RESEARCH NOTE

Effects of suspended inorganic matter on filtration and grazing rates of the invasive mussel *Limnoperna fortunei* (Bivalvia: Mytiloidea)

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The Asian mytiloid *Limnoperna fortunei* (Dunker, 1857) was first recorded in South America along the coast of the Río de la Plata estuary in 1991 (Pastorino *et al.*, 1993). Since then the mussel has expanded its range, colonizing most of the Río de la Plata basin, as well as several minor watersheds.

Because of its wide distribution, high densities and significant ecosystem engineering capabilities, *L. fortunei* has had sizeable impacts on the waterbodies colonized, including modification of nutrient concentrations and ratios, enhancement of water transparency, macrophyte growth and effects on cyanobacterial blooms, the abundance and diversity of benthic invertebrates, sedimentation rates and food availability for fishes (Sylvester, Boltovskoy & Cataldo, 2007; Boltovskoy *et al.*, 2009, 2013; Cataldo *et al.*, 2012a, b; Boltovskoy & Correa, 2015; Paolucci & Thuesen, 2015). Impacts on human activities have been especially marked: *L. fortunei* larvae enter raw water conduits of open cooling systems and develop large beds in pipes and other components, clogging them and causing pressure loss, overheating and corrosion (Boltovskoy, Xu & Nakano, 2015).

Northward expansion of *L. fortunei* is expected to continue beyond South America and into Central and North America (Karatayev *et al.*, 2015). Available data indicate that potentially colonizable areas include all continents except Antarctica (Kluza & McNyset, 2005; Karatayev *et al.*, 2015), but the fact that water bodies lacking mussels exist in watersheds where *L. fortunei* has been present for decades suggests that some environmental conditions may limit the expansion of this invader (Darrigran *et al.*, 2011). Among these, the concentration of suspended solids is of particular interest. Suspended matter can affect respiration, growth, parasite infestation and reproduction of the organisms (Robinson, Wehling & Morse, 1984; Alexander, Thorp & Fell, 1994; Rosewarne *et al.*, 2013), thereby restricting their geographic spread, but this constraint has not been explicitly addressed in models of the potential distribution of *L. fortunei* (Kluza & McNyset, 2005; Oliveira, Hamilton & Jacobi, 2010).

In order to estimate the tolerance of *L. fortunei* of inorganic suspended solids, thus providing data for analysis of its potential distribution worldwide, we assessed the species' capability of filtering water and retaining phytoplankton at different clay concentrations.

Individuals of *L. fortunei* were collected from Buenos Aires (34°32′S; 58°25′W) and stored in aerated aquaria filled with dechlorinated tap water at 23–25 °C. They were fed *ad libitum* on cultured algae (>95% *Scenedesmus* spp., mean biovolume 1300–1600 μm^3) known to be actively consumed by the mussel (Cataldo *et al.*, 2012a). Individuals 15–20 mm (mean 17.3 mm) in shell length were isolated from the clumps and placed in flat trays in order to verify their vitality. Actively filtering individuals were transferred from the trays to acclimation vessels at 27 °C for 48 h. All individuals were starved for 24 h and then stocked in cylindrical plastic netting cages (10 cm high, 6 cm in diameter) placed at mid-depth in the experimental 2-l containers (Fig. 1). In the latter, an air hose was attached laterally to a tube located vertically on the bottom, thereby suctioning settling sediment particles and returning them to the water column (Fig. 1).

Algal concentrations in the experimental containers ranged from 30 to 60 (mean 43.5 ± 6.3 SE) μg Chl *a* l^{-1}, mimicking usual values for eutrophic water bodies (Jones & Lee, 1982). Bentonite clay with a mean particle diameter of 9.45 μm, within the range of >90% of the inorganic suspended solids in the South American water bodies colonized by *L. fortunei* (Carignan, 1999; Sarubbi, Pittau & Menéndez, 2004), was used at different concentrations: 0 g l^{-1} (controls), 0.1, 0.5, 1, 2, 4, 6, and 8 g l^{-1}. All experiments were performed at 27 °C (typical of the lower Paraná River and Río de la Plata estuary during the summer) in a controlled temperature chamber. For each sediment concentration, three replicates without (controls) and three with 60 individuals of *L. fortunei* were used. Upon termination of each experiment (120 min) all individuals were measured to the nearest 0.01 mm with digital calipers, and their tissues extracted and dried at 60 °C to constant weight (DTW).

