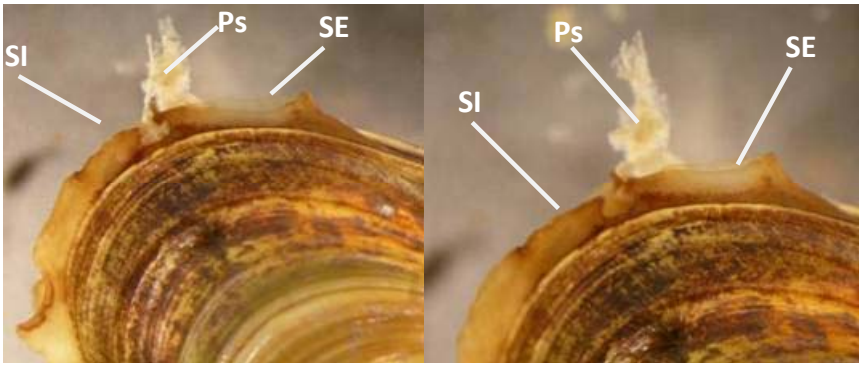

Figura 1.7: Padrão de liberação das pseudofezes de *L. fortunei* no tratamento fitoplâncton não-tóxico sob a forma de grandes aglomerados globulares. Sifão inalante (SI), sifão exalante (SE), pseudofezes (Ps).

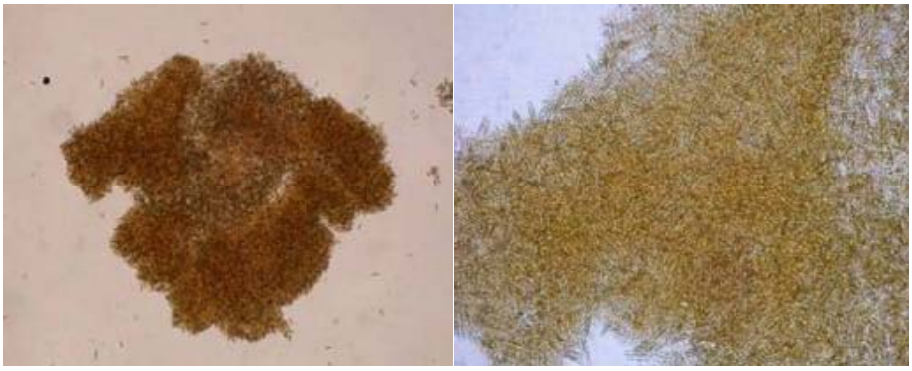

Figura 1.8: Pseudofezes de *L. fortunei* no tratamento fitoplâncton não-tóxico sob a forma de grande aglomerado globular (dimensões da célula de *N. palea*= diâmetro 4,5 µm; comprimento 22,5 µm).

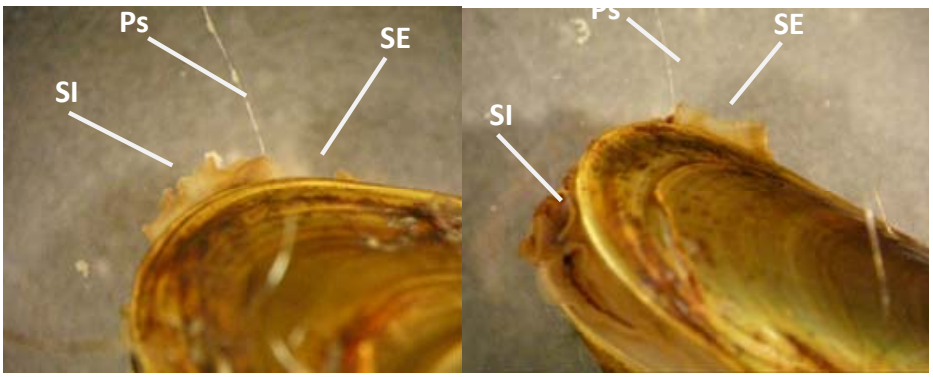

Figura 1.9: Padrão de liberação das pseudofezes de *L. fortunei* no tratamento fitoplâncton não-tóxico sob a forma de cordões. Sifão inalante (SI), sifão exalante (SE), pseudofezes (Ps).

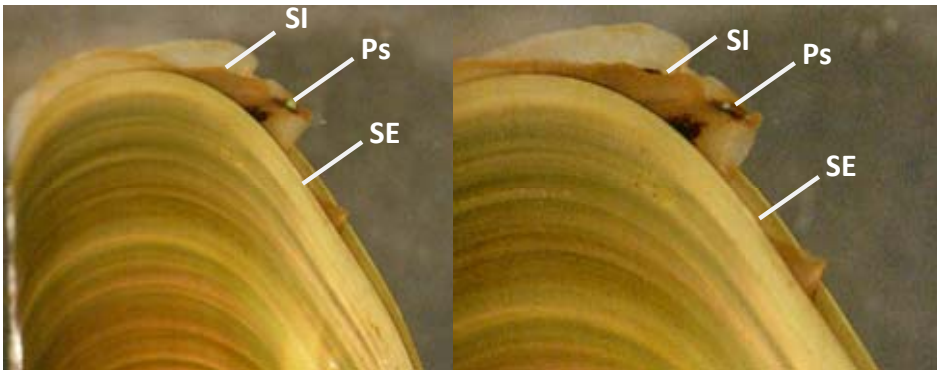

Figura 1.10: Padrão de liberação das pseudofezes de *L. fortunei* no tratamento cianobactéria tóxica, sob a forma de pequenos aglomerados globulares. Sifão inalante (SI), sifão exalante (SE), pseudofezes (Ps).

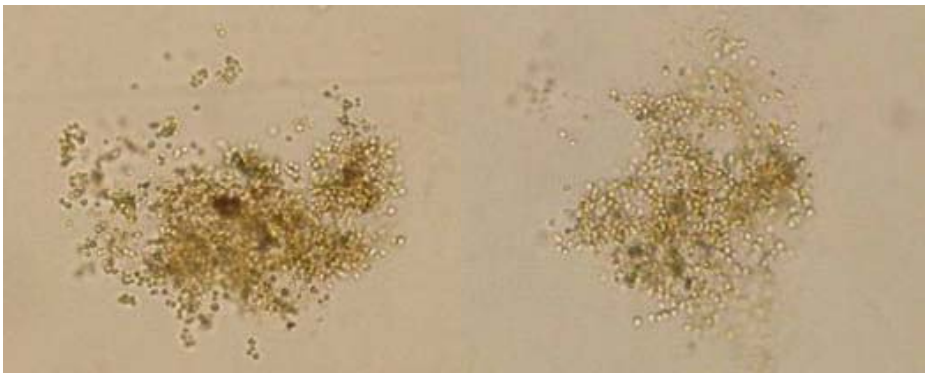

Figura 1.11: Pseudofezes de *L. fortunei* no tratamento cianobactéria tóxica (diâmetro celular de *M. aeruginosa*= 3,7 µm).

O padrão de liberação das fezes foi o mesmo em todos os tratamentos. As fezes apresentaram aspecto semelhante a "tiras achatadas" (formato do intestino do mexilhão) de coloração marrom (Figuras 1.12 e 1.13).

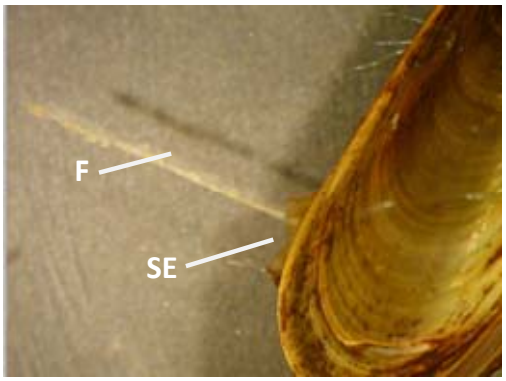

Figura 1.12: Padrão de liberação das fezes de *L. fortunei*. Sifão exalante (SE), fezes (F).

Figura 1.13: Fezes de *L. fortunei* no tratamento fitoplâncton não-tóxico *N. palea*.

1.5.5.Análise de microcistina (MC-LR)

A estimativa da concentração de microcistinas (MC-LR) nos diferentes experimentos foi realizada através do método ELISA (*Enzyme-Linked ImmunoSorbent Assay*), utilizando-se kit de Microcistina/Placa para determinação de microcistina em água da marca Beacon Analytical Systems Inc. (**Artigos I, II e III**). A faixa de detecção do método é de 0,1 a 2,0 µg L^{-1}.

1.5.6.Análises Estatísticas

Os resultados obtidos foram submetidos a Análises de Variância (*One-way* ANOVA) seguidas de teste Tukey de comparação múltipla para detectar diferenças

significativas (α= 0,05) entre os tratamentos utilizados (Artigos I, II e III). O teste de Tukey foi aplicado após a confirmação da normalidade dos dados através do teste de Kolmogorov-Smirnov (KS) (α= 0,05).

ARTIGO I

2. ARTIGO I

FEEDING BEHAVIOR OF THE INVASIVE BIVALVE *LIMNOPERNA FORTUNEI* (DUNKER, 1857) UNDER EXPOSURE OF TOXIC CYANOBACTERIA *MICROCYSTIS AERUGINOSA*

Vanessa Gazulha[1]*, Maria Cristina Dreher Mansur[2], Luiz Fernando Cybis[1], Sandra Maria Feliciano de Oliveira e Azevedo[3]

[1] *Institute of Hydraulic Research, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.*

[2] *Center of Ecology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.*

[3]*Laboratory of Ecophysiology and Toxicology of Cyanobacteria, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.*

*Author for correspondence: vanessagazulha@gmail.com

Keywords: golden mussel; filtration rates; selective grazing; microcystin; exotic species; phytoplankton.

