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# Fluctuating salinity improves survival of the invasive freshwater golden mussel at high salinity: implications for the introduction of aquatic species through estuarine ports

**Francisco Sylvester · Daniel H. Cataldo · Carolina Notaro · Demetrio Boltovskoy**

**Abstract** In order to evaluate the resistance to salinity as a factor enhancing freshwater invasiveness, we assessed the tolerance of the mussel *Limnoperna fortunei* to salinity conditions mimicking changes in an estuary. We tested mussel mortality in 30-day exposures to constant and fluctuating salinities at different temperatures in the laboratory. Test conditions simulated different seasons of the year and locations with increasing influence of marine waters in Río de la Plata, Argentina. Significant mortality (31 % after 30 days) was observed at a constant salinity of 2 ‰, increasing to 45 and 57 % at 5 and 10 ‰, respectively. In contrast, considerably greater tolerances were observed when conditions in the experimental chamber fluctuated between salt water and fresh water. No significant mortality was observed in mussels exposed to a salinity cycle with abrupt salinity changes ranging 1–23 ‰ (mean 2.68 ‰) over a month. Tolerance to this type of regime was unaffected by different temperatures within ambient ranges. Tests at constant salinity underestimate the tolerance of this and probably other freshwater nonindigenous species (NIS) to short-term saltwater exposures. Estuarine ports account for ca. 2/3 of non-marine ports globally, thus constituting donor and recipient hotspots for the spread of NIS propagules into continental aquatic ecosystems via shipping vectors. The tolerance of *L. fortunei* to estuarine conditions likely contributes to the species' remarkable invasive success. These results highlight the need to determine causes of invasiveness and to study NIS traits not alone but in combination with transport network properties.

**Keywords** *Limnoperna fortunei* · Salinity tolerance · Invasiveness · Ports · Estuaries · Propagule transport

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## Introduction

With the aim of building capacity to forecast and prevent new invasions, considerable effort has been devoted to elucidating the physiological and life history traits that confer invasiveness to organisms (e.g., Devin and Beisel 2007; Karatayev et al. 2009; Van Kleunen et al. 2010; Hui et al. 2011; Zalewski et al. 2011). While these surveys provided valuable

insight into traits related to invasiveness, the interpretation of results is often complicated by lack of adequate information on physiology and life history, differences between terrestrial and aquatic habitats, and the confounding effect of variation in propagule pressure (Colautti et al. 2006; Gordon and Gantz 2011).

The golden mussel, *Limnoperna fortunei* (Dunker 1857), is a highly invasive mussel native to rivers and lakes in Southeast Asia (Ricciardi 1998). Within a few decades, this species has been unintentionally introduced to over half a dozen countries across Asia and South America (Ricciardi 1998; Boltovskoy et al. 2006). *L. fortunei* is a fouling species for which a considerable number of economic and ecological impacts, comparable to those described for the zebra mussel *Dreissena polymorpha* (Pallas 1771) in Europe and North America, have been reported, such as fouling of industrial facilities and boat hulls, competition with other filter-feeding organisms, overgrowth of native bivalves, promotion of *Microcystis* blooms, changes in benthic communities, and nutrient cycling (Boltovskoy et al. 2009a; Karatayev et al. 2010; Cataldo et al. 2012). Given its tolerance to a wide range of environmental conditions (e.g., high temperature, pollution levels, low pH, calcium, and dissolved oxygen), further spread of *L. fortunei* into other continents is expected (Boltovskoy et al. 2006; Oliveira et al. 2010). Environmental tolerance has frequently been linked to invasiveness, and many studies have focused on the environmental and community interactions that come into play after a species has been released in a new habitat. Surprisingly, the implications of environmental tolerance for transport, an earlier stage in the invasion process, have been largely ignored (Colautti and MacIsaac 2004; but see Bailey et al. 2004; Ellis and MacIsaac 2009; Briski et al. 2011). Determining the causes favouring frequent transport of propagules into new habitats is needed for understanding the drivers of invasive success and designing more efficient prevention protocols, particularly for high profile invaders such as *L. fortunei*.

A large number of works have examined the salinity tolerance of nuisance NIS seeking to predict their potential introduced distributions (e.g., Strayer and Smith 1993; Wilcox and Dietz 1998). Some authors have further looked into the tolerance of NIS to salinity conditions in ballast water tanks vectoring

the immense majority of aquatic introductions, including *L. fortunei* (Ellis and MacIsaac 2009). Managing salinity in tanks by means of mandatory ballast water mid-ocean exchange and brine addition in destination ports currently represents the most effective way to prevent ballast water-mediated introductions (Bradie et al. 2010; Bailey et al. 2011). In contrast, few studies have investigated NIS tolerance to ambient conditions in propagule pickup and delivery hotspot locations such as estuaries (Ruiz et al. 1997). Because a large number of potential donor ports are located in transition areas from freshwater to marine environments where salinity values vary widely over short periods of time, we contend that survival in variable salinity conditions, rather than tolerance to high constant salinity levels per se, may confer an invasion advantage to many aquatic species. However, because investigations aimed at forecasting NIS potential distribution ranges usually focus on environmental match, rather than on introduction and transport-related mechanisms, studies of tolerance to variable salinity levels (e.g., Wilcox and Dietz 1998) strongly lag behind those of survival in constant salinity conditions (e.g., Deaton et al. 1989; Berezina 2003; Byrne and Dietz 2006).

