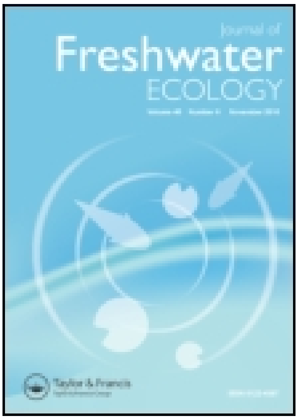

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The invasive freshwater bivalve *Limnoperna fortunei* is native to Chinese and Southeast Asian rivers and creeks. The impact of *L. fortunei* in South America involves both the human and the natural environments. Larvae and juveniles enter water systems of the drinking water plants and cooling systems of industries and power plants where they settle, mature, and produce macrofouling problems. Life cycle studies are undertaken in temperate region plants in order to gather basic information to develop strategies for control of *L. fortunei*. Individual growth of *L. fortunei* cohorts using experimental enclosures is recorded. The growth curve obtained shows that *L. fortunei* grows at a higher rate than recorded previously in works carried out in man-made facilities and natural environments along the coast of the Río de la Plata.

Keywords: individual growth; experimental enclosures; *Limnoperna fortunei*; bioinvasion

Introduction

The invasive freshwater bivalve *Limnoperna fortunei* (Dunker, 1857) (Mytilidae), the golden mussel, is native to Chinese and Southeast Asian rivers and creeks (Morton 1996). It invaded Hong Kong in 1968 (Morton 1973) and Japan (Kimura 1994) and Taiwan (Ricciardi 1998) in the 1990s. It was discovered in September 1991 in Bagliardi Beach (34°55′S–57°49′W), Río de la Plata, Argentina (Pastorino et al. 1993). Darrigran and Pastorino (1995) described the transport and release of this species into South America as a non-intentional introduction through ballast waters of ocean vessels.

Since 1991 the golden mussel has dispersed upstream in the Plata and Guaíba basins at a rate of 240 km/year (Darrigran and Damborenea 2005) and colonized about 1100 km of the Plata basin (Darrigran 2002). This species has become an important invasive species in South American freshwater environments and is currently found also in the Paraná River, Uruguay River, and Paraguay River

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(Boltovskoy et al. 2006). It also inhabits several lake environments, including Lagoa Guaíba and the Lagoa dos Patos (Mansur et al. 1999; Darrigran 2002; Capítoli and Benvenuti 2004; Darrigran and Pastorino 2004; Darrigran and Dreher Mansur 2006).

This has caused environmental damage to the native fish and benthic fauna (Darrigran et al. 1998; Penchaszadeh et al. 2000) and has had large economic impacts on man-made infrastructure (Darrigran and Damborenea 2009; Darrigran 2010) similar to those caused by *Dreissena polymorpha* in the northern hemisphere (Darrigran and Damborenea 2005). Differing from freshwater bivalves native to the region, *L. fortunei* has an epifaunal mode of life, living attached to a wide variety of hard substrates, both natural (ranging from tree trunks and aquatic plants to compact silt-sand) and artificial (e.g., docks, tubes, walls). Freshwater macrofouling is a new economic/environmental problem for South America. Industrial facilities that draw water from the Paraná River and Uruguay River and the Río de la Plata have suffered macrofouling-related problems.

Growth rate is particularly important for understanding the population biology and ecological impacts of *L. fortunei* because it seems probable that, as is the case for *D. polymorpha*, fecundity increases with body size (Karatayev et al. 2006). In this context, to achieve proper management of the golden mussel in water system intakes it is important to assess the growth of individual populations (i.e., maturity and reproduction times) in the environmental conditions of each water intake.

In the case of molluscs, it is generally accepted that growth rates depend on water temperature, season, depth of the water column, food availability, oxygen concentration, water velocity, and various other environmental factors (Coe and Fox 1942; Gilbert 1973; Seed and Suchanek 1992). However, it is very difficult to separate the independent effects of each of these factors, especially in natural water-bodies (Karatayev et al. 2006). The factors potentially intervening are varied and therefore the methods proposed by different authors to determine the growth rate also varied. These methods include: counting annual rings, analysis of size-frequency distributions, following growth under experimental conditions, and monitoring marked mussels under natural conditions without removing them from the substrate (Karatayev et al. 2006).