"This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to Hydrobiologia."

Abextract

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 feeding 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 possible vector to the transference of cyanotoxins to higher trophic levels.

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 (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 & 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 & 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 Pires & Van Donk, 2002). 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 & 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 & 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.

Material and methods

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.

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).

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):

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

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), C_0 is the food concentration (mm³ L^{-1}) at T= 0, C_t 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'_t concentration of food in the control flask at time T.

Mussel tissue was removed from shells and oven-dried at 60°C for 48h 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 concentration ($mm^3\ L^{-1}$) was calculated according to Hillebrand et al. (1999).

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 min to keep food particles in suspension during filtration time (1h). 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 in the present study to estimate accurately pseudofaeces production by mussels, as follows. Pseudofeces and feces were captured in the moment of expelling using a micropipette and preserved in 1% Lugol solution. Pseudofeces were disintegrated for 10 min 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 (1h). FRs, IRs, and PPRs were determined in the present experiment.

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 concentration of 2 $mm^3\ L^{-1}$ during 5 days (120h). Food suspension and water were replaced every 24h. 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 (plus feces) due to pseudofeces resuspension. IRs were estimated every 24h. Microcystin concentration in the water was analyzed by ELISA assay to compare toxins assimilated by mussels and toxins remaining in experimental aquaria.

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 (α= 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 (α= 0.05).

Results

Short term grazing of L. fortunei

Golden mussel FRs varied from 95.6 to 817.5 mL mussel^{-1} h^{-1}, and the mean value was 519.3 mL mussel^{-1} h^{-1}. Mean FRs varied from 608.6 mL mussel^{-1} h^{-1} on non-toxic *Nitzschia* to 356.1 mL mussel^{-1} h^{-1} on the mixture of *Nitzschia* + toxic *Microcystis* (Fig. 1). FRs in relation to body mass 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* (Fig. 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; Fig.1).

IRs of golden mussel ranged from 5.6 to 720.6 mL mussel^{-1} h^{-1}, and the mean value registered was 250.4 mL mussel^{-1} h^{-1}. Mean values in each food combination varied from 453.5 mL mussel^{-1} h^{-1} on non-toxic *Microcystis* to 13.5 mL mussel^{-1} h^{-1} on diatom

Nitzschia (Fig. 1). IRs in relation to body mass varied 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* (Fig. 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 17.4 to 726.9 mL $mussel^{-1}$ h^{-1}, and the mean value was 268.9 mL $mussel^{-1}$ h^{-1}. Golden mussel pseudofeces production in relation to body mass varied 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 (Fig.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* (Fig. 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).

Long term grazing of L. fortunei

Golden mussel IRs on toxic *Microcystis* ranged from 31.8 to 54.6 mL mussel^{-1} h^{-1} (Fig. 3) and on non-toxic *Microcystis* ranged from 36.3 to 62.5 mL mussel^{-1} h^{-1} (Fig. 4), with mean values of 40.9 and 48 mL mussel^{-1} h^{-1}, respectively. In terms of body mass, IRs on toxic *Microcystis* 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 72h in the presence of toxic *Microcystis* (Fig. 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 MCYST L^{-1} (Table 2). There were no significant differences between initial and final microcystin concentrations every 24h 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-hour 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.

Discussion

Short term grazing of L. fortunei

Sylvester et al. (2005) have registered *L. fortunei* FRs ranging from 125 to 350 mL 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*. In the present short term experiment, FRs were higher with mean values ranging from 356.1 to 608.6 mL mussel^{-1} h^{-1}. In terms of body mass, FRs registered in the present study (from 9 to 14.8 mL mgDW^{-1} h^{-1}) were comparable to that observed by Sylvester et al. (2005) (from 9.9 to 29.5 mL mgDW^{-1} h^{-1}).

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 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 Pires & Van Donk, 2002, 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 Pires & 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 (355.3 μm³) is larger than *Microcystis* cell volume (29.2 μm³). 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 *Mytilus 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 Pires & 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. An experiment has been carried out to test the effects of size and shape of particles on golden mussel feeding behavior (Gazulha et al., unpublished). Than, the hypothesis that golden mussel ingests preferentially *Nitzschia* and rejects toxic cyanobacteria has been rejected.

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 μg L^{-1}) (Amorim & 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 μg L^{-1}) (Dionisio-Pires 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 & Vasconcelos, 1999; Yokoyama & 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 (1h) than on long term (120h) 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 (1h); 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).

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

Species	**Strain**	**µg MC-LR L^{-1}**	**GLD***
Toxic *Microcystis aeruginosa*	NPLJ-4	7 µg L^{-1}	3,7 µm
Non-toxic *Microcystis aeruginosa*	NPCD-1	-	3,5 µm
Nitzschia palea	N	-	22,5 µm
50:50 mixture of toxic *M. aeruginosa* + *N. palea*	NPLJ-4 + N	3.5 µg L^{-1}	

* *Greatest Linear Dimension*

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

µg MCYST L^{-1}	**24h**	**48h**	**72h**	**96h**	**120h**
Initial concentration	2.0	2.1	1.9	2.2	2.6
Final concentration	1.8	2.0	2.0	2.4	2.5

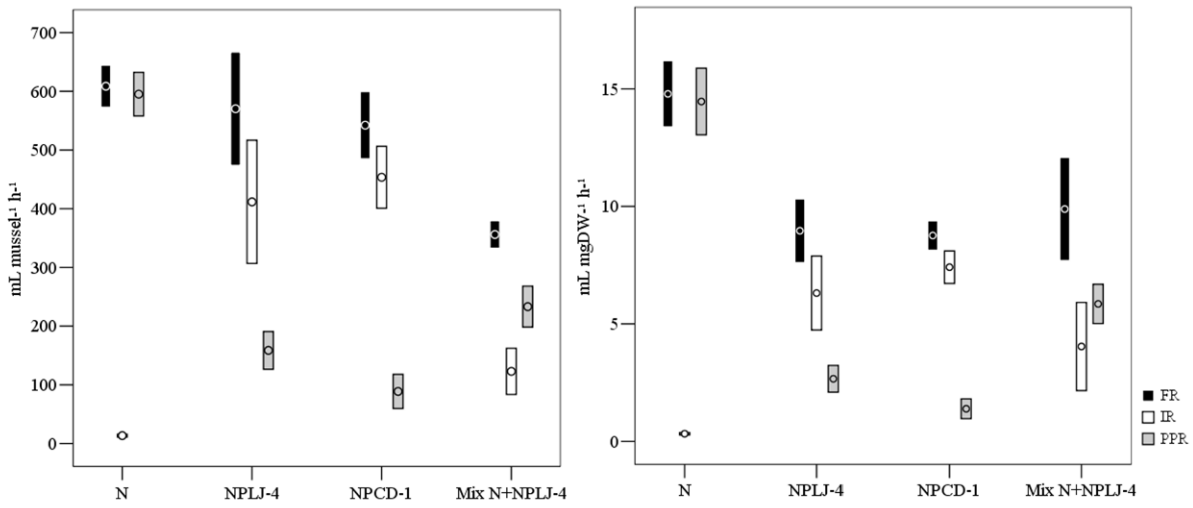

Fig. 1. Filtration Rates (FR), Ingestion Rates (IR) and Pseudofeces Production Rates (PPR) of *Limnoperna fortunei* (mL mussel^{-1} h^{-1}and mL mgDW^{-1} h^{-1}) on non-toxic *Nitzschia* (N), toxic (NPLJ-4) and non-toxic (NPCD-1) *Microcystis*, and mixture of *Nitzschia*+toxic *Microcystis* (Mix N+NPLJ-4)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

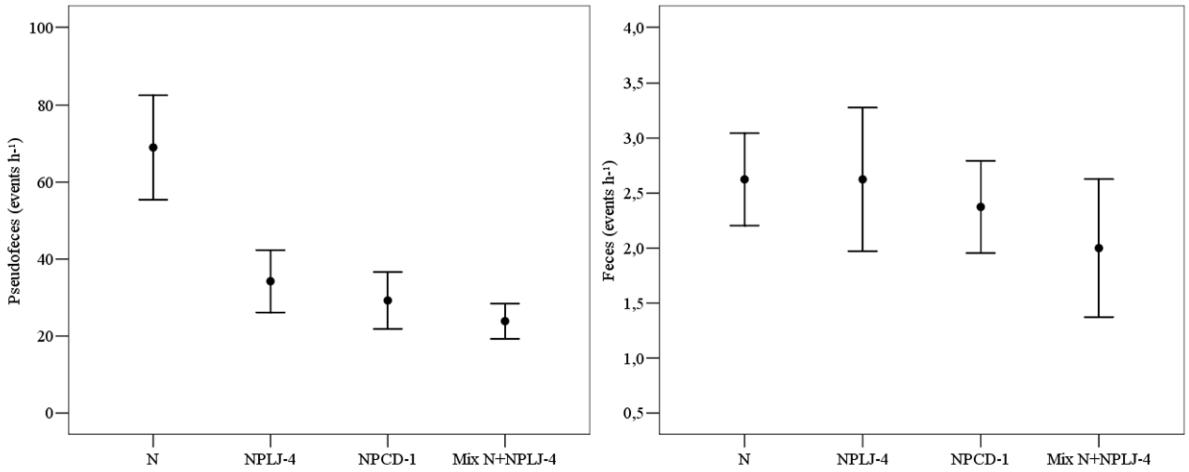

Fig. 2. Pseudofeces and feces of *Limnoperna fortunei* (events h^{-1}) on non-toxic *Nitzschia* (N), toxic (NPLJ-4) and non-toxic (NPCD-1) *Microcystis*, and mixture of *Nitzschia* + *Microcystis* (Mix N+NPLJ-4) (symbol= mean, and bar= SE).