Here we examine the tolerance of *L. fortunei* to a salinity cycle typical of an estuarine regime, a trait that may significantly enhance the invader's area of introduction and, consequently, its propagule pressure from transportation hubs. The primary objectives of this work are to: (1) assess the tolerance of *L. fortunei* to constant and fluctuating salinity conditions; (2) determine the effect of the thermal regime on salinity tolerance; (3) interpret the mechanisms responsible for the animal's salinity tolerance; and (4) evaluate the role of this tolerance in increasing invasiveness.

## Methods

We assessed the effects of salinity on mortality of *L. fortunei* mussels collected from Río de la Plata near the city of Buenos Aires (Fig. 1). The site where animals were collected has permanently freshwater conditions, and hence they had no prior history of exposure to saline waters. Within 24 h of collection, mussels were transported to the laboratory and stored in tanks containing aerated, dechlorinated tap water at

**Fig. 1** Mean November–
March salinity fields (‰) at
0 and 10 m depth during
normal Río de la Plata
discharge conditions
(between 17,370 and
28,000 m³ s⁻¹; the estuary
is dominated by higher
salinities at lower discharge
volumes). No isohalines
below 22.5 ‰ are indicated
in *bottom panel* because
farther into the estuary
bottom depths are below
10 m. Average values for
the area where *L. fortunei*
was recorded are, 0 m:
4–7 ‰, 10 m: 19–23 ‰
(bottom depths of the actual
sites where *L. fortunei* was
recorded are between 3 and
4 m, for which reason their
average salinities are most
probably somewhere
between the above values).
Salinity figures are based on
historical 1911–2003 data
from Guerrero et al. (2010).
Presence data for *L. fortunei*
after Giberto and Sardiña
(2009)
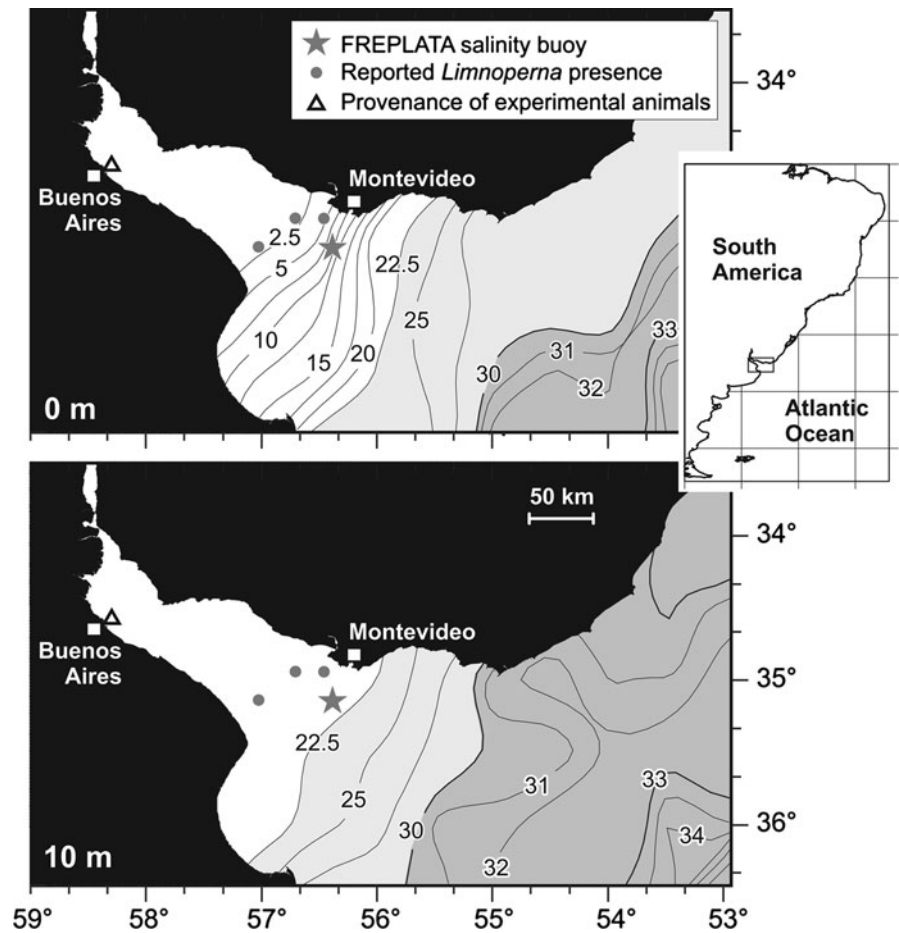


room temperature for a week to acclimate specimens. Acclimation to experimental temperatures (see below) was performed at 1 °C day⁻¹.

