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DOI: 10.4081/jlimnol.2016.1465

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Effect of starvation and subsequent feeding on glycogen concentration, behavior and mortality in the golden mussel *Limnoperna fortunei* (Dunker, 1857) (Bivalvia: Mytilidae)

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ABSTRACT

The success of *Limnoperna fortunei* as an invasive species is related to its physiological plasticity that allows them to endure adverse environmental conditions. Starvation tolerance is considered to be an important trait associated with bivalve invasiveness. In natural ecosystems, food resources can vary during the year, exposing mussels to variable periods of starvation or limited food availability. Thus, mussels have developed physiological strategies to tolerate fluctuations in food availability. Glycogen concentration has been used in different monitoring studies as an indicator of the nutritional condition of bivalves. The aim of this study was to investigate the physiological responses of *L. fortunei* based on the glycogen concentrations of specimens under four treatments, comprising different combinations of feeding and starvation, during 125 days. The experiment was carried out in two phases. In the phase I, mussels were divided in two treatments: starvation (S) and feeding (F). After 100 days, tissue samples were collected to quantify glycogen concentrations and, each phase I group was divided in two subgroups: starvation (S) and feeding (F), resulting in four treatments. In the phase II, that lasted 25 days, starvation specimens (S) from phase I were allowed to feed (starvation-feeding treatment, or S-F), or continued to undergo starvation (starvation-starvation treatment, or S-S) and the feeding specimens (F) continued feeding (feeding-feeding group, or F-F), or were subjected to starvation (feeding-starvation treatment, or F-S). Behavior (valve-closing) and mortality were recorded in 24 h intervals. After the 25 days (phase II) all specimens were killed, and their soft tissue was removed to quantify glycogen concentrations. The glycogen concentration of the S-F treatment was lower than that of the F-S treatment, which was initially allowed to feed (phase I) and then subjected to starvation (phase II). Stability in the glycogen concentrations was observed when the phase II feeding conditions were maintained during the experiments, as observed in the S-S (continued starvation) and F-F (continued feeding) treatments. Based on our glycogen concentrations results, the golden mussel shows a higher tolerance to starvation (125 days) than has previously been published, which suggests that its tolerance strongly influences its invasive behavior.

Key words: Bioinvasion; glycogen; golden mussel; starvation; comparative physiology.

Received: March 2016. Accepted: July 2016.

INTRODUCTION

Limnoperna fortunei (Dunker, 1857), the golden mussel, was first detected in Argentina at the Río de la Plata estuary (Darrigran and Pastorino, 1995), and it spread upstream of the Prata basin to invade five South American countries (Argentina, Paraguay, Brazil, Uruguay and Bolivia). Its macrofouling capacity makes the golden mussel an aggressive invasive species that has detrimental impacts on aquatic ecosystems, electrical power stations and water treatments plants (Darrigran, 2010; Rolla and Mota, 2010). Additionally, Darrigran and Damborenea (2011)

observed that *L. fortunei* is a very effective ecosystem engineer.

The success of *L. fortunei* as an invasive species is also related with its behavior and physiological tolerance for a wide range of ecological conditions. It can tolerate a wide range of temperatures and salinity and long periods of exposure to air, and it can survive in water up to 40 meters deep and in freshwater habitats with limited food availability (Oliveira *et al.* 2010; Darrigran *et al.*, 2011). Oliveira *et al.* (2010) suggested that freshwater habitats with limited food availability and suspended sediments can impair the filtration rates of the golden mussel and may

limit its abundance but does not preclude its establishment. In fact, starvation tolerance can be an important trait associated with the invasive ability of macrofouling bivalves such as *L. fortunei* (Chase and McMahon, 1995; Oliveira *et al.*, 2010). Starvation tolerance in the golden mussel has been observed over 63 days by Silva (2006), and the specimens that were not fed showed no weight loss. Starvation tolerance has also been observed in other invasive bivalves, such as the freshwater zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Bivalvia: Dreissenidae) and the marine blue mussel *Mytilus edulis* Linnaeus, 1758 (Bivalvia: Mytilidae) (Chase and McMahon 1995).

