Tolerance to salinities shocks of the invasive mussel *Limnoperma fortunei* under experimental conditions

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ABSTRACT. The golden mussel, *Limnoperna fortunei* (Dunker, 1857), has been found in the estuarine regions of South America, including the Patos Lagoon (Brazil), a huge choked lagoon with an estuarine region that is highly unstable chemically. *Limnoperna fortunei* space-temporal variability in the lagoon's estuarine region demonstrated the need to evaluate this species' ability to survive under salinity shocks. A set of experiments was conducted under controlled laboratory conditions. Specimens were tested under salinities of 2, 4, 6, 8 and 12 ppt, and were exposed for periods of 24, 48, 72, 96 and 240 hours. The mussel can survive (90%) up to a salinity shock of 2 ppt for periods of at least 10 days. Considering the influence of climatic and stochastic events and the chemical instability of the Patos Lagoon estuarine region, it's unlikely that populations could survive for longer periods (more than a year) in this area.

KEYWORDS. *Limnoperna fortunei*, golden mussel, salinity shocks, physiological tolerance, invasion.

RESUMO. Tolerância do bivalve invasor *Limnoperna fortunei* a choques de salinidade sob condições experimentais. O mexilhão-dourado *Limnoperna fortunei* (Dunker, 1857), tem sido encontrado nas regiões estuarinas da América do Sul, incluindo a Laguna dos Patos (Brasil), uma enorme laguna de choque com uma região estuarina altamente instável quimicamente. A variabilidade espaço-temporal de *L. fortunei* na região estuarina desta laguna evidenciou a necessidade da avaliação da sobrevivência desta espécie sob choques de salinidade. Experimentos foram conduzidos em condições controladas de laboratório. Os exemplares foram submetidos a choques de salinidade de 2, 4, 6, 8 e 12 ppt, por períodos de 24, 48, 72, 96 e 240 horas. O mexilhão pode sobreviver (90%) por períodos de no mínimo 10 dias após um choque de salinidade 2. Considerando a influência de eventos climáticos e estocásticos e a instabilidade química da região estuarina da Laguna dos Patos, é improvável que populações possam sobreviver por períodos maiores (mais de um ano) neste ambiente.

PALAVRAS-CHAVE. *Limnoperna fortunei*, mexilhão-dourado, choque de salinidade, tolerância fisiológica, invasão.

The Patos Lagoon is a huge choked lagoon (10,300 km²) with a long narrow entrance channel (750m width). The estuarine region (*sensu* Closs, 1965) is restricted to the southern portion of the lagoon and comprises 10% of its total area. The estuarine region has a highly variable salinity, which makes it a chemically unstable area (Niencheski & Baumgarten, 1997). This lagoon has been invaded by the golden mussel *Limnoperna fortunei* (Dunker, 1857) since the end of the 1990s (Mansur *et al.*, 1999, 2003).

Limnoperna fortunei (Mytilidae) is the only freshwater member of its family (Montalto & Drago, 2003) and it is indigenous to Southeast Asia, where it's found in rivers, streams (Morton, 1977) and near the mouths of estuaries (Hwang *et al*., 1981). It was first discovered in South America (La Plata river, Argentina) in 1991, when argentinian imports from Hong Kong and Korea had increased (Darrigran & Pastorino, 1995). The species probably entered the Argentine territory as larvae or young adults, carried in the ballast water of transoceanic freighters (Darrigran & Pastorino, 1995).

This species is apparently adapting to different environments along its invasion routes; between 1991 and 1999 invaded four countries: Argentina, Uruguay, Paraguay and Brazil. It traveled at a speed of about 240 km per year (Darrigran & Drago, 2000). Throughout its distribution range, *L. fortunei* has inhabited a wide variety of environments, from strictly fresh water to brackish waters in the La Plata river and Patos Lagoon estuaries (Darrigran, 2002; Capítoli & Bemvenuti, 2005).

At the end of 1998 and the beginning of 1999, the golden mussel was registered for the first time in the Guaiba Lake Basin, Patos Lagoon's northernmost region. After two years it had colonized the lagoon's limnic and pre-limnic region (Mansur *et al.*, 1999, 2003). The first appearance of the *Limnoperna* in northern estuarine region was in 2002, in the São Gonçalo channel. However, through the size of the specimens, the estimate of the period of the cohorts' establishment indicated that these individuals would have originated from cohorts in the spring of 2001 (Capítoli & Bemvenuti, 2004).