We conducted 30-day exposure experiments to both fixed and fluctuating salinity at two temperatures in the laboratory. In all the experiments (see below), we used 90 adult (valve length 5–27 mm) mussels equally divided among three containers for each salinity and temperature tested. Experiments were conducted under controlled temperature conditions (Fig. 2). Mussels were checked for mortality every 5–59 h (below) and dead individuals (as indicated by opened valves and lack of response to tactile stimuli) were recorded and removed. The endpoint was either 100 % mortality or termination of the experiment (30 days). Saltwater was produced from commercially available sea salts (Tetra Marine salt Pro) dissolved in dechlorinated tap water. Dechlorinated tap water was used for freshwater controls. Salinity changes in the

saltwater tests were accomplished by replacing the water in the experimental containers with water at the new salinity. Fresh water was renewed in the controls simultaneously with each saltwater change. Temperature was monitored throughout the experiments at 30-min intervals using autonomous programmable temperature data loggers in each experimental vessel.

We conducted three different mortality experiments. The first experiment was carried out between 4 June and 3 July 2010 at constant temperature (17 ± 1 °C), three fixed salinity concentrations: 2, 5, and 10 ‰, and a freshwater control. The objective of this trial was to explore *L. fortunei*'s tolerance to constant salinity at average ambient temperature (the water temperature in the lower Paraná-inner Río de la Plata estuary varies seasonally between ca. 11 and 28 °C).

With the aim of exploring the species' tolerance to conditions mimicking those in the estuary, we conducted

**Fig. 2** Scheme of experimental setup employed for 30-day mortality experiments conducted on *L. fortunei* mussels at three fluctuating salinities (salinity cycles), and freshwater controls under controlled laboratory conditions. See "Methods", Fig. 3, and Online Resource 1 for a detailed explanation of the salinity conditions in the fluctuating salinity experiments. Each container had 30 mussels (overall N = 360). Containers were distributed randomly in the bath, the spatial distribution shown being merely illustrative
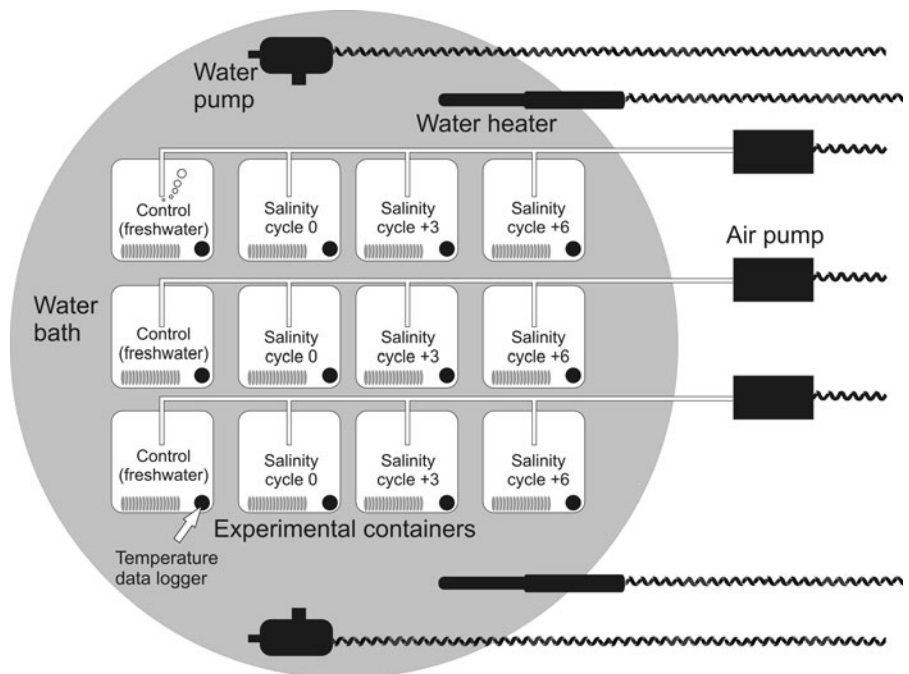