Limnoperna fortunei feed on phytoplankton, zooplankton and suspended organic particles by filtering water through their gills (Sylvester *et al.*, 2009). In natural ecosystems, the food resources can vary during the year, exposing mussels to short or long periods of starvation (seasonal variation) or limited food availability (Albentosa *et al.*, 2007). Thus, mussels have developed physiological strategies to survive these fluctuations in food availability (Oxford and Fish, 1979). In general, filter-feeding bivalves reduce their filtration rate, by closing their valves, when exposed to low food concentration, demonstrating that closing and gapping the valves, in this case, is a response to food availability (Riisgård and Larsen, 2015). In addition, valve closure promotes an immediate reduction in oxygen consumption (Famme, 1980), and the mussels save energy during stressful periods of starvation by reducing their valve-gap (Riisgård and Larsen, 2015). Valve movement is one the most important bivalve behaviors and has been monitored in experiments involving chemical (Di Fiori *et al.*, 2012; Montresor *et al.*, 2013) and nutritional stressors, such as limited food availability and starvation, under laboratory conditions (Famme, 1980; Higgins, 1980; Riisgård and Larsen, 2015).

The usual response to nutritive stress (starvation) in bivalves is to decrease metabolic rate to a minimum value (basal metabolism), which is maintained throughout the starvation period (Albentosa *et al.*, 2007). However, the energy needed to maintain vital functions in bivalves during periods of starvation or limited food availability differs according to species, sex and life stage (Albentosa *et al.*, 2007; da Costa *et al.*, 2012). The ability to store energetic substrates, such as glycogen and other carbohydrates, is also a critical trait that confers resistance to several stressors, such as temperature changes and transportation (Widdows and Bayne, 1971; Chen *et al.*, 2001). Da Costa *et al.* (2012) showed that during starvation experiments with larvae of the bivalve *Solen marginatus* (Pulteney, 1758) (Veneroida: Solenidae) there is a *point-of-no-return* that denotes the threshold above which larvae can no longer develop or survive even if food is provided again. In their study, after eight days of starvation, food was provided. However, three days later, all larvae died.

The monitoring of the nutritional condition of mussels has been performed by analyzing glycogen concentration (Chen *et al.*, 2001). According to Patterson *et al.* (1999), glycogen is the primary energy source stored by bivalves and is a good physiological parameter for assessing the health status of mussels (Patterson *et al.*, 1999; Anacleto *et al.*, 2013). Glycogen concentrations have been used in different monitoring studies, such as those conducted over the entire life cycle of bivalves in the field, during starvation under laboratory conditions, during relocation events, after transport *via* air and water (Cordeiro, *unpublished data*), and during and after the application of chemical or physical stressors (Thompson *et al.*, 1978; Hummel *et al.*, 1989; Patterson *et al.*, 1999; Chen *et al.*, 2001; Newton *et al.*, 2001; Almeida *et al.*, 2007; Patrick *et al.*, 2006; Yusufzai *et al.*, 2010; Anacleto *et al.*, 2013). According to Zwaan and Zandee (1972), *M. edulis* presented seasonally weight variations that were similar to changes in its glycogen concentrations, thus suggesting that the bivalve's weight is correlated with the accumulation and metabolic breakdown of glycogen. Because physiological stresses demand high consumption, the organism's metabolic rate increases and glycogen is quickly degraded and released, providing more energy to maintain vital processes (Mello-Silva *et al.*, 2011). Stressful situations, such as acute or chronic experiments, air exposure (Hummel *et al.*, 1989), starvation (Pinheiro, 1996), photoperiod changes, high population densities and parasitism (Pinheiro and Amato, 1994), can directly promote glycogen depletion in mollusks and, thereby, predispose the use of proteins and lipids as alternative sources of energy (Nandurkar and Zambare, 2012). However, the use of these alternative sources of energy (*e.g.*, protein catabolism) increases the production of nitrogenous wastes such as ammonia, which is a toxic substance that mussels normally excrete through their gills into the hemolymph fluid and tissues, that could contribute to the organism's stress and have toxic effects (Thompson *et al.*, 1978; Chen *et al.*, 2001).