Immediately before start and after termination, each experimental container was sampled (40–150 ml) to estimate chlorophyll *a* concentrations. Samples were filtered through fibreglass filters (Whatman GF/F) and pigment extraction was performed with hot (60–70 °C) ethanol in darkness two or three times in order to avoid underestimations due to chlorophyll adsorption upon sediment particles (Koyama, Shimomura & Yanagi, 1968). The extracts were clarified by centrifugation, their volume adjusted and the absorbance at 665 and 750 nm measured with a spectrophotometer before and after acidification with HCl (1 N). Pigment concentrations were calculated according to Marker *et al.* (1980).
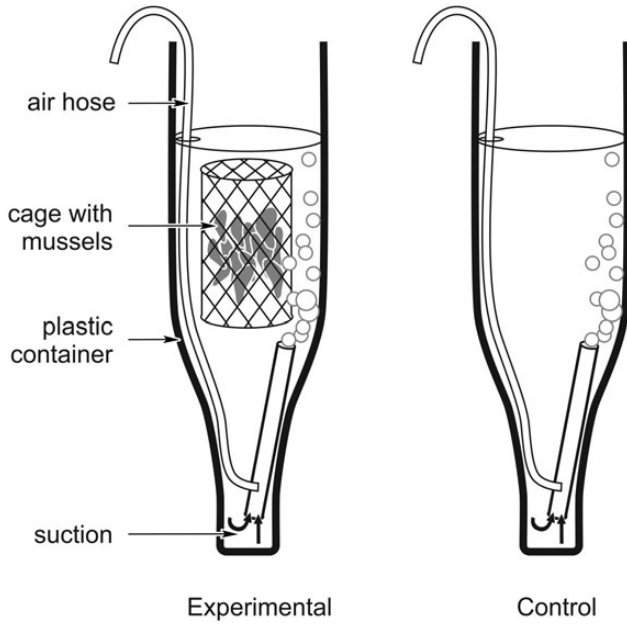
Figure 1. Scheme of the experimental units with individuals of *Limnoperna fortunei* in plastic netting cages (left side) and controls (without mussels, right).

Figure 2. Filtration rates of *Limnoperna fortunei* as a function of the concentration of suspended inorganic sediments (mean values for three replicates, error bars are standard deviations). Abbreviation: DTW, dry tissue weight. Significantly different values are denoted with different letters (ANOVA $P < 0.05$, Tukey's contrasts).

For estimates of filtration rates (FR, in ml g $TDW^{-1} h^{-1}$) and grazing rates (GR, in µg Chl a g $TDW^{-1} h^{-1}$) the following expression was used as proposed by Quayle (1948):

$$FR = \frac{V \cdot [\ln(C_i/C_f) - \ln(C'_i/C'_f)]}{\mathcal{N} \cdot T}$$

$$GR = \left(V \cdot \frac{r}{\mathcal{N}}\right) \cdot \left\{\frac{(C_f - C_i)}{[(k - r) \cdot T]}\right\}$$
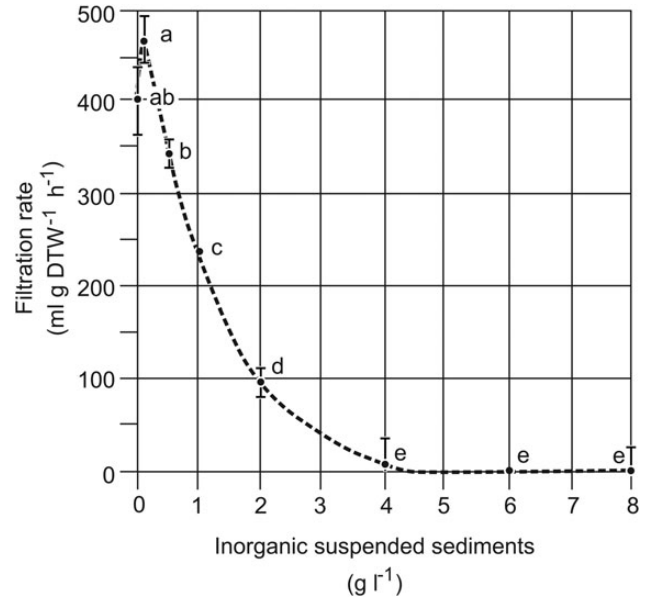
$$k = \frac{[\ln(C'_f/C'_i)]}{T}$$