a second experiment (1 August to 1 September 2010) at changing salinity concentrations. For this purpose, we built a fluctuating salinity cycle using actual in situ salinity profiles obtained from data collected between 1 December 2009 and 31 May 2010 by the FREPLATA project buoy moored in the Río de la Plata estuary off Montevideo (35.193°S, 56.397°W), slightly eastward from the most seaward records of *L. fortunei* (Giberto and Sardiña 2009; Figs. 1, 2). The buoy's sensors provide an hourly record of several parameters, including temperature and salinity (in Practical Salinity Units, or PSU, which in practice is identical to parts per thousand, or ‰; thus, for the sake of clarity throughout this work we use the latter unit). Data were recorded at a depth of 2 m (the bottom depth at the site of the mooring is 7 m). From this series, we extracted the values and frequencies of salinity peaks and troughs, and the length and frequency of the intervals between salinity shifts. These values were randomized and an experimental 30-day schedule was established where the frequency and duration of the different salinity levels followed those of the actual record (Fig. 3). Thus, we defined a salinity regime, herein referred to as "salinity cycle 0", with a range of 1–23 ‰, mean time-weighted salinity throughout the 30-day experiment of 2.7 ‰, and a mean interval between salinity changes of 13.3 h (maximum: 54 h, minimum: 3 h; see details in Online Resource 1)
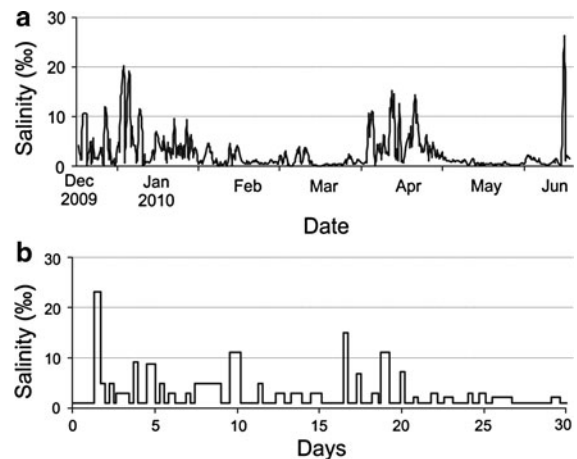


**Fig. 3** Six-month salinity profile obtained in the Río de la Plata estuary off Montevideo by the FREPLATA environmental monitoring buoy (**a**), and salinity settings used in salinity cycle 0 30-day experiments with *L. fortunei* (**b**)

that mimicked both the values and the timing of salinity changes in the estuary. On the basis of this salinity schedule, we established two other experimental regimes simulating sites progressively more influenced by marine waters. The first, "salinity cycle +3", had all salinities 3 ‰ higher than salinity cycle 0, with a range of 4–26 ‰ and a time-weighted mean of 5.7 ‰. The second, "salinity cycle +6", had all salinities 6 ‰

higher than salinity cycle 0, with a range of 7–29 ‰ and a time-weighted mean of 8.7 ‰. To simulate a spring-to-summer transition, when reproductive activity of the mussel peaks (Boltovskoy et al. 2009b), temperature in the experimental containers was raised gradually from 17 ± 1 to 21 ± 1 °C over the course of this experiment.

The third experiment (19 October to 19 November 2010) was aimed at examining the influence of winter and summer temperature extremes in the Río de la Plata estuary on mortality due to salt water. Because low mortality in the previous test was limited to salinities above those of the low salinity cycle (salinity cycle 0), but below those of the mid salinity cycle (salinity cycle +3; see "Results"), this experiment was conducted using salinity cycle 0 + 1.5 ‰ ("salinity cycle +1.5"; range: 2.5–24.5 ‰, time-weighted mean: 4.18 ‰) at 11 and 28 °C. Mortality in saltwater was compared with that in freshwater controls for the two temperatures tested.

## Data analysis

In all experiments, mortality differences were examined using an Analysis of Variance (ANOVA) and post hoc Tukey's tests on the proportion of mussels that remained alive in each container at the end of the experiment. Where necessary, we used $arcsin(x^{1/2})$ transformation of the variable to meet the statistical assumptions. Descriptive survival curves (Fig. 4) were constructed using individual mussel data. A significance level of $\alpha = 0.05$ was used for all statistical analyses.

## Results

Mortality in freshwater controls was always <10 % (Fig. 4). In all the experiments, L. fortunei's mortality increased with increasing average salinity (ANOVA, $P < 0.05$; Fig. 4).