Previous studies have estimated individual growth of *L. fortunei* on man-made infrastructure in a temperate region (33°58′S–59°12′W) (Boltovskoy and Cataldo 1999) and in natural environments (Darrigran and Maroñas 2002; Maroñas et al. 2003) along the coast of the Río de la Plata (34°55′S–57°49′W). These studies were based on analysis of size-frequency distributions. Considering that *L. fortunei* spawning occurs throughout the warm season, with several peaks of veliger densities during the year (Darrigran et al. 1999), the size classes are not easy to distinguish because of overlapping. Therefore the method of size-frequency distributions is not easy to apply, even if experimental substrates are used when the time of settlement is known (dos Santos et al. 2008). Taking into account this observation and the need for precise estimations of *L. fortunei* growth, we considered it important to evaluate an alternative method to measure the growth of the golden mussel under natural conditions. Thus, the aim of this study was to record growth of *L. fortunei* cohorts in natural waters but with use of enclosures.

Materials and methods

Study area

The study was performed in the semi-confined freshwater basin known as Río Santiago (34°51′1.46″ S–57°53′28.23″W) at the mouth of the Río de la Plata. Along these rivers there are different kinds of man-made facilities (ports, breakwaters, water system pipes).

In this area the climate is temperate with clear seasonal temperature variation of 7–30°C.

Experimental design and sampling

In order to estimate growth of *L. fortunei*, we installed in Río Santiago three enclosures (30 × 30 × 30 cm) made of stainless steel frames covered with a 1 mm plastic mesh. This mesh prevented escape or entrance of mussels larger than 1.5 mm but allowed circulation of water within the enclosure (Bij de Vaate 1991; Smit et al. 1992; Garton and Dolmer 1998; Johnson 2000).

In June 2006, one thousand specimens of juvenile *L. fortunei*, measuring 3.5 mm (0.97 SD), were placed in each enclosure. Each group was considered an experimental cohort given the similarity in size that they showed (Lévêque 1971; Vakily 1992). Monthly samples of 40 specimens belonging to the same cohort were taken from each enclosure until December without any kind of selection. Forty additional specimens were collected in December, but these included only small individuals that had entered the enclosures through the mesh openings. In the laboratory, maximum length (distance from umbo to posterior margin of valve) was measured with a precision of 0.01 mm. On each sampling occasion, water temperature was recorded and algal growth on the mesh taken off so that the water flow regime would not be impaired.

Statistical analysis

Measurements for each date and enclosure were grouped in class intervals of 1 mm. Size-frequency distributions were broken down into their unimodal components following the method described by Bhattacharya (1967) using FISAT II (Version 1.1.2, FAO-ICLARM Fish Assessment Tools) (Gayanilo et al. 1996). Each modal progression was confirmed with NORMSEP (Pauly and Caddy 1985).

Covariance analysis (ANCOVA) was used to compare growths obtained in each enclosure, using the maximum length as the dependent variable, time as an independent variable, and enclosures as factors (Garton and Johnson 2000; Navarrete 2001). Linear regressions estimated for the enclosures were compared by pairs with Student's T-test to determine the existence of differences between slopes and adjusted averages.

Growth curve models of *L. fortunei* published by other authors were also applied to the samples, starting from the average initial sizes of the specimens used. This was done in order to obtain the average estimated size for each time since the beginning of each sample. These values were adjusted with time-function linear models and then compared among each other and with the values that we obtained.