Thus, according to Chase and McMahon (1995) and to Oliveira *et al.* (2010), starvation tolerance is considered an important trait for invasive species and is likely associated with bivalves' invasiveness. Thus, considering the ecological importance of this trait, little attention has been given to starvation tolerance and feeding studies in invasive bivalves, such as *L. fortunei*, in natural ecosystems and/or under laboratory conditions. Furthermore, it is important to conduct different biological studies to increase our knowledge about the golden mussel and improve macrofouling control strategies (Darrigran, 2010; Mansur *et al.*, 2012; Boltovskoy, 2015). In the present study, to investigate the tolerance of *L. fortunei* to starvation, specimens were subjected to different combinations of feeding and starvation, and several responses were analyzed: i) glycogen concentration in the tissue; ii) behavior (valve-

closing); and iii) mortality. This study provides insights that improve our knowledge about the starvation and feeding of *L. fortunei* under laboratory conditions. The data presented here may contribute i) to the development of rearing protocols aiming to provide good quality animals for laboratory tests; ii) to better understand mechanisms related to their highly successful invasion strategies; iii) to improve prediction models of this species expansion.

METHODS

Specimens of *L. fortunei* were manually and carefully collected from a hard substrate, avoiding injury to the animals, at Refúgio Bela Vista of Itaipu's hydroelectric power plant (Paraná River), Foz do Iguaçu City, Paraná state, Brazil (25°26'48.9"S; 54°32'58.1"W). The specimens were manually and carefully washed using local water (at 25.01±1.67°C) to remove organic materials. Subsequently, some specimens had their soft tissue removed, stored and maintained at -20°C for later quantification of the glycogen concentration (see details in the *Glycogen concentration* section) at the laboratory (0.15±0.07 mg of glucose / g of tissue). This procedure was performed to evaluate the glycogen concentrations of *L. fortunei* specimens in their natural conditions and for comparison with specimens maintained under laboratory conditions.

The sampled specimens were packed in plastic bags that included 3 kg of mussels each without water (air transport). Subsequently, each bag was filled with O₂ using an air compressor, closed and kept in a transport box. The thermally insulated boxes (24 L each) were previously cooled and maintained at a low internal temperature (approximately 15 to 17°C) using iced gel during transport. Subsequently, the boxes were transported with the live specimens to the Laboratório de Estudos de *Limnoperna fortunei* (LELf) at Centro de Pesquisas Hidráulicas e Recursos Hídricos (CPH) of Universidade Federal de Minas Gerais (UFMG), Belo Horizonte city, Minas Gerais state (19°52'29.61"S; 43°58'03.41"W).

Once at the LELf (approximately eight hours of transport), the boxes were opened, the temperatures were individually measured, and 500 grams of mussels were placed in 40 L aquariums containing dechlorinated tap water (density: approximately 17 individuals L⁻¹). Water was dechlorinated with sodium thiosulphate. The temperature difference between the boxes and the water in the aquariums was less than 2°C to prevent thermal shock. The experiments were carried out in two phases describe above.

Experiment: phase I

Mussels were divided into two groups: mussels subjected to starvation (S) and mussels subjected to regular feeding (F) (Fig. 1). Both groups were maintained in their respective aquariums for 100 days with constant aeration and a controlled photoperiod of 12:12 h. No food was provided during the first five days to allow the organisms to acclimate to the laboratory conditions. After this five days period, the group F was daily fed with live algae *Scenedesmus* sp., Meyen, 1829 (Chlorophyceae: Scenedesmaceae) and *Ankistrodesmus*, Corda, 1838 (Chlorophyceae: Selenastraceae); 0.5 x 10⁵ cells mL⁻¹ (Owen, 1974; Montresor *et al.*, 2013), which were cultivated in the laboratory. Food was provided three hours before water replacement (50% or 100% daily water replacement). During this period, no food was provided to the treatment S. After 100 days, 25 specimens from each treatment of the phase I (S or F) were killed and their soft tissue was removed to quantify glycogen concentration (see the *Glycogen quantification* section).