All constant salinity concentrations tested yielded significantly higher mussel mortalities than fresh water (Tukey's test, $P < 0.05$). At the end of the 30-day exposures, between 31 % (at 2 ‰) and 57 % (at 10 ‰) of the animals died. In all cases there was no indication of a decreasing mortality rate with time, suggesting that maintenance of a viable population under these conditions was not feasible (Fig. 4a). At 10 ‰ mortalities were higher, albeit not significantly so, than at 5 ‰ (Tukey's test, $P = 0.417$).
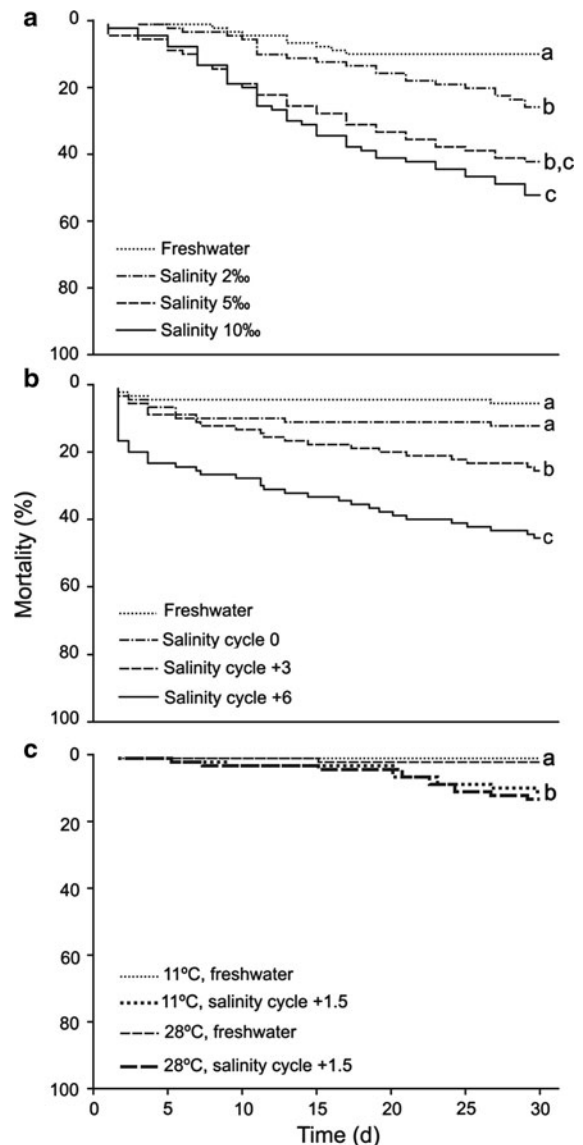


**Fig. 4** Survival of L. fortunei in constant salinity (**a**), and fluctuating salinity at 17–21 °C (**b**), 11 °C, and 28 °C (**c**). See "Methods", Fig. 3, and Online Resource 1 for a detailed explanation of the salinity conditions in the fluctuating salinity experiments. Curves with different letters denote significantly different survival rates

Mortality rates in exposures to fluctuating salinities at 17–21 °C were dependent on the range of values used. At the lowest salinities tested (salinity cycle 0) mortalities were only slightly higher than in the control, and did not differ significantly from the latter (Tukey's test, $P = 0.196$; Fig. 4b). The overall mortality trend was similar to that in fresh water,

suggesting that a sizable proportion of the animals could tolerate these conditions for extended periods. On the other hand, raising all salinity values by 3 and 6 ‰ yielded mortalities significantly higher than those in the control (Tukey's test, $P < 0.01$; Fig. 4b). The continued increase in mortality was sustained until termination of the experiment.

At 11 and 28 °C mortality of mussels exposed for 30 day to the salinity cycle +1.5 was significantly higher than in fresh water (ANOVA, $P < 0.01$), with gradual but sustained declines especially after day 20 (Fig. 4c). Water temperature did not affect mortality rates (ANOVA, $P = 0.111$; Fig. 4c).

## Discussion

This is the first experimental assessment of the long-term survival of a freshwater invasive bivalve in fluctuating salinity conditions. Our results indicate that tolerance to salinity extremes by *L. fortunei* is considerably higher than anticipated based on earlier studies of this and other closely related mussels (Table 1). Ours is likely a conservative estimate of the actual tolerance of *L. fortunei* to saline waters, since in our experiments salinity changes were instantaneous, while in the wild mussels benefit from short-term (minutes to hours) acclimation periods between salinity shifts (Fig. 3).

Tolerance to constant versus variable salinity conditions

Although *L. fortunei* is considered a freshwater animal (Brandt 1974; Morton 1977), it is common in estuarine habitats owing to its considerable tolerance to high salinity concentrations (Huang et al. 1981; Table 1). Our results indicate that ability of *L. fortunei* to survive in the middle section of the Río de la Plata estuary, periodically influenced by salinities in excess of 20 ‰ (Figs. 1, 3), is not due to its tolerance to high, constant salinities, but to its capacity to endure saline pulses of up to 23 ‰ for periods of up to several hours. The physiological tolerance and distribution potentials of invasive molluscs are underestimated when taking chronic tolerance values as indicators of their ability to withstand saline waters. Indeed, judging from previous studies (Angonesi et al. 2008; Barbosa and Melo 2009) and our own results at constant salinity

concentrations (Fig. 4a), *L. fortunei* should be unable to maintain a viable population in mixohaline habitats where it actually occurs (Fig. 1). Another likely source of misjudgement on the distribution of freshwater species is the assumption that isohalines based on synoptic surveys can be used for defining their geographic spread in mixohaline environments. Our results show that although *L. fortunei* cannot maintain a viable population at constant salinities above 2–3 ‰ (Fig. 4a), in the Río de la Plata estuary it lives in areas beyond the 5 ‰ isohaline (Fig. 1).