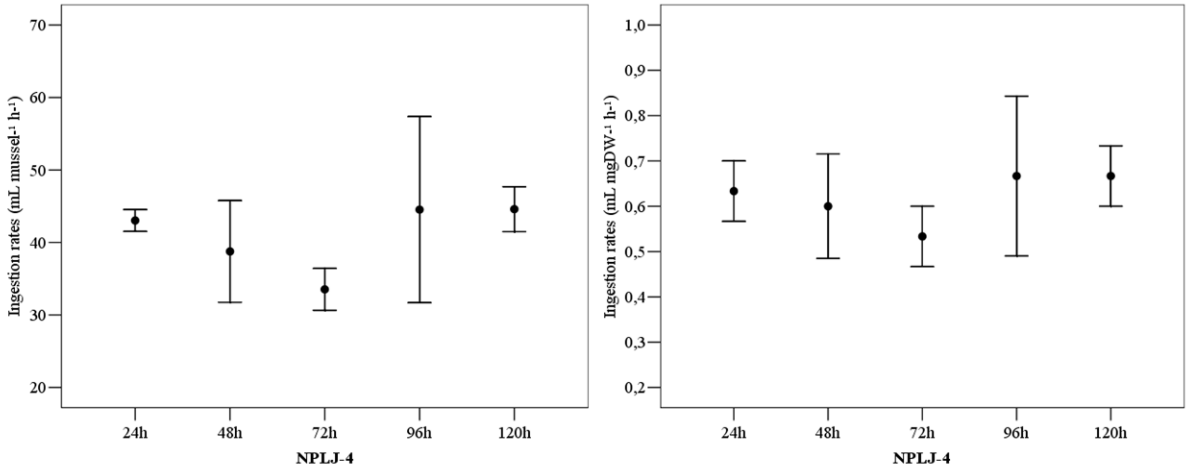

Fig. 3. Ingestion rates (IR) of *Limnoperna fortunei* (mL mussel^{-1} h^{-1} and mL mgDW^{-1} h^{-1}) on toxic *Microcystis* (NPLJ-4) (symbol= mean, and bar= SE).

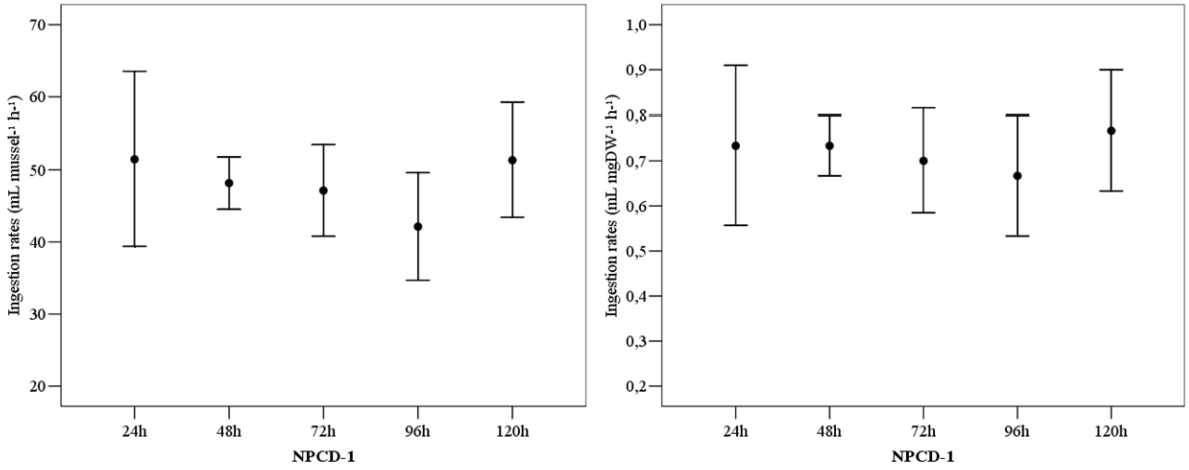

Fig. 4. Ingestion rates (IR) of *Limnoperna fortunei* (mL mussel^{-1} h^{-1} and mL mgDW^{-1} h^{-1}) on non-toxic *Microcystis* (NPCD-1) (symbol= mean, and bar= SE).

ARTIGO II

3. ARTIGO II

GRAZING IMPACTS OF THE INVASIVE BIVALVE *LIMNOPERNA FORTUNEI* (DUNKER, 1857) ON SINGLE-CELLED, COLONIAL, AND FILAMENTOUS CYANOBACTERIA

Vanessa Gazulha[1]*, Maria Cristina Dreher Mansur[2], Luiz Fernando Cybis[1], Sandra Maria Feliciano de Oliveira e Azevedo[3]

[1] *Institute of Hydraulic Research, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.*

[2] *Center of Ecology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.*

[3]*Laboratory of Ecophysiology and Toxicology of Cyanobacteria, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.*

*Author for correspondence: vanessagazulha@gmail.com

Keywords: selective feeding, golden mussel, *Microcystis*, *Planktothrix*, filtration rates, exotic species.

Abextract

Feeding behavior of the invasive bivalve *Limnoperna fortunei* in the presence of single-celled, colonial, and filamentous cyanobacteria was tested in laboratory experiments to evaluate the effects of size and shape on mussel feeding. The first hypothesis holds that golden mussel filters more efficiently smaller particles, which could be more easily assimilated by its filtering apparatus. The second hypothesis sustains that *L. fortunei* filters more efficiently rounded colonies, such as *Microcystis*, which would be more easily ingested than lengthy filamentous, such as *Planktothrix*. Filtration rates of golden mussel in the presence of single-celled, colonial, and filamentous cyanobacteria were similar. Nevertheless, there was a great difference on ingestion and pseudofeces production rates. Single cells were widely accepted as food, while filamentous and colonial cyanobacteria were massively expelled as pseudofeces. The results confirmed the first hypothesis that golden mussel prefers to ingest smaller particles. The second hypothesis was rejected since filamentous were preferentially ingested than colonial cyanobacteria. The presence of golden mussel may result in a dominance of large, colonial and filamentous, over small cyanobacteria. Massive densities of *L. fortunei* in association to its powerful filtering capability point out to the potential of this invasive species to promote great changes in the structure of trophic chains from invaded ecosystems.

Introduction

Bivalve feeding mechanisms by filtering particles can strongly influence on pelagic and benthic communities, especially when some species occur in large densities. Bivalves play an important role in regulating dynamics of aquatic ecosystems through processes such as removal of particles, nutrient cycling, and biodeposition (Milke & Ward, 2003).

Invasive species have the potential to promote changes in the structure of aquatic ecosystems because of their rapid establishment and high densities. Zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Bivalvia, Dreissenidae) is an example of an invasive bivalve altering ecosystem and community structure of lakes and rivers. This mussel is invasive in many freshwater ecosystems in Europe and North America. In places where dense populations of *D. polymorpha* were established, phytoplankton biomass was changed (Holland, 1993, Fahnenstiel et al., 1995, Caraco et al., 1997) as well as seston composition (Vanderploeg et al., 1996, Smith et al., 1998, Strayer et al., 1999).

Studies have pointed out to the fact that zebra mussel can change cyanobacteria density and promote the occurrence of cyanobacteria blooms (Lavrentyev et al., 1995, Vanderploeg et al., 2001, Nicholls et al., 2002). Cyanobacteria dominance is a global problem, especially in freshwater ecosystems, due to bloom formation and toxins production. Poisoning and even death of wild animals, domestic animals, and humans may be linked to cyanobacteria toxicity (Carmichael et al., 2001). Nevertheless, the effects of zebra mussel on cyanobacteria densities remain contradictory. Several studies have documented that zebra mussel filtration promoted the decrease of cyanobacteria densities (Bastviken et al., 1998, Dionisio-Pires Pires & Van Donk, 2002). Other studies have found that the presence of zebra mussel in invaded ecosystems has led to increased cyanobacteria

densities (Lavrentyev et al., 1995, Vanderploeg et al., 2001, Juhel et al., 2006, Naddafi et al. 2007).

Most studies about bivalve filtration were carried out with laboratory cultures of single-celled cyanobacteria. However, bloom forming cyanobacteria occur mainly as colonies or filaments in nature. Colonies eventually formed in laboratory conditions are usually small and without mucilage with the predominance of single cells (Dionisio-Pires Pires & Van Donk, 2002). Laboratory studies under controlled conditions of mussel feeding in the presence of colonial and filamentous cyanobacteria are scarce (Dionisio-Pires et al., 2005, Bontes et al., 2007). Studies performed with natural seston containing natural colonies of *Microcystis* have found that *D. polymorpha* promoted the increase of cyanobacteria densities by low filtration on colonies and their rejection as pseudofeces (Baker et al., 1998, Makarewicz et al., 1999, Vanderploeg et al., 2001, Nicholls et al., 2002, Naddafi et al., 2007). Natural colonies of *Microcystis* are usually large, therefore non-preferentially ingested (Vanderploeg et al., 2001).

The invasive bivalve *L. fortunei* (Dunker, 1857) (Bivalvia, Mytilidae), known as golden mussel, has similar behavior to zebra mussel *D. polymorpha*. Since its invasion in South America in 1991, golden mussel has been proliferating and dominating aquatic ecosystems such as rivers, lakes, and reservoirs. This invasive species has a great potential to change the structure of invaded environments due to its ability to quickly proliferate and turn into massive populations.