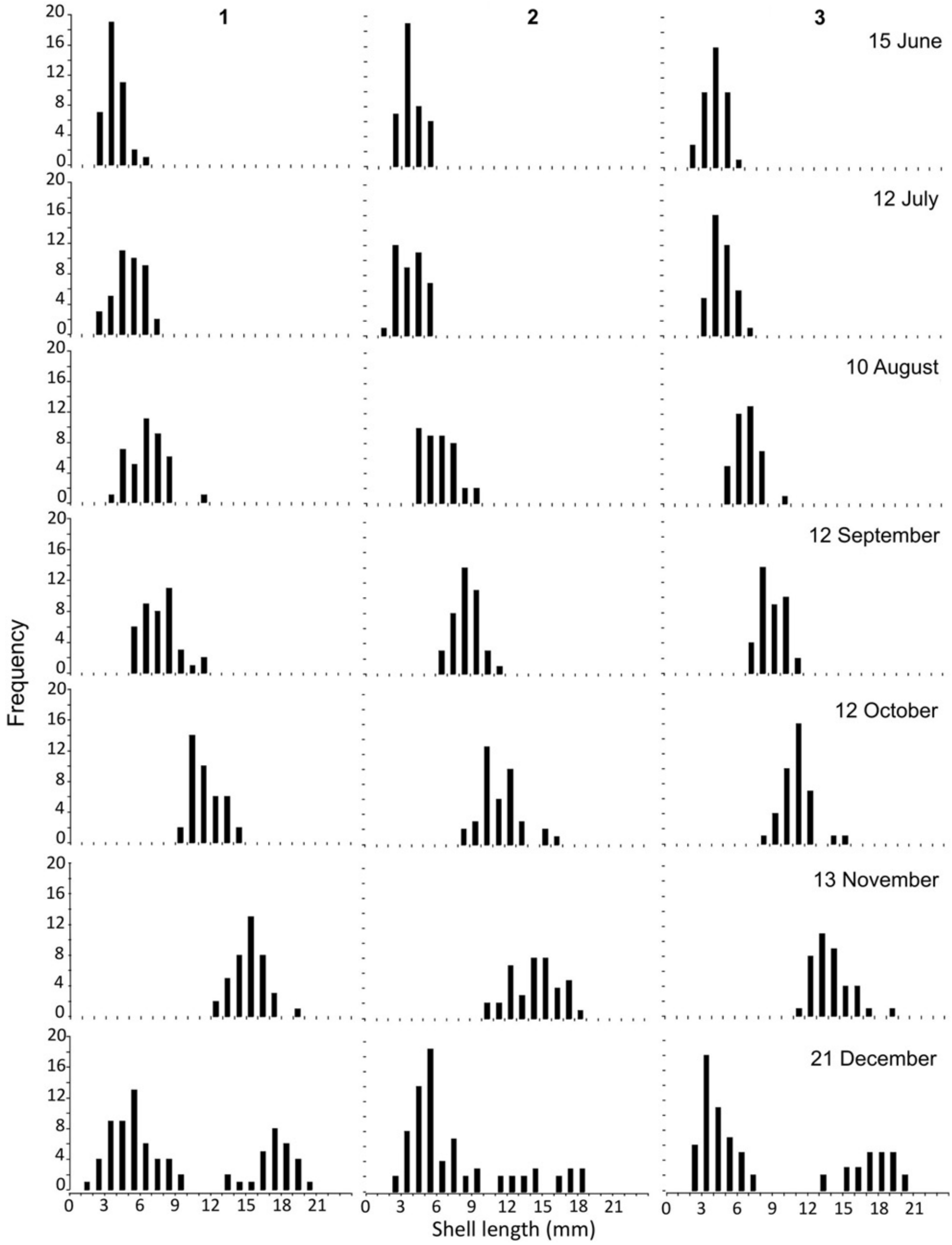

Figure 1. Size-frequency distribution of *L. fortunei* collected in experimental enclosures (1, 2 and 3) held in the Río Santiago (La Plata, Argentina) from June until December 2006.