The tolerance of *L. fortunei* to constant salinity (<2 ‰) is substantially lower than that reported for invasive dreissenid mussels, known to inhabit marshes, coastal lagoons, canals, and inland lakes at salinities of up to 6 ‰ (*D. polymorpha*), 14 ‰ (*D. polymorpha andrusovi*), 17.6 ‰ (*D. polymorpha aralensis*), and 18.4 ‰ (*D. polymorpha obtusecarinata,* probably extinct) (Table 1). Higher tolerance ranges have been obtained for mussels in laboratory studies when salinity changes take place gradually (Kilgour et al. 1994; Dietz et al. 1996). Deaton et al. (1989) reported low mortality of *L. fortunei* at constant salinities of up to 7 ‰ preceded by periods of acclimation at intermediate salinities. This result likely represents an exception where unusually high tolerances might be the result of acclimation and the use of mussels that had survived successive stepwise salinity increases thus reflecting the behaviour of a selection of the most resistant individuals (Deaton et al. 1989; Table 1). We anticipate that, unlike some other freshwater invasive mussels, *L. fortunei* will most probably be excluded from habitats dominated by permanently high salinities (Table 1; Fig. 4a).

Tolerance to sudden salinity fluctuations is a trait likely shared by other invasive mussels, such as *Mytella charruana*, a South American brackish water mussel introduced to coastal and estuarine habitats of the southern US (Yuan et al. 2010; Table 1). Surprisingly, the abundant literature on the zebra and quagga (*D. rostriformis bugensis*) mussels has rarely focused on their tolerance to intermittent salinity (but see Casper 2007). Yet evidence for it is provided by recent laboratory experiments reporting unexpectedly low mortality rates of zebra and quagga mussels exposed to a salinity peak of 30 ‰ for 5 h (Ellis and MacIsaac 2009). Field and laboratory studies have found excellent survival of zebra mussels exposed to diurnal cycles of salinity between 0.45 and 5 ‰

**Table 1** Salinity tolerance of freshwater and brackish NIS mussels (*L. fortunei*, dreissenids, and *Mytella charruana*) in field and laboratory conditions simulating the ionic composition of natural seawater

| Species | Type of survey/ location | Duration of the study (day) or 20 % mortality (h) | Salinity regime[c] | Tolerance to fluctuating (range) or constant (upper limit) salinity (‰) | References |
|---|---|---|---|---|---|
| *L. fortunei* | Laboratory | 30 | Variable, w/o acclimation | 1–23 | This work |
| | Laboratory | 30 | Constant, w/o acclimation | <2 | This work |
| | Laboratory | 21 | Constant, with acclimation | 7 | Deaton et al. (1989) |
| | Laboratory | 10 | Constant, w/o acclimation | 2 | Angonesi et al. (2008[f]) |
| | Distribution/South America | Long-term | Variable | fw-3 | Barbosa and Melo (2009) |
| | Distribution/Asia | Long-term | Variable | fw-8 | Huang et al. (1981) |
| *D. polymorpha* | Laboratory | Long-term | Constant, w/o acclimation | 3.5 | Dietz et al. (1996) |
| | Laboratory | Long-term | Constant, w/o acclimation | 4 | Kilgour et al. (1994) |
| | Laboratory | 14 | Constant, w/o acclimation | 5 | Wilcox and Dietz (1998) |
| | Laboratory | 15 | Constant, w/o acclimation | 8.1 | Berezina (2003) |
| | Laboratory | Long-term | Constant, with acclimation | 8.5 | Dietz et al. (1996) |
| | Laboratory | Long-term | Constant, with acclimation | 8 | Kilgour et al. (1994) |
| | Laboratory | 29 | Constant, with acclimation | 8.8 | Wilcox and Dietz (1998) |
| | Laboratory | 11 | Variable, w/o acclimation | fw-4.5 | Berezina (2003) |
| | Distribution/Europe | Long-term | Constant? | 6.2 | Karatayev et al. (1998[a]) |
| | Distribution/North America | Long-term | Variable | fw-5 | Strayer (2006) |
| | Distribution/North America | Long-term | Variable | fw-5 | Walton (1996) |
| | Distribution/Europe | Long-term | Variable? | 6–9 | Strayer and Smith (1993[a]) |
| | Laboratory | Short-term (4 h) | Constant, w/o acclimation | 30 | Ellis and MacIsaac (2009) |
| | Distribution/Europe | Long-term | Variable | fw-5 | Karatayev et al. (1998[a]) |
| | Distribution/Europe | Long-term | Variable | fw-2 (likely up to 5.5) | Orlova and Panov (2004) |
| *D. rostriformis bugensis* | Laboratory | Short-term (3 h) | Constant, w/o acclimation | 30 | Ellis and MacIsaac (2009) |
| *D. polymorpha andrusovi* | Distribution/Europe | Long-term | Constant | 13 | Karatayev et al. (1998[a]) |