Aquariums were cleaned every two days. Before replacing the water in each aquarium, organic residues and feces were removed using dechlorinated tap water and a thin paint brush. The tap water used in the cleaning procedure was first dechlorinated with sodium thiosulfate and then used in each aquarium to maintain the mussels in the laboratory. To reduce stress on the organisms, none of the specimens were removed from their aquarium, and each

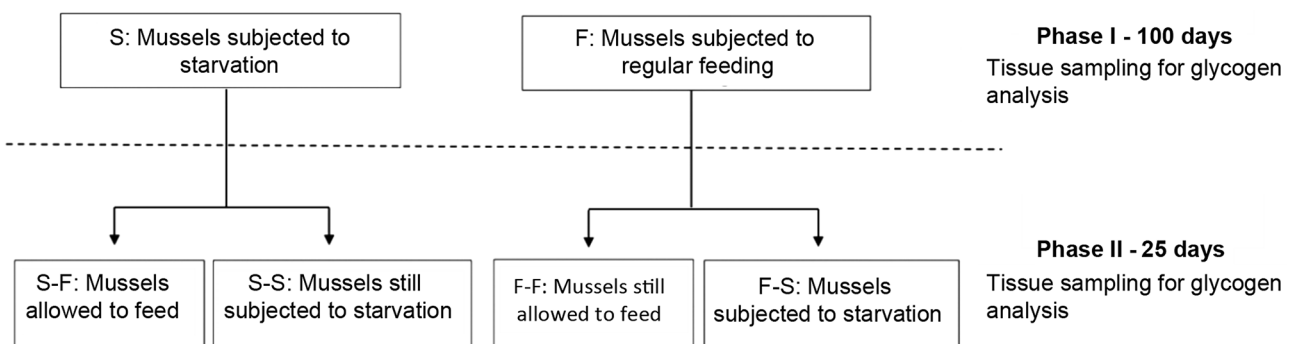


Fig. 1. Schematic representation of the experimental design.

cleaning procedure was carefully performed to avoid touching them. The physicochemical parameters in the aquariums, including temperature, pH, dissolved oxygen and total ammonia, were monitored daily using a pH meter (HI 3221, HI 1131B, Hanna Instruments, Woonsocket, RI, USA), an optical oximeter (ProODO, YSI Inc., Yellow Springs, OH, USA), and an ammonia-selective electrode (HI 4101, Hanna), respectively. The aquariums were maintained under the following conditions: $20.03 \pm 2.98^\circ\text{C}$, pH 7.7 ± 0.39 , $7.63 \pm 0.67 \text{ mg L}^{-1}$ of dissolved oxygen and total ammonia-N $\leq 0.6 \text{ mg L}^{-1}$.

Two days prior to the phase II of the experiment, 150 mussels (specimens $19.81 \pm 4.38 \text{ mm}$ in shell length) were removed from phase I groups (S and F) and kept in sterile plastic containers for an acclimation period with dechlorinated water and constant aeration in an incubator at $25 \pm 2^\circ\text{C}$ and with a controlled photoperiod of 12:12 h light:dark cycle). After 24 hours, specimens that did not attach to the containers and did not present extended siphon were excluded. After 24 h, groups of twenty-five individuals were randomly distributed into 12 plastic containers (4 L each) containing 2 L of dechlorinated water each under constant aeration. Mussel density in the plastic containers was equivalent to the density in aquariums (approximately $17 \text{ individuals L}^{-1}$). The specimens were placed in an incubator and the water was maintained at a pH of 7.73 ± 0.11 , $17.54 \pm 0.38^\circ\text{C}$, $92.86 \pm 0.68\%$ oxygen saturation, $8.86 \pm 0.11 \text{ mg L}^{-1}$ of dissolved oxygen and $0.020 \pm 0.02 \text{ mg L}^{-1}$ of ammonia. The plastic containers selected for the phase II were those with all twenty-five specimens attached to the container or to other mussel.