Results

Figure 1 shows the frequency distribution of sizes of specimens collected from the experimental enclosures during the study period. The bimodal distribution in December reflected the presence of specimens that were recruited through the enclosure mesh. However, this group of mussels was clearly distinguishable from the

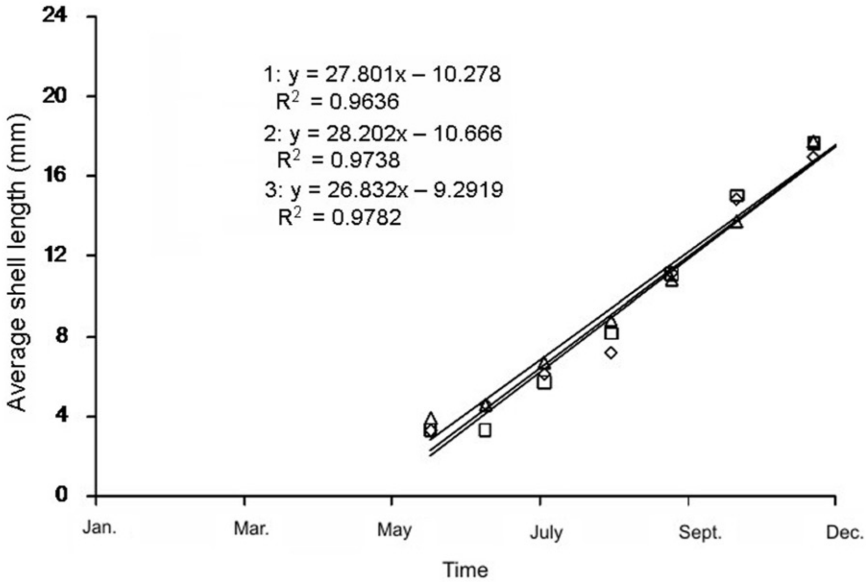

Figure 2. Lineal regressions adjusted to average values of valve length for each enclosure (1, 2 and 3) and sampling time.

experimental cohorts only in the last month of sampling. The average sizes and tracking through time of the initial cohorts in each sampling enclosure and sampling date followed an increasing linear pattern (Figure 2). The slopes of linear regressions of average valve length with time were not significantly different among enclosures (ANCOVAS; $F_{2,16} = 0.1176$; $p = 0.8898$); the same held true for adjusted averages ($F_{2,18} = 0.3383$; $p = 0.7174$)

Water temperature was lowest between June and September ($13 \pm 0.4°C$) and highest in December ($26 \pm 1°C$). Growth of small *L. fortunei* within the analyzed period was adjusted to a straight line; this implies a constant growth rate. Thus, under experimental conditions, temperature appears to have had little influence on growth.

Figure 3 depicts the growth models suggested by Maroñas et al. (2003) along the coast of the Río de la Plata; they differed significantly from our model (ANCOVA; $F_{4,30} = 86.112$; $p < 0.0001$) and from that of Boltovskoy and Catalado (1999) on man-made facilities in the Paraná River (33°58′S–59°12′W). Our model was not significantly different from that of Boltovskoy and Cataldo (1999) ($F_{4,34} = 10.202$; $p < 0.0001$). Adjusted measurements of the lineal regressions were all similar.

Discussion

Although growth in mollusks can be measured by different methods (Bij de Vaate 1991), each method has its own advantages and disadvantages (Bayne and Worrall 1980). Our study is the first in which enclosures were used to assess growth of *L. fortunei* in the Plata basin. Sàra et al. (2009) suggested that the enclosures induced changes in growth performance of mussels, but according to Garton and

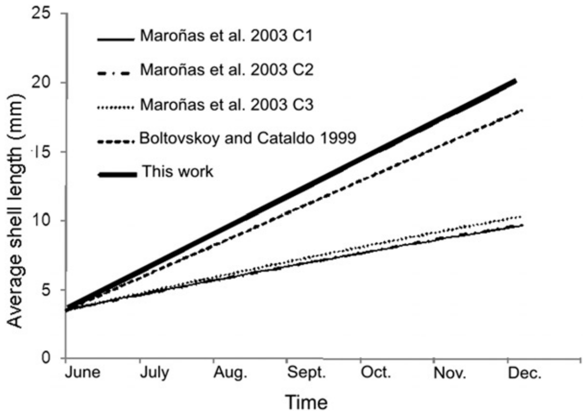

Figure 3. Lineal regressions estimated based on average values of valve lengths related to experiments with experimental enclosures, and those estimated based on growth models described by Boltovskoy and Catalado (1999) and Maroñas et al. (2003).