**Table 1** continued

| Species | Type of survey/location | Duration of the study (day) or 20 % mortality (h) | Salinity regime[c] | Tolerance to fluctuating (range) or constant (upper limit) salinity (‰) | References |
|---|---|---|---|---|---|
| | Laboratory | Short-term | Constant | 20 | Karatayev et al. (1998[a]) |
| *D. polymorpha aralensis* | Distribution/Europe | Long-term | Constant | 17.6 | Karatayev et al. (1998[a]) |
| *D. polymorpha obtusicarinata*[b] | Distribution/Europe | Long-term | Constant | 18.4 | Karatayev et al. (1998[a]) |
| *M. charruana* | Laboratory | 28 | Constant, with acclimation | Min.: 2; max.: 23[e] | Yuan et al. (2010) |
| | Laboratory | 12 | Constant, w/o acclimation | Min.:14; max.: 31 | Yuan et al. (2010) |
| | Laboratory | 12 | Variable, w/o acclimation[d] | fw-45 | Yuan et al. (2010) |

Lower tolerance limits are only shown for the brackish-water species

*Long-term* sustained population in the wild (distribution studies) or unspecified reported long-term survival (laboratory studies); *fw* fresh water

[a] And references therein

[b] Extinct

[c] The salinity regime was classified according to whether the salinity was constant or variable and to the type of change (with or without acclimation)

[d] Mussels were exposed to a 6-h salinity shock and subsequently transferred back to fresh water for the rest of the experiment

[e] Mortality was 40–55 %; survival was >20 % for all other reports included

[f] No information given on the type of salts used

(Walton 1996; Wilcox and Dietz 1998). Unfortunately, tolerance to wider fluctuations has not been tested. These results suggest that very significant differences in the tolerance to constant versus variable salinity are not exclusive to *L. fortunei* (Table 1).

Salinity tolerance and temperature

Osmotic balance in mussels is maintained using free amino acids and inorganic ions (Deaton et al. 1989; Dietz et al. 1996). Similar to other physiological processes, *L. fortunei*'s resistance to high salinities was expected to be influenced by temperature. Surprisingly, we did not find any differences between salinity tolerances at quite different temperatures (11 and 28 °C). A possible explanation for this might be that our observations lie at the two tails of a Gaussian tolerance curve. Salinity exposure experiments have shown that mortality of *D. polymorpha* is lowest at 10–12 °C, increasing below 4 °C and above

18 °C (Kilgour et al. 1994). The salinity tolerance of *L. fortunei* may change with temperature, with a maximum tolerance somewhere between 11 and 28 °C. Alternatively, our data might simply indicate the lack of any influence of temperature on salinity resistance of *L. fortunei* over that temperature range (which represents the yearly range of environmental conditions in the Río de la Plata estuary).

Tolerance mechanisms

We hypothesize that valve gaping is the main mechanism by which *L. fortunei* can withstand swift salinity changes. The use of valve closure to prevent exposure to harmful environmental conditions is well known for bivalves (Jørgensen 1990). Toxicity experiments have shown that, by closing its valves, *L. fortunei* can survive several weeks of continuous exposure to concentrations of up to 100 mg L$^{-1}$ of chlorine (Cataldo et al. 2003). Upon cessation of the adverse

conditions, mussels open and reassume their normal activity (Jørgensen 1990; Cataldo et al. 2003). Valve closure is so effective in preventing exposure to the environment that, in long-term experiments, mussels exposed to toxicants are affected by starvation and debilitation of byssal attachment as much as by the toxicant (Cataldo et al. 2003; Rajagopal et al. 2003).

## Tolerance to variable salinity conditions and invasiveness

The ability to survive in estuaries has very important implications for the invasion ecology of aquatic ecosystems. In the first place, it involves an increase in the geographical range where tolerant species can be delivered and picked up by transport vectors. Most importantly, the habitats involved in this areal range increase are particularly significant in terms of human-mediated species dispersion, because they host a large number of ports. Several workers have recently modelled the spread of aquatic NIS in terms of a network of ports functioning as transport hubs connected by shipping vectors (vessel ballast water and hull fouling) (e.g., Drake and Lodge 2004; Floerl et al. 2009). Transport hubs play a key stepping-stone role in the spread of biological invasions providing species that can make use of a greater number of hubs with increased transport opportunities (Muirhead and MacIsaac 2005; Floerl et al. 2009). According to a broad classification of ports based on their salinity and distance to the ocean, estuarine areas include about 20 % of the ports worldwide (Keller et al. 2011). Considering that freshwater ports barely amount to about 10 % of the total, tolerance to estuarine conditions potentially triples the number of transport hubs for the spread of *L. fortunei*.