Studies about *L. fortunei* impacts on phytoplankton biomass and food chain structure are rare. In a previous study, it was tested the hypothesis that *L. fortunei* prefer to intake non-toxic phytoplankton (diatom *N. palea* and non-toxic strain of *M. aeruginosa*) and reject toxic cyanobacteria (toxic strain of *M. aeruginosa*) as pseudofeces (Gazulha et al., 2007; Gazulha et al., submitted). Golden mussel has shown preferential acceptance of

Microcystis, independently of being toxic or non-toxic, and great rejection of diatom as pseudofeces. The long-term grazing experiment showed there were no negative effects of toxic *Microcystis* on filtration and survival of golden mussel suggesting other factors than toxicity influence selective feeding of golden mussel (Gazulha et al., submitted).

The aim of this study was to compare filtration rates and feeding behavior of golden mussel (*L. fortunei*) when exposed to food of different size and shape: single-celled (*M. aeruginosa*), colonial (*M. aeruginosa*), and filamentous (natural bloom of *Planktothrix* sp.) cyanobacteria. The first hypothesis holds that golden mussel filter more efficiently smaller particles, which could be more easily assimilated by bivalve filtering apparatus. The second hypothesis sustains that *L. fortunei* filter more efficiently rounded shapes, such as colonies of *Microcystis*, which would be more easily ingested than length filamentous cyanobacteria. To test these hypotheses it was used non-toxic food and from the same nutritional group (*i.e.* cyanobacteria) to avoid possible effects of these factors in the results.

Material and Methods

L. fortunei sampling and acclimation

Golden mussels used in the experiments were collected from Guaíba Lake, southern Brazil. In the laboratory, mussels were kept in aquaria containing natural water from the sampling site to acclimatize for 24h. The aquaria were kept in acclimatized room (24º C) at constant aeration. Those specimens selected for the experiments were apparently healthy as indicated by their filtration activity. Mussels selected were washed and brushed to remove microorganisms attached to their shells and placed in flasks filled with mineral water for a 4-hour period without food to evacuate and clear their guts.

Cyanobacteria strains

Non-toxic (NPCD-1) strain of *Microcystis aeruginosa* was 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. NPCD-1 strain was 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. Bloom of *Planktothrix* sp. was sampled from Guaíba Lake. Non-toxicity of *Planktothrix* natural bloom was confirmed by analyses of microcystins (MC-LR) using ELISA test kit (Beacon).

Filtration, Ingestion and Pseudofeces Production Rates

Feeding behavior of golden mussel was evaluated by assessment of Filtration Rate (FR) or Clearance Rate (CR), Ingestion Rate (IR), and Pseudofeces Production Rate (PPR). FR was assessed by considering the amount of particles captured by the mussels. IR equaled FR less 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.

Filtration rates of golden mussel were estimated using the following equation based on Coughlan (1969):

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

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), C_0 is the food concentration (mm³ L^{-1}) at T= 0, C_t is the food concentration at time T in flasks with mussel, C'_0 is the food concentration in the control flask (without mussel) at T= 0 and the C'_0 is the food concentration in the control flask at time T.

The samples taken to estimate food concentration (mm³ L^{-1}) were preserved in 1% Lugol solution and counted in a Sedgewick-Rafter chamber. Biovolume (mm³) was calculated according to Hillebrand et al. (1999). Mussel tissue was removed from shells and oven-dried at 60°C for 48h to assess the dry weight (mgDW).

Experimental design

Limnoperna fortunei was fed with non-toxic strains of single-celled, colonial, and filamentous cyanobacteria to evaluate the effects of size and shape on selective feeding. This experiment was conducted using 3 types of food (Table I) with 10 replicates each at an initial biovolume of 2 mm³ L^{-1}. A 60 μm-plankton net was used to separate colonial from single *Microcystis* in NPCD-1 strain. Each replicate was performed in a flask containing 400 mL of mineral water, food suspension, and one mussel. Flasks were kept in acclimatized room (24º C) during total filtration time (1h) and gently stirred each 15 minutes to keep food particles in suspension. Flasks were prepared in the same conditions, with no mussel, to assess possible phytoplanktonic growth during filtration time. Each specimen of golden mussel was monitored under a stereomicroscope during total filtration time (1h). The number of feces and pseudofeces released by mussels was scored and expressed as events h^{-1}.

A method was applied in the present study to estimate accurately pseudofaeces production by mussels, as follows (Gazulha et al., submitted). All feces and pseudofeces produced were captured using a micropipette in the moment of expelling and preserved in 1% Lugol solution. To assess PPRs samples were disintegrated in an ultrasound Bandelin Sonorex RK 100H for 10 min to separate cells from the mucus and counted in a Sedgewick-Rafter chamber (mm^3 L^{-1}). The application of ultrasound was efficient to separate cells from mucus and did not damage the cells. Individuals used in this experiment kept their filtering ability with the valves opened and the inhalant siphon exposed during the total filtration time (1h). FRs, IRs, and PPRs were assessed in the present work.

Table I: Food types (species, strain, shape, and size) used in *Limnoperna fortunei* feeding experiment.

Cyanobacteria species	**Strain**	**Shape**	**Size (GLD*)**
1) Non-toxic *Microcystis aeruginosa*	NPCD-1	Single cells	3.7 µm
2) Non-toxic *Microcystis aeruginosa*	NPCD-1	Colonial	60-100 µm
3) Non-toxic bloom of *Planktothrix* sp.	NB	Filamentous	100-1000 µm

*Greatest Linear Dimension

Statistical analysis

Data on golden mussel feeding were submitted to analyses of variance (One-way ANOVA) with Tukey's test for multiple comparison to detect significant differences (α= 0.05) of FRs, IRs and PPRs among food types. Tukey's test was performed after confirming the normality of data using Kolmogorov-Smirnov (KS) test (α= 0.05).

Results

FRs of golden mussel varied from 404.6 to 848 mL mussel^{-1} h^{-1}. The mean value was 606.6 mL mussel^{-1} h^{-1}. Mean FRs slightly varied from 562.1 on filamentous to 663.2 mL mussel^{-1} h^{-1} on colonial *Microcystis* ($p>0.05$; Figure 1). *L. fortunei* FRs in relation to body mass ranged from 6.8 to 26.1 mL mgDW^{-1} h^{-1} at a mean value of 14.8 mL mgDW^{-1} h^{-1}. There were no significant differences on mussel FRs among different types of food ($p>0.05$). Mean values barely varied from 13.7 mL mgDW^{-1} h^{-1} when mussel fed on filamentous *Planktothrix* to 16.2 mL mgDW^{-1} h^{-1} when it fed on colonial *Microcystis* (Figure 1).

IRs ranged from 7.1 to 610.5 mL mussel^{-1} h^{-1}. Golden mussel ingestion greatly varied among food types ($p<0.05$; Figure 1). Single cells of *Microcystis* were highly ingested, at a mean rate of 502.2 mL mussel^{-1} h^{-1}. Filamentous *Planktothrix* were less accepted than single cells, being ingested at a rate of 135.1 mL mussel^{-1} h^{-1}. Colonial *Microcystis* were scarcely ingested at a rate of 10.3 mL mussel^{-1} h^{-1}. In terms of body mass, IRs varied from 0.2 to 18.8 mL mgDW^{-1} h^{-1} and the mean value was 5.3 mL mgDW^{-1} h^{-1}. Single cells were preferentially ingested by golden mussel and the mean rate was 12.3 mL mgDW^{-1} h^{-1}, while colonies were ingested at a very low rate of 0.3 mL mgDW^{-1} h^{-1} ($p<0.05$; Figure 1). *L. fortunei* preferentially ingested single-celled and greatly rejected filamentous and colonial cyanobacteria.

PPRs varied from 42.7 to 838.7 mL mussel^{-1} h^{-1}. Golden mussel greatly rejected colonial *Microcystis* ($p<0.05$) at a mean rate of 652.9 mL mussel^{-1} h^{-1} (Figure 1). Filamentous cyanobacteria were rejected at a mean rate of 427 mL mussel^{-1} h^{-1}. Single-celled *Microcystis* was the food type less rejected as pseudofeces ($p<0.05$) at a mean rate of 92.4 mL mussel^{-1} h^{-1} (Figure 1). PPRs in body mass varied from 0.9 to 25.8 mL mgDW^{-}

^{1} h^{-1}. Colonial *Microcystis* were most rejected by golden mussel at a mean rate of 15.9 mL $mgDW^{-1}$ h^{-1}, while single-celled *Microcystis* was hardly rejected at a mean rate of 2.3 mL $mgDW^{-1}$ h^{-1} ($p<0.05$; Figure 1).

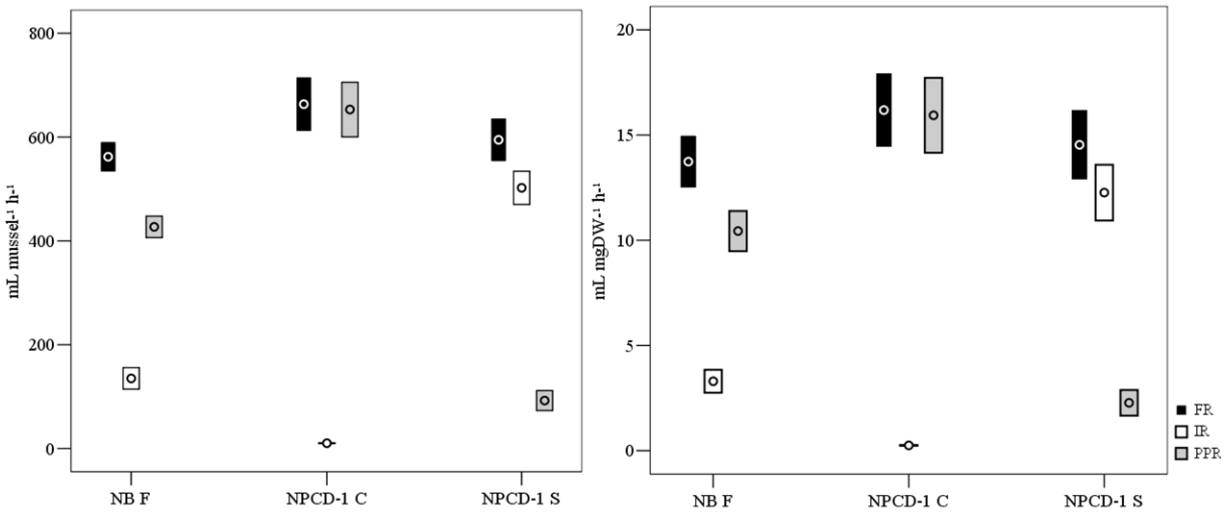

Figure 1: Filtration Rates (FR), Ingestion Rates (IR), and Pseudofeces Production Rates (PPR) of *L. fortunei* (mL $mussel^{-1}$ h^{-1} and mL $mgDW^{-1}$ h^{-1}) in the presence of filamentous, colonial, and single-celled cyanobacteria (symbol= mean, and bar= SE). Natural bloom of filamentous *Planktothrix* (NB F), colonial *M. aeruginosa* (NPCD-1 C), and single-celled *M. aeruginosa* (NPCD-1 S).