$$r = \frac{(FR \cdot \mathcal{N})}{V}$$

where C_i and C_f are Chl a concentrations (in µg l^{-1}) at the beginning and the end of the experiments (respectively) in containers with mussels, and C'_i and C'_f are concentrations in the controls; V is the volume of liquid in the experimental container (in ml); T is total filtration time (in h); $\mathcal{N}$ is the total DTW of the experimental mussels (in mg) or the number of experimental individuals; r is the feeding coefficient; and k is the algal growth rate.

Our results indicate that filtration and grazing rates are strongly (and significantly) affected by inorganic sediment loads in the water column. Maximum FR (467 and 403 ml g $DTW^{-1} h^{-1}$) occurred at the lowest sediment concentrations (0.1 and 0 g l^{-1}, respectively), falling by 50% at 1 g l^{-1} (236.6 ml g $DTW^{-1} h^{-1}$) and over four times at 2 g l^{-1} (95 ml g $DTW^{-1} h^{-1}$) (Fig. 2). At ≥ 4 g of clay l^{-1}, filtration rates were negligible. The values shown in Figure 2 differed significantly (ANOVA, $P < 0.0001$). Tukey's contrasts indicated that significantly different ($P < 0.05$) FR occurred at 0–0.5, 1, 2 and 4–8 g of clay l^{-1} (Fig. 2). The highest FR per individual was 11.6 ml $ind^{-1} h^{-1}$ (at 0.1 g l^{-1}).

Grazing rates generally followed the same pattern and were significantly affected by clay concentrations (ANOVA, $P < 0.0001$) (Fig. 3). Maximum values occurred at 0 g l^{-1} (13.5 µg Chl a g $DTW^{-1} h^{-1}$), falling gradually to 9.24 µg Chl a g $DTW^{-1} h^{-1}$ at 1 g l^{-1} and then collapsing to 2.58 µg Chl a g $DTW^{-1} h^{-1}$ at 2 g l^{-1}. At sediment loads ≥ 4 g l^{-1} grazing rates
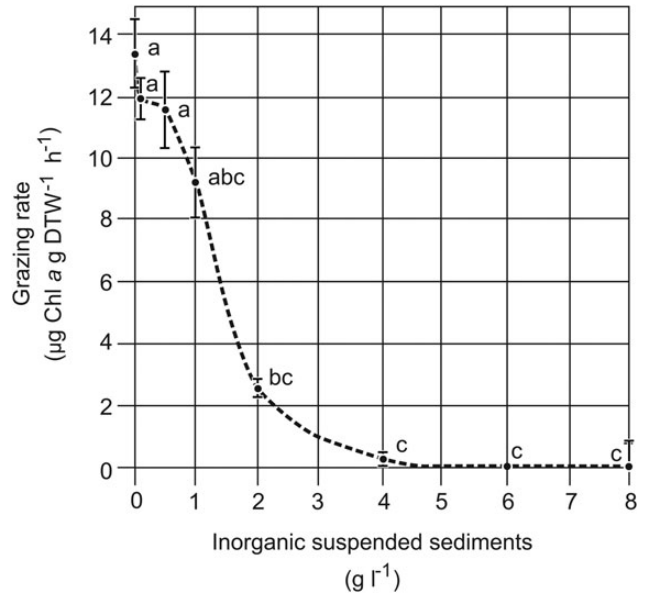
Figure 3. Grazing rates of *Limnoperna fortunei* as a function of the concentration of suspended inorganic sediments (mean values for three replicates, error bars are standard deviations). Abbreviation: DTW, dry tissue weight. Significantly different values are denoted with different letters (Kruskal-Wallis ANOVA $P < 0.05$).

were almost undetectable. GR per individual were highest at 0 g l^{-1} (0.37 µg Chl a $ind^{-1} h^{-1}$).