Limnoperna fortunei was recorded in the Patos Lagoon's southern estuarine region, near the entrance channel, in 2003, after a long period of freshwater preponderance, due to the meteorological event *El Niño*, which increased the pluviosity in the Patos Lagoon basin. In the following year, after an intense estuarine salinization, the mussel was no longer found in Patos Lagoon's southernmost estuarine region. However, this species keeps persistent population stocks inside the Pelotas Stream and in the pre-limnic and limnic regions of the lagoon (Capítoli & Bemvenuti, 2004, 2005).

Being Patos Lagoon a choked lagoon, where the salinity of the estuarine region can vary up to twenty units in a one-hour period (Cruz *et al*., 1982), and considering *L. fortunei*'s space-temporal variability in the lagoon's estuarine region, this study aims to evaluate the species' ability to survive under salinity shocks.

MATERIAL AND METHODS

In order to evaluate the tolerance of *L. fortunei* at different salinity levels, a set of experiments was conducted in a laboratory under controlled conditions. The specimens were collected in June 2005 from a Patos Lagoon bay called Lagoa Pequena (Fig. 1), located between the cities of Pelotas and Turuçu. The specimens, attached to wooden logs, were placed in aerated tanks containing freshwater from the Santa Bárbara Barrage (Fig. 1) for approximately fifteen days before the experiments began. Pumping water through the barrage provided a constant source of food.

The specimens used in the tests had size ranges between fifteen and twenty millimeters, and were carefully separated by cutting their byssus with a scissors. For each treatment, forty specimens were equally spaced in four aerated aquariums (four replicates), and, in the same way, forty other mussels were placed in four aerated aquariums as controls. Water was not renewed during the tests, limiting the specimens' food during the experiment.

Mussels were kept in fresh water under controlled conditions, while the *L. fortunei* specimens under salinity treatments were transferred from fresh water directly to the saline solution to be tested. The mussels were submitted to salinity shocks of 2, 4, 6, 8 and 12 ppt during periods of exposure of 24, 48, 72, 96 and 240 hours. Water temperature, dissolved oxygen, oxygen saturation (YSI 55), pH (PHTEK pH100), conductivity (Corning CD55), CO_2 and alkalinity (titulation) were registered at the initial and final periods in each test. The mussels that survived were never used in subsequent experiments. Specimens were considered alive when they closed their valves in response to a stimulus or when valve-closed mussels resisted forcible valve opening. The dead mussels always showed these valves open. A Mann-Whitney U Test was applied to analyze the differences in the survival time between treatments and respective controls. A significance level of $p < 0.05$ was used in the statistical test.

RESULTS

During the tests, water temperature varied from 15 to 22 ºC, and the oxygen saturation was always above 75%. Pronounced variations in the other variables between treatments and controls were not observed.

The relationship between salinity and exposure time was evident, which coincided with the number of