Experiment: phase II

The mussels selected from each phase I group (100 days at S or F) were subjected to one of two treatments (25 days at S or F), resulting in four treatments in the phase II: S-F, S-S, F-F and F-S (Fig. 1). Phase II experiments were carried out in triplicate. The S-F specimens, after 100 days of starvation, began to receive food; the S-S specimens remained under starvation conditions. The F-F group continued to be fed, and the F-S specimens, after 100 days feeding were subjected to starvation. Experiments were carried out using a 2×2 factorial design (two previous diets and two new diets throughout the experiment, see the *Statistical analysis* section for further details) in triplicate ($n=75$ per treatment). The mussels subjected to feeding in the phase II (S-F and F-F groups) (Fig. 1) were fed daily three hours before 100% water replacement. Before the daily water replacement, physicochemical parameters were monitored as described in previous section. During the phase II mortality and valve-closing responses were recorded over 24-h intervals, and dead individuals were removed from the containers twice a day to prevent water quality degradation.

At the end of the phase II (Fig. 1), all specimens, from the four treatments (S-F, S-S, F-F and F-S) were killed and their soft tissue was removed to quantify glycogen concentration (see the *Glycogen quantification* section).

Glycogen quantification

The mussels' soft tissues were removed from the shells at the end of each phase. For each group of each phase, three samples of one gram of pooled tissue were collected and stored at -20°C . Glycogen concentration from tissues was determined using a spectrophotometer, by 3,5-dinitrosalicylic acid technique (Pinheiro and Gomes, 1994; Sumner 1944) and concentrations were expressed as milligrams of glucose per gram of tissue ($\text{mg glucose g tissue}^{-1}$, wet weight).

Statistical analysis

To check for differences and interactions between the treatments (S and F) received in phase I and II, a factorial analysis of variance (factorial ANOVA) was performed (Zar, 2009). Considering only phase II results, one-way analysis of variance (ANOVA) were performed (Zar, 2009) to test the differences among the treatments S-F, S-S, F-F and F-S over the responses i) valve-closing, and ii) mortality. Comparisons were made *a posteriori* using 95% confidence intervals, and differences were considered significant for treatments in which there was no overlap in the confidence intervals. All statistical analyses were performed using STATISTICA 8.0 software (StatSoft, 2007).

RESULTS

Glycogen quantification

No interaction was found between the diets provided in the phase I and phase II within the tested groups (treatments) (factorial ANOVA: $F_{3,9}=1.45$, $P=0.29$). However, a significant difference was found among phase II treatments (diets) (factorial ANOVA: $F_{3,13}=4.15$, $P=0.04$). These differences were observed between the treatments F-S and S-F at the end of the 25-day phase II. Mussel under the treatment F-S ($0.44 \pm 0.01 \text{ mg glucose g tissue}^{-1}$) had a glycogen concentration that was 72.72% higher than those under S-F treatment ($0.12 \pm 0.05 \text{ mg glucose g tissue}^{-1}$). However, those treatments that had the same diet in both phases (S-S and F-F) remained stable when compared to their phase I period and did not show significant differences in their glycogen concentrations from those of the other treatments (Fig. 2 and Tab. 1).

Behavioral response (valve closing) and mortality

The diet provided in the phase II affected the valve-closing response of *L. fortunei* (ANOVA: $F_{3,211}=32.40$,

$P < 0.001$). Specimens subjected to starvation in the phase I had closed valves throughout almost the entire phase II (48.81% and 49.18% for S-F and S-S, respectively). In contrast, specimens that were provided food in phase I (F-F and F-S) had a lower occurrence of valve closure in the phase II. Interestingly, the treatments that were fed daily before the phase II showed an opposite behavioral valve response during the phase II. In fact, the mussels from the F-F and F-S treatments had opened valves throughout almost the entire phase II (22.03% and 29.55% with closed valves, respectively, Fig. 3).