Golden mussel pseudofeces releasing ranged from 16 to 233 events h^{-1}, and the mean value was 106.6 events h^{-1}. Both colonial *Microcystis* and filamentous cyanobacteria were greatly rejected as pseudofeces ($p<0.05$; Figure 2). Golden mussel when fed with single-celled *Microcystis* expelled a very low quantity of pseudofeces when compared to colonial and filamentous ($p<0.05$; Figure 2). These results corroborate with the present data on PPRs. Feces releasing by golden mussel varied from 1 to 4 events h^{-1} and did not significantly changed among different types of food ($p>0.05$; Figure 2).

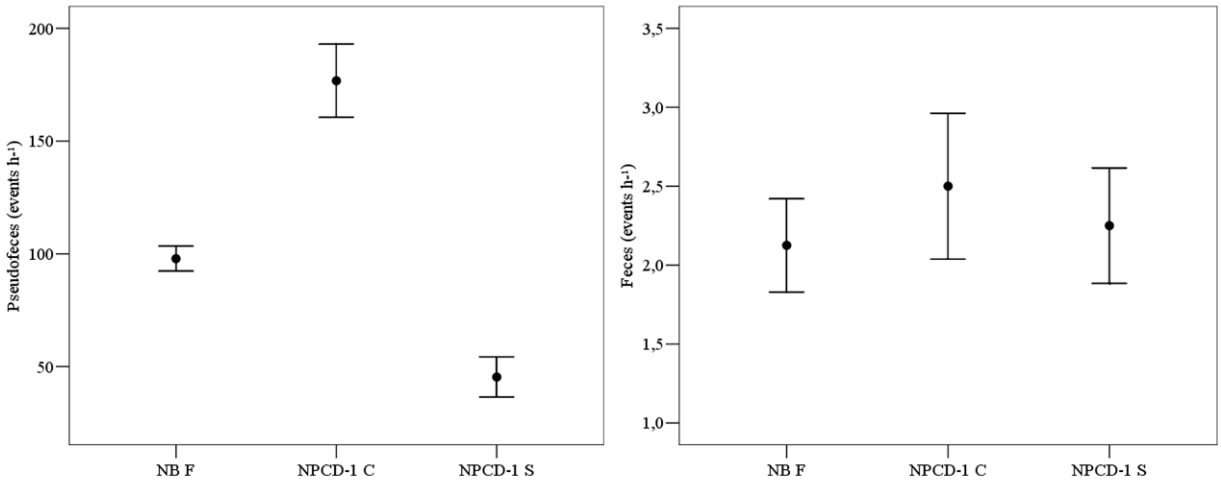

Figure 2: *L. fortunei* pseudofeces and feces (events h^{-1}) in the presence of filamentous, colonial, and single-celled cyanobacteria (symbol= mean, and bar= SE). Natural bloom of filamentous *Planktothrix* (NB F), colonial *M. aeruginosa* (NPCD-1 C), and single-celled *M. aeruginosa* (NPCD-1 S).

Discussion

FRs of golden mussel in the presence of single-celled, colonial, and filamentous food were similar. Despite that, there was a great difference between IRs and PPRs among food types. Single cells were widely accepted as food, while filamentous and colonial cyanobacteria were massively expelled as pseudofeces and barely ingested. The first hypothesis was accepted given that golden mussel prefers to ingest smaller particles, such as single cells of *Microcystis*. The second hypothesis was rejected, since filamentous were preferentially ingested than colonial cyanobacteria, although filamentous *Planktothrix* was highly rejected, as well as *Microcystis* colonies, when compared to single cells.

Some studies performed with other bivalves, especially *Dreissena*, corroborate with the present data indicating mussels preferentially ingest smaller particles. Vanderploeg et al. (2001) have shown zebra mussel predominantly ingested small colonies and single cells of *M. aeruginosa* from laboratory cultures, while *Microcystis* colonies from natural seston were mostly rejected. Baker et al. (2000) have revealed that zebra

mussel when fed with a mixture of *Scenedesmus* and *Microcystis* ingested preferentially cyanobacteria, while green algae were commonly incorporated into a mucus string and rejected as pseudofeces. Smaller particles, such as single cells of *Microcystis*, were ingested and larger ones, such as *Scenedesmus*, were rejected. Nadafi et al. (2007) studying the effects of *Dreissena* on natural seston have shown zebra mussel greatly expelled in pseudofeces large phytoplankton.

In experiments about the impact of *Dreissena* on natural seston from Hudson River, Bastviken et al. (1998) have found that FRs on different phytoplankton species were similar, even large filaments or colonies were cleared, nevertheless their rejection as pseudofeces varied greatly. They have also observed that zebra mussel ingested preferentially single-celled *Microcystis* while *Microcystis* colonies were immensely rejected as pseudofeces. Bastviken et al. (1998) data agree with the present results, similar FRs were registered on different sizes and shapes of food, whereas PPRs varied hugely.

In a previous work it was found that *L. fortunei* preferably ingested *Microcystis* cells, independently of being toxic or not, and highly expelled diatom *Nitzschia palea* as undesirable food (Gazulha et al., submitted). Low ingestion of *Nitzschia* was attributed to their larger cell volume in comparison to *Microcystis* as well as to stiff silicate frustules of diatoms which would not make them as a suitable food. Their findings are in agreement with the present results wherein larger particles, such as colonial and filamentous, were commonly expelled while small single cells were widely accepted by golden mussel.

There are few experiments focused on ingestion of filamentous algae by bivalves (Dionisio-Pires-Pires et al., 2005, Bontes et al., 2007). Bontes et al (2007) have studied feeding of bivalve *Anodonta anatina* on filamentous (*Planktothrix*) and colonial (*Microcystis*) cyanobacteria. They have concluded mussels can filter and ingest colonial as well as larger filamentous cyanobacteria independently of their toxicity. They have also

observed high pseudofeces production by *Anodonta* when fed with filamentous *Planktothrix* suggesting this species is not a very suitable food, which agrees with the present data.

In a feeding experiment with zebra mussel Dionisio-Pires et al. (2005) have registered high FRs on single-celled, colonial and filamentous cyanobacteria. However, it was not observed a significant difference on pseudofeces releasing among different cyanobacteria. Dionisio-Pires et al. (2005) results corroborate with the present data since it was not observed significant differences on FRs in the presence of different cyanobacteria sizes and shapes. Nevertheless, it disagrees with the present data since it was not found a rejection of filamentous and colonial cyanobacteria. The absence of differences on pseudofeces releasing observed by Dionisio-Pires et al. (2005) could be related to the method used to evaluate pseudofeces production. They have evaluated pseudofeces after the filtration time, though mussels actually keep releasing pseudofeces while are filtering, which could have led to an underestimation of excreted products.

There are many differences among methods used to estimate feeding of mussels, making it difficult to compare results. Several studies do not effectively separate filtered or cleared from ingested and rejected food making comparisons among works not always valid. In the present study, feeding activity of each *L. fortunei* specimen was carefully observed under a stereomicroscope. All feces and pseudofeces released during total filtration time have been captured and quantified, which makes data more accurate.

The selective feeding of golden mussel could promote the returning of non-selected food to sediments and favor species rejected that possibly remain alive in the bottom of systems, as suggested by Baker et al. (1998). The present results have shown feeding selection of golden mussel do not occur during clearing, but in the labial palps and gills after the filtration of particles. Some studies have registered that food particles have

remained alive after being expelled by mussels. Nadafi et al. (2007) have observed that colonial cyanobacteria *Gloeotrichia echinulata* were viable after rejection by zebra mussel and could return unharmed to the water column and migrate vertically due to their possession of gas vacuoles. Baker et al. (1998) have showed that pseudofeces from zebra mussel containing diatoms can resuspend under turbulent mixing during seasonal circulation and autumn turnover (Baker et al., 1998). They have also observed that algae from zebra mussel pseudofeces when cultured in laboratory continued to grow.

The impacts of mussels on trophic chains will probably be related to system mixing regime (Bastviken et al., 1998). In well mixed systems, phytoplankton would not be able to avoid contact with mussels. On the other hand, in systems with low mixing, phytoplankton would be able to avoid this contact by locomotion or buoyancy regulation. Thus, mussel impact on plankton community will be dependent on the ecosystem mixing regime among other indirect factors, such as light and nutrients (Bastviken et al., 1998).

L. fortunei is an efficient filter-feeding indicating its potential in removing particles from water column and causing great changes on trophic structure of food chains. The presence of golden mussel may result in a dominance of large phytoplankton (colonies and filaments) over small phytoplankton in ecosystems where this invasive bivalve occurs in massive densities.