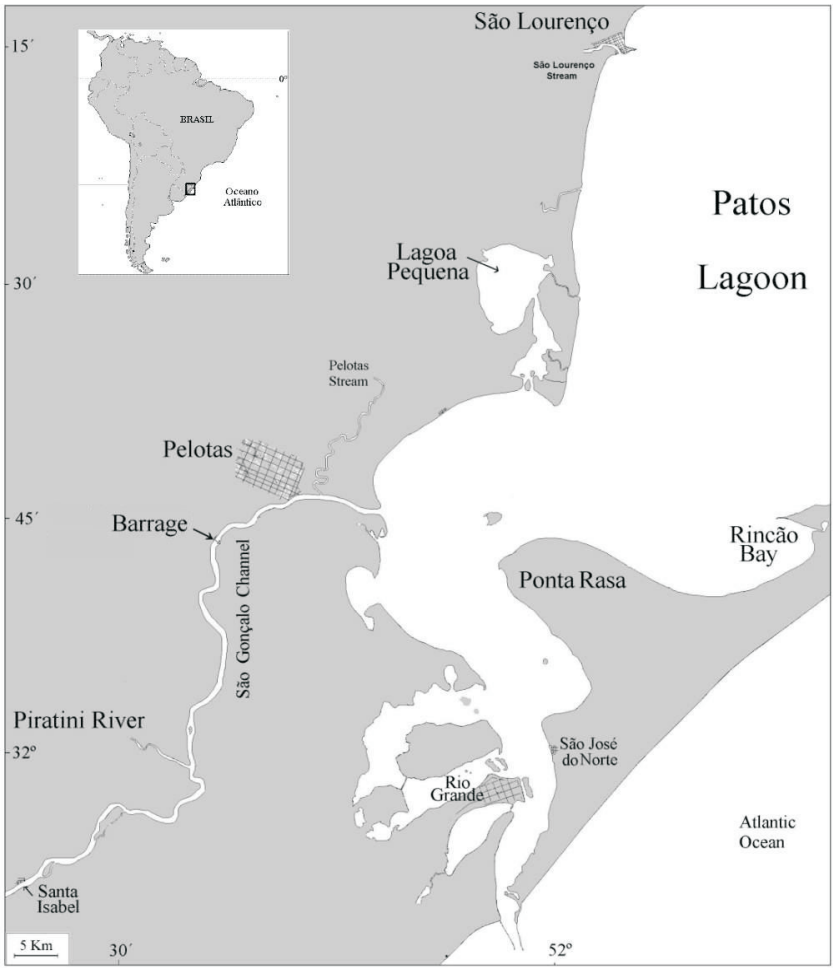

Fig. 1. Patos Lagoon southern region showing Lagoa Pequena bay where the specimens of *Limnoperna fortunei* and water to the experiment were collected.

survivors in the treatments. Less *L. fortunei* survivors were registered in the treatments combining high salinity and long exposure times (Tab. I). It was observed a high significant mortality of *L. fortunei*, in all treatments, after 240 hours of experiment (Tab. II).

Survival rates were greater than or equal to 85% for mussels under salinities of 2, 4, and 6 ppt in periods up to 96 hours. In the 240-hour period, the mussels' survival rates were seriously affected in salinities of 4 and 6 ppt, with more than 20% of the individuals not surviving (Fig. 2). In the 8 and 12 ppt salinity tests, *L. fortunei* survival was affected in the first 24 hours, when the number of live animals declined gradually. After 240 hours in 6, 8 and 12 ppt salinity levels, no live specimen was registered. Control treatments invariably showed 100% survival (salinity 0, for all time periods).

Table I. Mean survival (%) of *Limnoperna fortunei* (Dunker, 1857) at different salinity levels and periods of exposure (Salinity 0, control; SD, standard deviation).

Time/ Hours	24h		48h		72h		96h		240h	
Salinity	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	100	0	100	0	100	0	100	0	100	0
2	100	0	100	0	100	0	100	0	90	0
4	100	0	100	0	100	0	95	5,7	20	8,1
6	100	0	100	0	95	5,7	85	13	0	0
8	97,5	5	92,5	5	65	5,7	57,5	5	0	0
12	97,5	5	87,5	5	55	10	25	5,7	0	0

Table II. Significance level of *Limnoperna fortunei* (Dunker, 1857) survival (Mann-Whitney U Test) in salinity (S) treatments in relation to 100% survival of the control (Zero Salinity).

Time	S - 2ppt	S - 4ppt	S - 6ppt	S - 8ppt	S - 12 ppt
24h	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
48h	p>0.05	p>0.05	p>0.05	p=0.04	p=0,02
72h	p>0.05	p>0.05	p>0.05	p=0,02	p=0,02
96h	p>0.05	p>0.05	p=0,047	p=0,02	p=0,02
240h	p=0,008	p=0,02	p=0,008	p=0,008	p=0,008

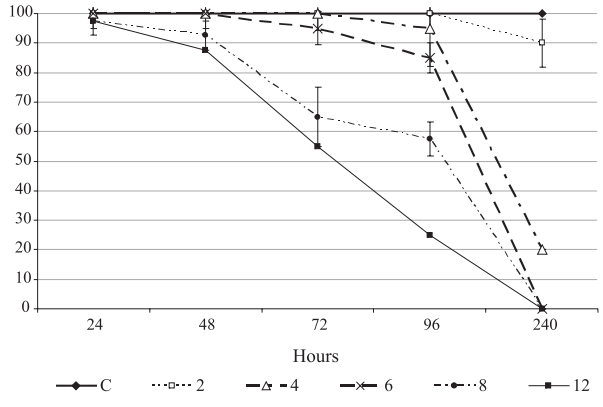

Fig. 2. Survival (%) of *Limnoperna fortunei* (Dunker, 1857) in laboratory experiments under salinities treatments 2, 4, 6, 8, and 12 ppt in the experiment control (C, zero salinity).