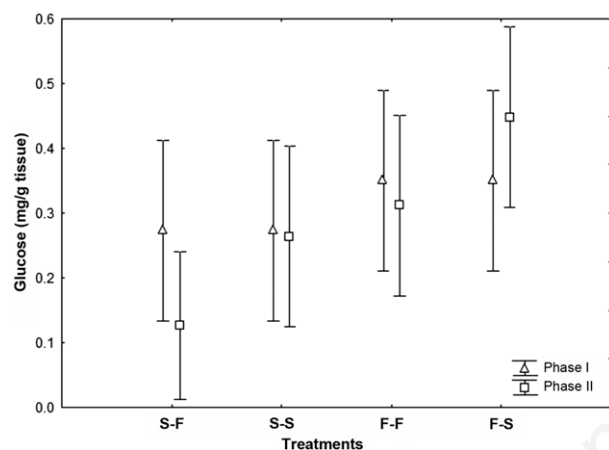


Fig. 2. Average glycogen content (mg glucose/g tissue, wet weight) of *Limnoperna fortunei* with 95% confidence intervals for the phase I and phase II.

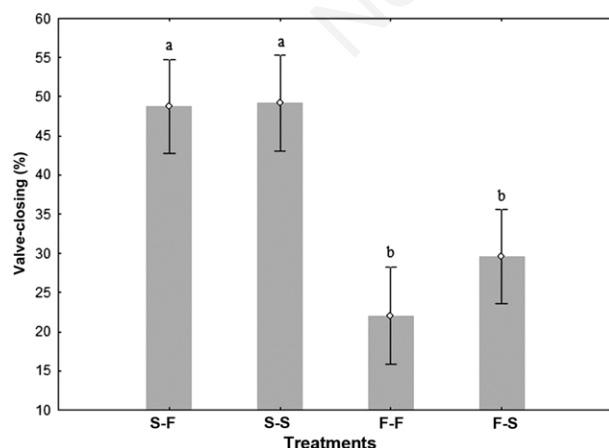


Fig. 3. Valve closing behavior (%) for the 25 days of the phase II for the different treatments of *Limnoperna fortunei* with 95% confidence intervals. The means followed by different letters in the columns are significantly different ($P < 0.05$).

Significant differences in mortality were observed (ANOVA: $F_{3,8} = 10.72$, $P = 0.003$). The treatments S-F and S-S showed the highest mortality in the phase II, and this mortality was not dependent on whether the mussels were allowed to start feeding or if starvation was continued (Fig. 4).

DISCUSSION

Bivalves are considered to be evolutionarily adapted to changes in food abundance and present characteristics (e.g., physiological traits) of animals that inhabit ecosystems in which the scarcity or even total absence of food during variable periods of time is very common (Chase and McMahon, 1995; Albentosa et al., 2007; Riisgård and Larsen, 2015). In fact, bivalves are exposed to an annual cycle of accumulation and use of energy reserves, which is influenced by food availability (Albentosa et al., 2007). Riisgård and Larsen (2015) showed that mussels reduce their oxygen uptake and save energy during starvation pe-

Tab. 1. Mean glycogen concentration values \pm standard deviation (mg glucose/g tissue, wet weight) of *Limnoperna fortunei* for each treatment group (phase I and phase II).

Treatments	Phase I	Phase II
S-F	0.27 \pm 0.07	0.12 \pm 0.05*
S-S	0.27 \pm 0.07	0.26 \pm 0.08
F-F	0.35 \pm 0.13	0.31 \pm 0.08
F-S	0.35 \pm 0.13	0.44 \pm 0.01*

S, starvation; F, feeding; * $P < 0.05$.