Acknowledegments

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ARTIGO III

4. ARTIGO III.

EFFECTS OF TOXIC CYANOBACTERIA *MICROCYSTIS AERUGINOSA* ON FILTRATION AND SURVIVAL OF GOLDEN MUSSEL LARVAE

Vanessa Gazulha[1]*, Maria Cristina Dreher Mansur[2], Luiz Fernando Cybis[1], Sandra Maria Feliciano de Oliveira e Azevedo[3]

[1] *Institute of Hydraulic Research, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.*

[2] *Center of Ecology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.*

[3]*Laboratory of Ecophysiology and Toxicology of Cyanobacteria, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.*

* Author for correspondence: vanessagazulha@gmail.com

Keywords: bivalve, *Limnoperna fortunei*, invasive species, green algae, natural seston, microcystins.

Abextract

The aim of this study was to assess filtration and survival of golden mussel larvae in the presence of toxic and non-toxic cyanobacteria, and non-toxic phytoplankton. Two hypotheses were tested: first, that cyanobacteria toxicity affects larvae survival; second, that toxic cyanobacteria have a negative effect on filtration rates leading larvae to filter less efficiently. The present study is the first to estimate filtration rates of *L. fortunei* larvae, as well as to evaluate the effects of toxic cyanobacteria on their feeding and survival. High survival of larvae was registered in the presence of green algae and natural seston during all experiment. After four days of exposure, larvae survival decreased in the presence of toxic *Microcystis*, followed by toxic *Microcystis* extract + natural seston, and non-toxic *Microcystis*. Low survival of larvae might have been influenced by food toxicity and also by the food quality due to high mortality observed in all treatments containing cyanobacteria, including the non-toxic strain. Low survival in treatments containing cyanobacteria could be related to their low lipid concentration causing a nutritional deficiency to larvae. Golden mussel larvae ingested *Monoraphidium* as well as non-toxic and toxic *Microcystis* at similar filtration rates. It indicates cyanobacteria toxins had no effect on filtration activity of *L. fortunei* possibly relating to larvae incapability to detect food toxicity.

Introduction

Cyanobacteria blooms are becoming increasingly common in freshwater ecosystems in the world. Major problems are related to the production of toxins by some species. Cyanobacteria toxins can cause poisoning, and even death by ingestion of contaminated water in animals and humans (Carmichael et al., 2001). Several studies have shown that these toxins affect aquatic communities mainly zooplankton (Lampert, 1982; DeMott, 1999). Cyanobacteria toxicity may decrease reproduction, feeding rates, as well as survival of zooplankton (Ferrão-Filho & Azevedo, 2003).

The effects of cyanobacteria on zooplankton are not fully understood. The negative effects of cyanobacteria may be related to their toxicity or shape (Ferrão-Filho & Azevedo, 2003). Some filter-feeding zooplankton, such as copepods, are able to differentiate toxic and non-toxic food, making them less susceptible to cyanobacteria toxicity (DeMott & Moxter 1991). The small-bodied cladocerans are not able to ingest large colonies or filaments, which allows them to coexist with toxic cyanobacteria (Kirk & Gilbert, 1992).

Cyanobacteria toxins could also have a negative effect on bivalve larvae. Nevertheless, the effects of cyanobacteria on bivalve larvae were barely studied. Dionisio-Pires Pires et al. (2003) have estimated filtration rates of zebra mussel larvae, *Dreissena polymorpha* (Pallas, 1771), under exposure to toxic cyanobacteria. Zebra mussel larvae were fed with green algae *Chlamydomonas reinhardtii*, toxic and non-toxic strains of the cyanobacteria *Microcystis aeruginosa*. Filtration rates on the non-toxic strain were significantly higher than on the toxic strain, suggesting cyanobacteria toxicity affected negatively the feeding of larvae. The size of food particles is also a factor influencing filtration rates of bivalve larvae. In laboratory studies on feeding behavior of *D. polymorpha*, Sprung (1989) noted that only particles of 1-4 μm (diameter) have served as

food for larvae. Further studies reported that bivalve larvae also ingest larger particles (>l0 μm) (Baldwin & Newel1 1991) and as they grow, they can feed on a wider size range of food particles (Baldwin 1995).

The golden mussel *Limnoperna fortunei* (Dunker, 1857) (Bivalvia, Mytilidae) is an invasive bivalve in South America. It was first recorded in Argentina in 1991 (Pastorino et al., 1993) and seven years later in southern Brazil, especially in Guaíba Lake (Mansur et al., 2003). This invasive species is spreading quickly in South America due to its fast growth rates, high filtration capacity, and planktonic larval stage (Ricciardi, 1998). Golden mussel larvae feed on suspension material as well as other bivalve larvae, and then are susceptible to possible effects of cyanobacteria toxins on their feeding and survival.

So far, the studies about *L. fortunei* larvae focused only aspects related to the development time of each larval stage and morphological description of larval stages. Choi & Shin (1985) and Choi & Kim (1985) described different larval stages for the first time in populations from Korea. Santos et al. (2005) have studied larval stages of *L. fortunei* in populations from Guaíba Lake, southern Brazil.

The aim of this study was to assess filtration rates and survival of golden mussel larvae (*L. fortunei*) in the presence of toxic and non-toxic cyanobacteria (*M. aeruginosa*), and non-toxic phytoplankton (green algae *Monoraphidium* sp. and natural seston). Two hypotheses were tested: first, that cyanobacteria toxicity affects larvae survival; second, that toxic cyanobacteria have a negative effect on filtration rates leading larvae to filter less efficiently. The present study is the first to estimate filtration rates of *L. fortunei* larvae, as well as to evaluate the effects of toxic cyanobacteria on their feeding and survival.

Material and Methods

L. fortunei larvae sampling

L. fortunei larvae were sampled in Guaíba Lake, by filtering about 1 m^3 of water in a 30-µm plankton net. In the laboratory, larvae were separated under observation on a stereomicroscope in order to be used in the experiments. Larvae were selected at the veliger stage, in which the ciliated velum responsible by feeding and locomotion is formed and developed (Figure 1).

Figure 1: *Limnoperna fortunei* larvae (length 150-200 µm) at the veliger stage sampled in Guaíba Lake, southern Brazil. V= velum; S= shell.

Cyanobacteria and phytoplankton

Toxic (NPLJ-4) and non-toxic (NPCD-1) strains of *Microcystis aeruginosa*, and non-toxic green algae *Monoraphidium* sp. (MONO) were provided by the Laboratory of Toxicology and Ecophysiology of Cyanobacteria from the Federal University of Rio de Janeiro, Brazil and cultivated in ASM-1 growth medium. 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*

and natural seston from Guaíba Lake were performed using an ELISA assay test kit Beacon.

Survival of L. fortunei larvae

Survival experiment of *L. fortunei* larvae was carried out in the presence of 5 food strategies (Table I) with 5 replicates each. Food strategies were: 1) MONO (mineral water + *Monoraphidium* + larvae), 2) NPCD-1 (mineral water + non-toxic *M. aeruginosa* + larvae), 3) NPLJ-4 (mineral water + toxic *M. aeruginosa* + larvae), 4) NS (Natural Seston <60µm + larvae), and 5) NPLJ-4 + NS (toxic *M. aeruginosa* crude extract + Natural Seston <60µm + larvae). Crude extract of NPLJ-4 was obtained by a sequence of at least three freeze-thaw cycles. The experiment was carried out in Erlenmeyers containing water, food suspension, and approximately 70 mussel larvae by replicate at final volume of 50 mL and initial food concentration of 2 mm^3 L^{-1}. The flasks were kept on a shaker at 30 rpm in acclimatized room (24°C).

Table I: Food strategies (species, strain, size and MCYST) used in *L. fortunei* larvae survival experiment.

Species	**Strain**	**Size (GLD*)**	**µg MCYST L^{-1}**
1) non-toxic *Monoraphidium* sp.	MONO	26.4 µm	-
2) non-toxic *Microcystis aeruginosa*	NPCD-1	3.7 µm	-
3) toxic *Microcystis aeruginosa*	NPLJ-4	3.7 µm	2.0 **
4) non-toxic Natural Seston	NS	< 60 µm	-
5) toxic *Microcystis* crude extract + Natural Seston	NPLJ-4 + NS	< 60 µm	2.5 ***

* Greatest Linear Dimension; ** intracellular toxin; *** free-toxin.

The effects of toxic and non-toxic food on larvae were checked out in each replicate every 24 hours during 96 hours. Live and dead larvae were counted. Live larvae were transferred to flasks containing fresh food suspension to avoid possible effects related to

starvation. Larvae lethality criteria were lack of locomotion, of opening and closing of valves, and of crystalline style rotation.

Feeding of L. fortunei larvae

Golden mussel feeding was estimated for the following food strategies from survival experiment: 1) MONO (mineral water + *Monoraphidium* + larvae), 2) NPCD-1 (mineral water + non-toxic *M. aeruginosa* + larvae), and 3) NPLJ-4 (mineral water + toxic *M. aeruginosa* + larvae) (Table I), with 5 replicates each. Flasks under same food strategies were prepared, but without larvae, to assess possible phytoplanktonic growth during filtration time.

Filtration rates (FR) or clearance rates (CR) are 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. In the present study, the water was stirred to get the pseudofeces resuspended prior sampling for final food concentration, therefore FR equaled IR. Filtration rates (FRs) of golden mussel larvae were estimated using the following equation based on Coughlan (1969):

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

where FR is the filtration rate (µL larva^{-1} h^{-1}), V is the volume of water in the experimental chamber (µL), N is the number of larvae per chamber, T is the total filtration time (h) which was of 24 hours in this experiment, C_0 is the food concentration (mm³ L^{-1}) at T= 0, C_t is the food concentration at time T in flasks with larvae, C'_0 is the food

concentration in the control flask (without larvae) at T= 0 and the C'_t is the food concentration in the control flask at time T. Food concentration before and after filtration ($mm^3\ L^{-1}$) was estimated by Sedgewick-Rafter chamber counting. Samples were preserved in 1% Lugol solution. Food concentration (mm^3) was calculated according to Hillebrand et al. (1999).