In the second place, the capability of withstanding salinity fluctuations can substantially increase effective colonization rates. Estuarine and freshwater ports serviced by oceanic vessels, usually located relatively close to the ocean, are invariably subjected to seaward water flow. Thus, a major challenge for the NIS released in these ports is to avoid being flushed out into the sea before having established a self-sustaining population capable of withstanding the high expatriation rates typical of these environments. The ability to survive in mixohaline waters significantly enlarges the area suitable for colonisation, which facilitates initial introduction and subsequent secondary spread.

The saltwater tolerance of embryos and larvae, which likely differs from that of adult mussels, need to be studied before we can confidently determine settling boundaries of *L. fortunei* in the estuary (Wright et al. 1996; Barnard et al. 2003). Yet, the ability of *L. fortunei* larvae to settle and survive in saline regions is evidenced by the presence of colonies (unlikely formed by accumulation of drifting adults) near the Río de la Plata salinity front (Fig. 1).

It might seem surprising that in the case of Ponto-Caspian dreissenid mussels, it was *D. polymorpha*, the species with the lowest tolerance to high constant salinity levels, that resulted in widespread invasions (Karatayev et al. 1998; Table 1). As previously observed, the tolerance of *L. fortunei* to constant salinity is also quite modest, yet this was not an obstacle to the species' invasive success. This suggests that mere resistance to high constant salinity values is not necessarily translated into invasive success. Rather, resistance to abrupt salinity changes seems to be more advantageous in some situations. In the case of *L. fortunei*, the advantage provided by this resistance to salinity changes is not restricted to the environmental tolerance during establishment, but perhaps more importantly, also at an earlier stage in the invasion process during the transport of propagules (Colautti and MacIsaac 2004). We thus contend that tolerance to a wide range of salinities, and likely many other 'invasive traits', are not only (and perhaps not primarily) advantageous features per se but advantageous in association with transport network characteristics.

## Implications for vessel-mediated NIS dispersal

Ballast water is the most likely vector of introduction of *L. fortunei* to over half a dozen countries across South America and Asia (Ricciardi 1998; Boltovskoy et al. 2006). Current international ballast water regulations require mid-ocean exchange (MOE), at least 200 miles (370 km) off-shore, of all ballast originated from freshwater to coastal sources (IMO—International Maritime Organization 2004). Although MOE is effective in reducing the number of larvae transported in ballast water (Bailey et al. 2011), adult freshwater mussels may survive attached to the walls of exchanged tanks (Carlton and Geller 1993). While for some mussels the window for adult survival after MOE seems to be small (<5 h for zebra and quagga mussels: Ellis and MacIsaac 2009), we have found

90 % survival of *L. fortunei* exposed to a salinity peak of 23 ‰ for 9 h. In terms of the distance covered by a commercial vessel typically sailing at 13–24 knots (24–44 km h$^{-1}$), 9 h represents anywhere between 217 and 400 km, which in many areas exceeds the distance between the destination port and the site of MOE. International and national regulations in most countries, including Argentina, require salinities of at least 30 ‰ of all ballast to be discharged—e.g., IMO and regulations for Canada and the US (IMO 2004; Saint Lawrence Seaway Development Corporation 2008). Further research is needed to determine *L. fortunei*'s ability to withstand these salinity levels, and whether vessels in strict compliance with these regulations may still pose an invasion threat.

Hull fouling, on the contrary, does not seem to be a viable option for intercontinental introduction of freshwater species with a salinity tolerance similar to that observed for *L. fortunei*. This is consistent with the absence of freshwater bivalves from extensive hull fouling inventories for marine and freshwater environments (Sylvester and MacIsaac 2010; Sylvester et al. 2011).

## Conclusions

Physiological tolerance to chronic exposures in adverse settings and distribution studies may underestimate NIS capabilities to colonize habitats characterised by fluctuating conditions, in particular salinity. In estuaries, the freshwater mussel *L. fortunei* appears to be able to tolerate very high salinity concentrations provided saltwater pulses are interrupted by periods of freshwater conditions. The ability of *L. fortunei* to live in estuaries not only expands the potential distribution range of the species, but also greatly increases the likelihood of its vessel-mediated transport and effective introduction to new habitats. This invasive trait may be shared by other successful aquatic NIS around the world. Given the prevalence of human-mediated introductions, with a prime influence of propagule pressure on NIS invasive success, research on species invasiveness should examine species characteristics in the light of vector and transport network properties.

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