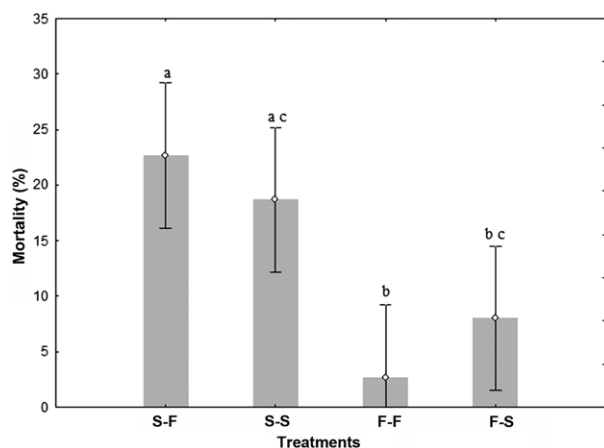


Fig. 4. Total mortality (%) during the 25 days (phase II) for the different treatments of *Limnoperna fortunei* with 95% confidence intervals. The means followed by different letters in the columns are significantly different ($P < 0.05$).

riods by closing their valves. Considering each treatment in the phase I and II this study, the feeding regimes tested (feeding or starvation) did not affect the glycogen content of *L. fortunei* specimens. Starvation is considered an extreme nutritive stressor, and the usual physiological response is a decrease in metabolic rate to a minimum value (required to vital functions) throughout the starvation period. Thus, this strategy allows the animal to reduce its energy consumption and save energy during the starvation period – when energy gain is nonexistent or only very minimal (Albentosa *et al.*, 2007). Based on our data, after a starvation period of 100 days (phase I), golden mussels in the S-F treatment, which were provided food during the phase II, most probably used this strategy to maintain its metabolism at a low level, and a reduction of its energy reserve was observed. The glycogen concentration in S-F mussels was lower than in F-S mussels, which were fed during the 100-day phase I and subjected to starvation during the 25-day phase II (Fig. 2).

The ability to survive during periods of food scarcity or even a total absence of food for variable periods of time is related to the physiological condition of the animal before the starvation period. In fact, the most generalized model for this is based on the accumulation of energy during the periods when food is abundant. Thus, using their accumulated energy throughout the starvation period, animals can survive this nutritive stress without a significant impact (Chen *et al.*, 2001). In fact, the absence of significant differences in the glycogen concentrations of the F-S treatment over a short period of time (25 days) and the high mortality rate of the mussels in the S-F group over 100 days are in accordance with the ideas mentioned above. Stable glycogen concentrations were observed when the feeding regimes of the phase I remained the same during the phase II, as observed in treatments S-S (continued starvation) and F-F (continued feeding). Thus, our data show that when no changes occur in the mussel's diet between the phase I and II, the glycogen concentrations were stable (*i.e.*, no significant difference was observed). However, it is important to emphasize that glycogen concentrations were maintained in S-S mussels, which were maintained under adverse conditions (starvation stress) for the entire trial. Hummel *et al.* (1989) mentioned that bivalves undergo a latent period (3 to 7 days in *Mytilus edulis*), which means that mussels can go through some period of stress during which glycogen is not used. Based on our results, golden mussels showed a higher latency period to nutritive stress (125 days) than *M. edulis*.

Studies on bivalves have shown that they have a high tolerance to starvation, as long as 63 days for *L. fortunei* (Silva, 2006), 159 days for *M. edulis* (Riisgård and Larsen, 2015). The tolerance to starvation can be influenced by the temperature and *D. polymorpha* can survive for 125.9 days when maintained at 25°C, and for 229 days at 5-15°C,

without mortality (Chase and McMahon, 1995). The differences among the ranges of tolerance to starvation periods of these bivalves may be a consequence of several aspects, such as species-specific resistance traits of these mussels, environmental conditions (*e.g.*, temperature) and the nutritional status of the bivalves prior to the starvation test (Chase and McMahon, 1995; Albentosa *et al.*, 2007; da Costa *et al.*, 2012). However, based on our results of glycogen concentrations, the golden mussel shows a higher tolerance to starvation (125 days) than previously published, and this tolerance could strongly influence its invasive ability. Moreover, an understanding of the starvation tolerance of *L. fortunei* allows us to be more informed about the ecology of this species.