Statistical analyses

Data on golden mussel larvae were submitted to analyses of variance (One-way ANOVA) with Tukey's test for multiple comparison to detect significant differences (α= 0.05) on filtration rates and survival of larvae among food types used in feeding and survival experiments. Tukey's test was employed after confirming the normality of data using Kolmogorov-Smirnov (KS) test (α= 0.05).

Results

Survival of L. fortunei larvae

There were no significant differences in larvae survival during the first 72h in all performed treatments ($p>0.05$, ANOVA; Figure 2). After this period, there was a great decrease in larvae survival in the presence of toxic *Microcystis* (8,1%; $p<0.05$, ANOVA) and natural seston containing toxic extract of *Microcystis* (12,6%; $p<0.05$, ANOVA). Non-toxic *Microcystis* have also promoted a significant decline in the survival of larvae (23%; $p<0.05$, ANOVA). Survival of golden mussel larvae was significantly high (>80%; $p<0.05$,

ANOVA) in the presence of natural seston and green algae *Monoraphidium* during all experiment (Figure 2).

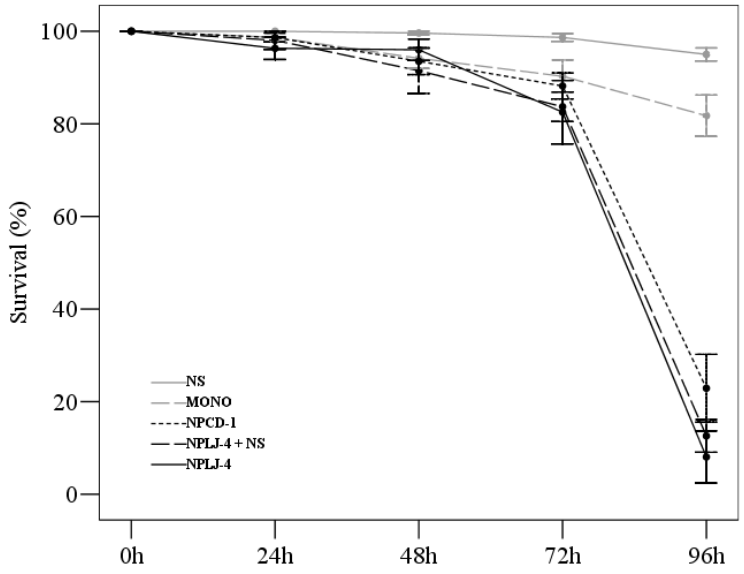

Figure 2: Survival (%) of *L. fortunei* larvae in the presence of non-toxic *Monoraphidium* (MONO), non-toxic *Microcystis* (NPCD-1), toxic *Microcystis* (NPLJ-4), Natural Seston (NS), and toxic *Microcystis* extract + Natural Seston (NPLJ-4 + NS) (symbol= mean, and bar= SE).

Feeding of L. fortunei larvae

FRs of golden mussel varied from 9.9 to 44.5 µL $larva^{-1}$ h^{-1} (Figure 3). Highest FRs were registered in the presence of non-toxic *Monoraphidium*, with a mean value of 28.2 µL $larva^{-1}$ h^{-1}. Mean FRs on toxic and non-toxic *Microcystis* were of 21.2 and 24.7 µL $larva^{-1}$ h^{-1}, respectively. Larvae FRs were higher on green algae than on toxic and non-toxic cyanobacteria, although differences were not significant ($p>0.05$, ANOVA), indicating there was no negative effect of cyanobacteria toxins on larvae feeding. It was not observed a significant decrease or increase of larvae FRs in different treatments during the 4 days of experiment ($p>0.05$, ANOVA).

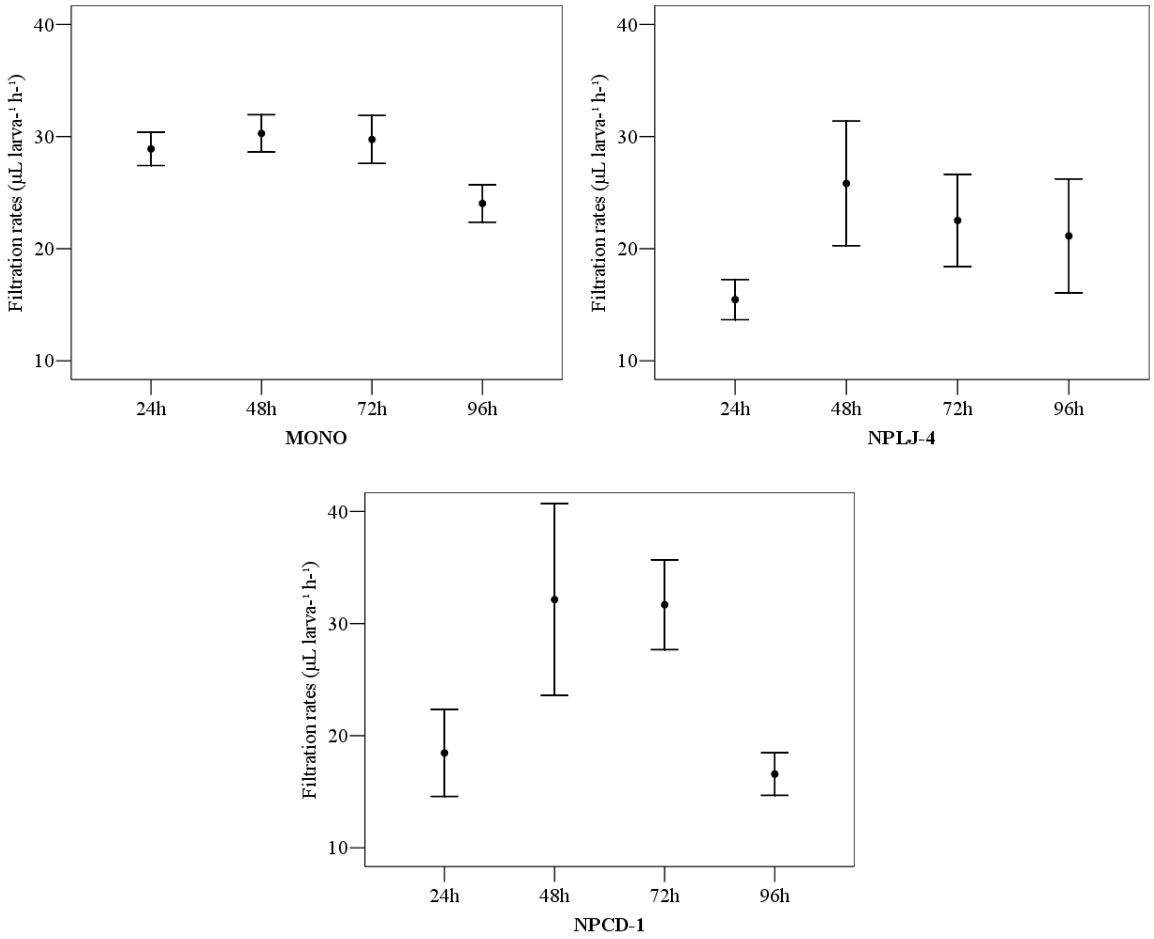

Figure 3: Filtration rates of *Limnoperna fortunei* larvae on non-toxic *Monoraphidium* (MONO), toxic *Microcystis* (NPLJ-4) and non-toxic *Microcystis* (NPCD-1) (symbol= mean, and bar= SE).

Discussion

Survival

High survival of golden mussel larvae was registered in the presence of green algae and natural seston during all experiment. Studies have shown that cladocerans have better performance feeding on natural seston and green algae than feeding on cyanobacteria (Ferrão-Filho & Azevedo, 2003), which corroborates with the present results. After four

days of exposure, survival of golden mussel decreased in the presence of toxic *Microcystis*, followed by toxic *Microcystis* extract + natural seston, and non-toxic *Microcystis*.

Boltovskoy et al. (2009) have registered a decrease in larvae (*L. fortunei*) production in mid-summer period coincident with a *Microcystis* bloom. This decrease of reproduction in summer is not commom according to the authors. They have found that larvae production decreased mainly with the decrease of temperature in winter; whereas the decrease in summer was attributed to the toxicity of *Microcystis* bloom in the studied reservoir.

Present data on feeding and survival indicated golden mussel larvae were not able to identify and then avoid toxic food. Filtration rates have shown *L. fortunei* larvae were ingesting both toxic and non-toxic cyanobacteria. Thus, indicating low survival in cyanobacteria presence was not related to a possible starvation or a decrease of filtration activity. In the presence of cyanobacteria, adult golden mussel has also grazed on *Microcystis* cells (toxic and non-toxic strains) independently of their toxicity (Gazulha et al., submitted). Both larvae and adult golden mussel have grazed on toxic *Microcystis*.

Although, cyanobacteria were lethal to larvae, it did not have a negative effect on adult survival even after 5 days of exposure (Gazulha et al., submitted); therefore, suggesting *L. fortunei* adults are more resistant than larvae. Dionisio-Pires Pires et al. (2003) have observed zebra mussel larvae are more sensitive to food type (quality and toxicity) than adults, which agrees with the present results. Dionisio-Pires Pires et al. (2003) registered a significantly higher survival when zebra mussel larvae fed on non-toxic compared to toxic *Microcystis* in the first day of exposure. After two days, survival was low in both toxic and non-toxic *Microcystis*, which was attributed to methodological difficulties in raising *Dreissena* larvae. However, it could be an indicative the low quality of food had also affected larvae survival.