DISCUSSION

Ninety percent of the individuals of *Limnoperna fortunei* survived for at least 10 days after a salinity shock of 2 ppt. While in salinity shocks of 4 and 6 this species showed high tolerance only in the first 96 and 72 hours, respectively. After this period the species survival was greatly affected .

This tolerance can be found in *L. fortunei* in the Asian and South American estuarine regions, as in the Changjiang River (China) (HWANG *et al.*, 1981), La Plata river (Argentina) (DARRIGRAN, 2002), and in the Patos Lagoon southern region (Brazil) (CAPÍTOLI & BEMVENUTI, 2004, 2005).

In the present study, *L. fortunei* survival differs from the findings in DEATON *et al.* (1989), where 80% of the animals survived in a salinity of 6.8 ppt (200 mOsm) for time periods of three weeks or more, and less than a week in a salinity of 13.6 ppt (400 mOsm). In our study, *L. fortunei* specimens could only tolerate a salinity of 2 ppt for a period of up to ten days. For this same time period, salinities starting at 4 ppt were fatal for at least 80% of the organisms. The golden-mussel's higher tolerance, found by DEATON *et al.* (1989) through salinity and osmotic regulation experiments (hemolymph osmotic and ionic composition and tissue amino acid content) may be related to the species' ecological adaptation from Asia, the species' natural habitat.

Our results concerning *L. fortunei*'s salinity tolerance coincided with what was observed in the golden mussel distribution in the La Plata river estuary, where colonies were registered in areas where the mean salinity does not exceed 3 ppt (DARRIGRAN & PASTORINO, 1995; DARRIGRAN, 2002; BRUGNOLI *et al.*, 2005).

An invasive species should have several characteristics to be successful in the new environment: a short lifespan (two to three years), rapid growth, high fecundity, the ability to colonize a wide range of habitats, a wide range of physiological tolerances, wide genetic variability, phylogenetic plasticity, among others (MORTON, 1996). *Limnoperna fortunei* has been reported under a variety of synonyms, including subspecies (RICCIARDI, 1998). This plethora of species' synonyms reflects the high phenotypical variation that allows *Limnoperna* to thrive in a broad range of aquatic environments (RICCIARDI, 1998) and probably tolerate wide salinity variations, as well as occurs in this species' expected lifespan (which can vary from one to more than four and a half years, depending on the location studied) (MAGARA *et al.*, 2001).

According to the present study, the *L. fortunei* populations in the Patos Lagoon probably do not tolerate salinities above 2 or 3 ppt, but in salinities lesser than or equal to this value, it's likely that the populations will persist for an indefinite time and retain feeding, growth and reproductive abilities. At salinities around 4 ppt, the organisms could survive for a few days without, however, tolerating the harsh environmetal conditions, and as a result, it would become extinct.

In agreement, CAPÍTOLI & BEMVENUTI (2004, 2005) registered *L. fortunei* colonies in the estuarine region of Patos Lagoon during the high precipitation levels due to

the 2002/2003 *El Niño* (between August 2002 and February 2003), which caused a prevalence of low salinities in the estuarine region. Between the summer of 2004 and the autumn of 2005, the most severe dryness in the last forty years in the state of Rio Grande do Sul was registered, resulting in a high salinity period in the Patos Lagoon estuarine region. The surface salinity records oscillated between 10 and 30 ppt in the southern region and between 5 and 17 ppt in the northern region of the estuarine area, causing the mortality of the golden mussel populations (CAPÍTOLI & BEMVENUTI, 2005). The species wasn't registered in the estuarine region of the Patos Lagoon in the last census of *L. fortunei* (CAPÍTOLI & BEMVENUTI, 2005; BEMVENUTI, march 2006 – pers. obs.).

Considering the influence of climatic and esthocastic events and the chemical instability of the Patos Lagoon estuarine region (NIENCHESKI & BAUMGARTEN, 1997), it's unlikely that *L. fortunei* populations will survive for long periods (more than a year) in this area. However, due to *L. fortnunei* opportunistic lifestyle, cannot be discarded that in the future, this specie might increase its tolerance to the salinity limits in the South American estuaries, which reinforces the need for studies on its physiological tolerance.

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