Our data also show that the F-F group, which were fed throughout the phase II, did not present signs of nutritive stress and no significant alterations in glycogen concentration were observed, which suggests that the energetic metabolism of this treatment was not altered during the 125 days of the experiment (phase I and phase II). This information is compatible with the observations of Patterson *et al.* (1999) that in freshwater bivalves fed throughout the experiment, glycogen concentrations were constant over time. Valve-closing behavior was more frequent in the S-F and S-S treatments, which were subjected to starvation. In bivalves, valve closure is considered an important behavior to save energy to maintain vital functions during stressful periods. In fact, valve-closing is a common behavioral response in bivalves to maintain low rates of oxygen uptake (Famme, 1980; Riisgård and Larsen, 2015). These observations have also been made in the mytilid *M. edulis*, which is also able to survive for weeks without food by keeping valves closed and controlling glycogen deposits (Riisgård and Larsen, 2015). In fact, in our study, the valve-closing response was smaller in the F-F and F-S treatments, which were fed, than in the S-F and S-S treatments, which were kept under starvation conditions during the phase I. The bivalve filtration mechanism is controlled by changes in food availability. The neuroreceptors in the edge of their mantle are capable of detecting suspended particulates, which promote valve movement during filtration (Higgins, 1980). Thus, the organisms that regularly fed spend more time with open valves and exposed siphon (Higgins, 1980). Riisgård and Larsen (2015) showed that the minimum algal concentration needed to stimulate *M. edulis* to keep its valves open was similar to the algal concentration needed to meet the mussel's maintenance energy demand (*e.g.*, its basal metabolism). Thus, keeping valves open is a behavioral response, which expends energy obtained by feeding, as occurred in the F-F and F-S treatments. When energetic reserves are exhausted, mortality can be observed (Albentosa *et al.*, 2007; da Costa *et al.*, 2012). In fact, our results show that significantly higher mortality rates were ob-

served in the treatments that were subjected to 100 days of starvation independent of whether they were subsequently fed (S-F and S-S). The animals had a poor survival rate independent were provided food, which is supported by the *point-of-no-return* proposed by da Costa *et al.* (2012). Indeed, according to Famme (1980) and Higgins (1980), the initiation of feeding after starvation can increase oxygen consumption and protein catabolism, causing an increase in nitrogenous excretion that could contribute to additional stress (Thompson *et al.*, 1978; Chen *et al.*, 2001) and/or toxic effects in the specimens via ammonia buildup (Montresor *et al.*, 2013).

CONCLUSIONS

Our data show that *L. fortunei* has an ability to endure periods of starvation with minimal impact on glycogen concentrations and mortality and suggest that this species has a *point-of-no-return* below 125 days. Our results provide useful information that may contribute to different issues regarding this invasive species. Knowledge on the physiological response to feeding and starvation is useful to the development of better rearing protocols, that will result in good quality animals for laboratory tests, essential to achieve reliable results. The extreme tolerance to starvation showed here reinforces the need for great efforts in order to prevent this species range expansion and indicates its capability to invade water bodies with low concentration of organic matter. This information improves our understanding of the degree of its invasiveness and the process by which it establishes itself in new areas. Incorporating this parameter in prediction models may help to obtain more reliable estimates on the species expansion.

ACKNOWLEDGMENTS

We thank Dr. A.P. Paglia (UFMG) for statistical support, Dr. D.R. Fernandez (Itaipu Binacional) for logistical support during sampling and especially Dr. S. Ludwig for her valuable help in the correction of this paper. This work was supported by grants from the foundation for research from the State of Minas Gerais (Fapemig) and Vale S.A (CRA - RDP-00097-10); CNPq and Capes.

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