Low survival of *L. fortunei* larvae in treatments containing cyanobacteria might be influenced by two factors: 1) food toxicity due to high larvae mortality registered in the presence of toxic food (NPLJ-4 and NS+NPLJ-4); 2) food quality due to high mortality observed in all treatments containing cyanobacteria, including the non-toxic strain (NPCD-1). Cyanobacteria negative effects were also observed for cladocerans because of their low nutritional quality. Negative effects in the reproduction of *Ceriodaphnia cornuta* were attributed to low nutritive value of *Microcystis* for this cladoceran as it was observed by Ferrão-Filho & Azevedo (2003). Several studies have shown 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 (Vanderploeg et al., 1996, Naddafi et al., 2007). Cyanobacteria contain no or little EPA and DHA (Naddafi et al., 2007), which makes them low quality food.

Dionisio-Pires Pires et al. (2003) have observed a survival decline of zebra mussel larvae in the presence of both toxic and non-toxic *Microcystis*, which was attributed to their lack of essential fatty acids. It corroborates with the present study, where it was observed low survival in different treatments containing cyanobacteria could be related to low lipids concentration of this group, causing a nutritional deficiency to larvae. Based on the present results, the hypothesis of cyanobacteria toxicity affect golden mussel survival has been accepted. Despite that, food nutritional quality was the main factor influencing larvae survival in the present experimental conditions.

Most of bivalve larvae researches have focused on filtration rates (FRs) and particle selection mechanisms (Crisp et al., 1985; MacIsaac et al., 1992; Gallager et al., 1994). Studies about the effects of toxic cyanobacteria to bivalve larvae are scarce (Dionisio-Pires Pires et al., 2003). However, there are some studies about toxic effects of cyanobacteria on zooplankton (DeMott & Moxter, 1991; Kirk & Gilbert, 1992; Ferrão-Filho & Azevedo,

2003). Watanabe et al. (1992) showed that cladoceran *Bosmina fatalis* ingested *Microcystis* despite their toxicity, and cladoceran *Diaphanosoma* and copepod *Cyclops vicinus* did not ingest *Microcystis*. Some zooplankton species are able to identify food particle toxicity and avoid them, while others ingest toxic food becoming a risk of transferring toxins to higher trophic levels. It was observed in the present work that golden mussel larvae were not able to avoid toxic cyanobacteria.

Feeding

Filtration rates of bivalve larvae have been estimated in several studies (Sprung, 1985; MacIsaac et al., 1992; Gallager et al., 1994; Tomaru et al., 2000). The present study is the first to estimate FRs of *L. fortunei* larvae. Riisgard et al. (1981) have found FRs of marine bivalve *Mytilus edulis* feeding on marine phytoplankton *Isochrysis galbana* ranging from 10 to 100 μL $larva^{-1}$ h^{-1}. Crisp et al. (1985) have shown that oyster *Ostrea edulis* FRs on *I. galbana* varied from 8 to 16 μL $larva^{-1}$ h^{-1}, and on green algae *Nannochlorus* sp. from 3 to 11 μL $larva^{-1}$ h^{-1}. Marine bivalve *Mercenaria mercenaria* feeding on cyanobacterium *Synechococcus* sp. presented FRs from 2 to 23 μL $larva^{-1}$ h^{-1} (Gallager et al., 1994). Japanese pearl oyster *Pinctada fucata martensii* FRs feeding on algal picoplankton have ranged from 0.12 to 1.5 μL $larva^{-1}$ h^{-1} (Tomaru et al., 2000). FRs of *D. polymorpha* fed with cyanobacteria and green algae have ranged from 0.05 to 0.28 μL $larva^{-1}$ h^{-1} (Dionisio-Pires Pires et al., 2003). MacIsaac et al. (1992) have compared filtration rates of bivalve *D. polymorpha* from different studies and shown that mean FRs ranged from 10.3 to 17.5 μL $larva^{-1}$ h^{-1}. FRs for golden mussel ranged from 9.9 to 44.5 μL $larva^{-1}$ h^{-1}. Golden mussel showed higher FRs compared to values registered to the invasive *D. polymorpha*.

There is evidently a great variation among FRs of bivalve larvae. This variation can be related to different conditions and methods used in experiments, mollusk species, larvae size, food type**,** and intraspecific disparities. These factors implicate in a great variability in FR results, making it difficult to compare different works. FRs of golden mussel also presented high variation in agreement with most of bivalve larvae studies. FRs registered to *L. fortunei* larvae are in the range of variation observed for other bivalves.

Golden mussel larvae ingested *Monoraphidium* as well as toxic and non-toxic *Microcystis* at similar filtration rates. It indicates cyanobacteria toxins had no effect on filtration activity of *L. fortunei* possibly due to larvae incapability to detect food toxicity. Dionisio-Pires Pires et al. (2003) have found that FRs of zebra mussel larvae on non-toxic *Microcystis* were higher than on toxic *Microcystis*, although it was also observed that FRs on both *Microcystis* strains were not significantly different from FRs on *Chlamydomonas*. The present study did not corroborate with Dionisio-Pires Pires et al. (2003), which may be an indicative that golden mussel larvae are less sensitive to cyanobacteria toxicity. Therefore, the hypothesis that toxic cyanobacteria have a negative effect on filtration rates leading larvae to filter less efficiently has been rejected.

Acknowledegments

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5. CONSIDERAÇÕES FINAIS

Os efeitos das cianobactérias tóxicas sobre a alimentação e sobrevivência de *L. fortunei* foram avaliados no presente estudo na presença de células intactas de *Microcystis*. Os resultados demonstraram que as cianobactérias tóxicas não exerceram efeito negativo na alimentação e sobrevivência dos indivíduos adultos do mexilhão dourado. Recomenda-se em trabalhos futuros a exposição de indivíduos adultos do mexilhão dourado a extratos de cianotoxinas para verificar a ocorrência de possíveis efeitos tóxicos das toxinas livres na alimentação e sobrevivência do bivalve invasor. O decaimento de florações tóxicas nos ecossistemas naturais seria simulado em testes nestas condições, podendo gerar resultados interessantes a respeito dos efeitos das cianotoxinas no mexilhão dourado e possíveis mecanismos de sobrevivência do bivalve em exposição a estas toxinas.

Os indivíduos adultos de *L. fortunei* foram capazes de se alimentar e sobreviver na presença da cianobactéria tóxica *Microcystis aeruginosa*. Estes resultados demonstram o potencial deste bivalve invasor como vetor para a transferência das cianotoxinas para os níveis tróficos superiores, aumentando o risco de bioacumulação destas nos ecossistemas aquáticos. Além disso, o mexilhão dourado vem sendo registrado entre os principais itens alimentares de diversas espécies de peixes do Rio da Prata, inclusive espécies utilizadas para consumo humano (Garcia & Protogino, 2005), evidenciando a possibilidade de bioacumulação de toxinas na cadeia trófica.

Os impactos do mexilhão dourado sobre as comunidades planctônicas podem ser elevados, visto que este bivalve invasor ocorre em densidades massivas nos ecossistemas invadidos e apresenta elevadas taxas de filtração em comparação com outros bivalves invasores de água doce. Além disso, como demonstrado no presente estudo, *L. fortunei* tem a capacidade de selecionar as partículas alimentares, e desta forma, promover a dominância

de determinadas espécies do plâncton nos ecossistemas aquáticos. A presença do mexilhão dourado poderia levar a uma redução de células solitárias de cianobactérias (*Microcystis*), e favorecer o aumento em densidade de cianobactéiras coloniais (*Microcystis*) e filamentosas (*Planktothrix*), e de diatomáceas (*Nitzschia*). O mexilhão dourado apresenta potencial para remover células tóxicas de cianobactérias (*Microcystis*), entretanto, este potencial ficaria reduzido em eventos de floração, onde as formas coloniais, preferencialmente rejeitadas por *L. fortunei*, são predominantes. Neste caso, a presença do bivalve no ambiente poderia ainda potencializar a ocorrência da floração via rejeição das cianobactérias coloniais nas pseudofezes.

As larvas demonstraram maior sensibilidade à natureza da partícula em comparação com os adultos de *L. fortunei*. As cianobactérias não tiveram efeito negativo nas taxas de filtração das larvas, indicando a incapacidade destas de detectar e evitar as partículas tóxicas. Entretanto, ocorreu um aumento significativo da mortalidade larval na presença das cianobactérias tóxicas e não-tóxicas em longo prazo (96h). Estes resultados demonstram que além da toxicidade da partícula alimentar, a qualidade desta pode ser um fator importante na seletividade alimentar das larvas. O predomínio das cianobactérias no ambiente poderia ter como conseqüência o aumento da mortalidade das larvas de *L. fortunei*.

No presente estudo, foram abordados apectos relacionados à seletividade alimentar de *L. fortunei* na presença de cianobactérias tóxicas e não-tóxicas, e fitoplâncton não-tóxico. Algumas questões foram esclarecidas, entretanto ainda existe uma lacuna no entendimento dos mecanismos de seleção de partículas do mexilhão dourado, especialmente no que diz respeito à qualidade do alimento, como a seleção de partículas orgânicas ou inorgânicas, a capacidade de ingestão e a eficiência de digestão. Além disso, estudos sobre os efeitos da filtração do mexilhão dourado na estrutura trófica dos

ecossistemas são raros. A continuidade de estudos desta natureza é de grande importância no sentido de se compreender a interação existente entre esta espécie invasora, o plâncton e o bentos nos ecossistemas invadidos.

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