Comparison of the FR yielded by these experiments with literature data indicates that our values (up to 466.5 ml g $DTW^{-1} h^{-1}$) at low sediment loads are within the range of—but generally lower than—previously reported figures. However, the latter span over three orders of magnitude, from 100 to 29,500 ml g $DTW^{-1} h^{-1}$ (Boltovskoy *et al.*, 2015), suggesting that differences in

the experimental settings used (e.g. temperature, food type, starvation times, size of the mussels and experiment duration) strongly influence the results. Our estimates of the FR unencumbered by suspended sediments are therefore seemingly within 'normal' values for *L. fortunei*.

At sediment loads $>2–3$ g l^{-1} filtration and consumption of phytoplankton dropped to $5–10\%$ of the rates in 'clean' water, suggesting that above these values feeding is almost totally suppressed and therefore that survival of *L. fortunei* is unlikely.

High inorganic sediment loads decrease the quality of the suspended matter as food, not only because they decrease the proportion of organic material in the suspension, but also because they involve much higher energy expenditures in sorting out and eliminating the energetically unprofitable particles (Jorgensen, 1990; Velasco & Navarro, 2005; Safi & Hayden, 2010). Our visual observations of experimental animals indicate that water-pumping activity *per se* does not differ noticeably at different clay concentrations, but whereas at low sediment loads the production of pseudofaeces is moderate, at high concentrations mussels expel mucus-embedded strings of material at noticeably higher rates, which indicates that their ability to sort and ingest organic particles is reduced severely (Robinson *et al*., 1984; Berg, Fisher & Landrum, 1996; Baker *et al*., 1998) and may also affect their oxygen consumption rates, growth and reproduction (Alexander *et al*., 1994). Comparison of our results with literature data suggests that $0.5–1.0$ g of inorganic particles l^{-1} is the threshold value above which feeding is strongly hampered in many marine and freshwater filter-feeding bivalves (Robinson *et al*., 1984; Jorgensen, 1996; Lei, Payne & Wang, 1996; Cheung & Shin, 2005; Velasco & Navarro, 2005).

In South America, several tributaries of the Paraguay River have very high concentrations of inorganic suspended solids. The Bermejo and Pilcomayo rivers, for example, have suspended sediment concentrations of up to >40 g l^{-1}. Interestingly, although these rivers form part of the largely invaded Río de la Plata watershed, *L. fortunei* has not been recorded from them (Darrigran *et al*., 2011; Blettler *et al*., 2014). Values ≥1 g of suspended sediments l^{-1} are not uncommon in South America and throughout the world (Meade, 1994; Guyot *et al*., 1996; Walling & Webb, 1996; Guyot, Jouanneau & Wasson, 1999; Milliman, 2001; Meybeck *et al*., 2003). Meybeck *et al*. (2003) compiled data for suspended sediment loads in 62 rivers worldwide, recording that 29% of the mean values are >1 g l^{-1}.

We conclude that suspended inorganic sediments can represent a major limiting factor for the expansion of *L. fortunei* to many major rivers in South America and elsewhere. Although high sediment loads are usually restricted to the rainy season and when discharge rates are high (Guyot *et al*., 1996), the duration of these periods is likely long enough to wipe out any colonization that occurred during the low-water flow phase. Consideration of this variable in predictive models of the geographic expansion of *L. fortunei* and, by extension, of other freshwater invasive bivalves (e.g. *Dreissena polymorpha* and *D. rostriformis bugensis*) is, therefore, recommended.

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