Johnson (2000) the enclosure design did not have a significant effect on mussel shell growth. However, other authors stated that mortality was much higher in enclosures due to mud accumulation (e.g., Bij de Vaate 1991) or overgrowth by periphyton, which reduces water flow (Karatayev et al. 2006). In our case, we did not have those problems. Our only major problem was the progressive colonization of enclosures by young specimens, arising from a September–October reproductive peak (Darrigran et al. 1999, 2007). They were conspicuous by the sixth month when they became dominant. Despite samplings and the natural mortality, individuals belonging to experimental cohorts were always clearly recognizable, and the results for each enclosure were the same.

The enclosure method allowed us to observe the growth of the same group of golden mussels in the Río de la Plata in a more precise and detailed way than before. However, partial isolation of the individuals could have affected the results due to the fact that partly artificial conditions may have masked effects linked to density, competition, and predation, as was pointed out by other authors (Coe and Fox 1942; Gilbert 1973; Seed and Suchanek 1992). In this sense it is important to note that in the Río de la Plata the golden mussel lacks an appropriate natural substrate to colonize. For this reason enclosures can be considered a model to assess risks and maintenance timing of facilities such as pipes or filters. Results of this study indicate that the use of enclosures is a reliable alternative way of keeping a group of individualized golden mussels under wild conditions not only to assess growth but also for other kinds of studies.

Another remarkable result was that temperature did not substantially modify growth rates, although it varied greatly along the experiment. This contrasts the generally accepted concept that growth ceases during the winter months in temperate latitudes because growth of mussels is closely related to water temperature (Vakily 1992). But this disagreement could be linked to the fact that the mussels used in the experiment were juveniles which naturally have the maximum potential growth rate expression. After reaching the adult stage, important amounts of energy are directed to reproduction and also metabolic demands increase. Under these conditions growth rates surely are more susceptible to temperature than in the juvenile stage.

When controlling golden mussels in man-made facilities in temperate climates there is a tendency to underestimate macrofouling during low temperature seasons (end of fall and winter). Results of this study reveal that this trend is mistaken. According to Darrigran et al. (1999), gonadic tissue of specimens of *L. fortunei* longer than 5 mm carries gametes during the entire year, and water temperature change can induce spawning in adult specimens. Based on the independence of individual growth in relation to temperature in addition to the rapid larval development in the golden mussel (Cataldo et al. 2005; Ezcurra de Drago et al. 2006) it seems likely that a short and sudden change in temperature can result in entrance of subsequently gradual cause larvae into man-made facilities. The presence of larvae in these human environments; which show similar conditions to those of experimental enclosure, would be followed by settlement and growth of juveniles of *L. fortunei* and macrofouling. The relative independence of juvenile *L. fortunei* growth from temperature is a point to be considered when designing management strategies for this invasive species.

The growth curve obtained applying this alternative methodology indicates that *L. fortunei* grows at a higher rate than recorded previously in works carried out in man-made facilities (Boltovskoy and Cataldo 1999) and natural environments along the coast of the Río de la Plata (Maroñas et al. 2003). This is the first time that monitoring of a group of clearly differentiated individuals of *L. fortunei* could be undertaken, instead of applying statistic methods to break down complex size-frequency distributions in order to define age or size groups. Regardless of previous growth estimations and taking into account the experimental design, the results obtained should be considered a precise assessment of *L. fortunei* growth in the wild. Further studies simultaneously using the referred methods and monitoring marked mussels under natural conditions, inside and outside of enclosures, could be useful to determine the limitations of both enclosures and frequency analysis methods.

Acknowledgements

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