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EXPLORING THE SURVIVAL THRESHOLD: A STUDY OF SALINITY TOLERANCE OF THE NONNATIVE MUSSEL *MYTELLA CHARRUANA*

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ABSTRACT In this study, our objective was to understand life history attributes of *Mytella charruana*, a newly introduced species to the southeastern Atlantic coast of the United States, that would enable its survival in the introduced range. We therefore addressed the following questions regarding the range of salinities in which *M. charruana* can survive: First, in what range of salinities is survival possible for *M. charruana* if slowly adjusted to test salinities? Second, in what range of salinities can these mussels survive when experiencing rapid changes of salinity? Third, in what range of salinities can *M. charruana* survive with temporary, rapid changes of salinity (6-h duration)? We tested survival in salinities ranging from freshwater to hypersaline conditions (0–45 ppt) and determined whether mussel size affected experimental results. All experiments examined survivorship of mussels by increasing or decreasing the salinity from the field value under laboratory conditions. Mortality in each tank was recorded daily for 43 days for the gradual adjustment trials, and 12 days for permanent and 6-h shock trials. Large *M. charruana* (20–54 mm) survived best in salinities from 2–23 ppt, with 100% mortality at 0 ppt and 45 ppt with gradual adjustment. Small *M. charruana* (3–19 mm) survived in a wider range of salinities (2–40 ppt) with gradual adjustment to new salinities. However, survival of both large and small mussels was significantly lower in permanent shock trials at salinity extremes. Six-hour shock trials had no effect on survival at any of the test salinities (0–45 ppt) for both large and small *M. charruana*. Overall, the data indicate that these mussels could invade a wide variety of saline environments with significant freshwater or saltwater input.

KEY WORDS: introduced species, estuary, marine mussels, bivalve, southeastern United States

INTRODUCTION

As global transportation has become more common, large numbers of species have been unintentionally introduced worldwide (Carlton & Geller 1993, Collinetti et al. 2001, Pimentel et al. 2005, Baker et al. 2007). When a newly introduced species becomes established and causes ecological or economic impacts, it is considered an invasive species (Lockwood et al. 2007). Introductions of aquatic species are hard to control because of the difficulties associated with identification, monitoring, and eradication. To date, at least 88 mollusc species (freshwater and marine) have become established in the United States as a result of unintentional transport via ballast water and hull fouling (Office of Technology Assessment 1993, Pimentel et al. 2005).

Recently, *Mytella charruana* (d'Orbigny, 1846), was found on the southeastern Atlantic coast of the United States. This mussel is commonly known as "sururu" in Brazil. The native distribution of *M. charruana* includes the Atlantic coast of South America and the Pacific coast of Central and South America from Mexico to Ecuador (Keen 1971, Carlton 1992, Szefer et al. 1998, Boehs et al. 2004, Boudreaux & Walters 2006). This mussel can reach up to 65 mm in shell length and is distinguished by its exterior dark-brown/black shell with radial patterns. The interior shell color ranges from light to dark purple. *M. charruana* was first discovered in the United States in Jacksonville, FL, in 1986 at the Northside Generator Power Plant (Lee 1987). *M. charruana* were considered extirpated after a freeze in 1987. In 2004, however, a small number of individuals were found in Mosquito Lagoon, along the east coast of central Florida (212 km south of Jacksonville, FL) (Boudreaux & Walters 2006). Since 2004, *M. charruana* have been documented along the southeastern Atlantic coast of the United States from Jupiter, FL, to South Carolina (Gillis et al. 2009, Walters & Hoffman, pers. obs.). *M. charruana* have the potential to cause problems in the introduced range because it can reach high densities (11,036 mussel/m^2) in its native range (Pereira et al. 2003).

The extent of the damage *M. charruana* may create is currently unknown, but bivalve mussels are notorious invasive species. For instance, the Asian green mussel *Perna viridis* was initially documented clogging intake pipes in the cooling system of an electrical power plant in Tampa Bay, FL, in 1999 (Benson et al. 2001, Ingrao et al. 2001, Baker et al. 2007). In India, *P. viridis* has also caused numerous problems to power plants such as clogging intake pipes and causing financial losses (Rajagopal 1997). Likewise, the zebra mussel, *Dreissena polymorpha*, and the quagga mussel, *Dreissena bugensis*, have both had dramatic impacts on the Great Lakes ecosystem (Ludyanskiy et al. 1993, Benson & Boydstun 1995, Pimentel et al. 2005) as well as southward and westward in the United States (Drake & Bossenbroek 2004, Lockwood et al. 2007). These species have completely altered the native system by outcompeting many native Great Lakes species (Ludyanskiy et al. 1993, Ricciardi et al. 1998, Lockwood et al. 2007), in addition to *D. bugensis* outcompeting *D. polymorpha*. In addition, *D. polymorpha* has caused significant economic impacts; for example, they have been estimated to cause approximately $1 billion each year in damage to power plants (Ludyanskiy et al. 1993, Power et al. 2004, Pimentel et al. 2005). Sites where these species are found cannot be restored back to their original state, nor can these mussels be completely eradicated (Lockwood et al. 2007).

The testing of salinity tolerance can help to determine whether a new, nonnative aquatic species such as *M. charruana* could thrive once introduced to a new ecosystem. To understand the invasion potential of *M. charruana*, we sought to understand environmental thresholds for this species. For marine species in general, salinity is one of the most important abiotic factors that contribute to a species' distribution (e.g., Dame 1996). The distribution of adult bivalve molluscs is particularly affected by

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salinity as a result of their limited mobility (Castagna & Chanley 1973). Many marine bivalves are thus able to withstand minor changes in osmotic concentration in the environment (Shumway 1977a). According to Dame (1996), salinity-driven changes in (1) total osmotic concentration (Shumway 1977b), (2) relative proportions of solutes, (3) coefficients of adsorption and saturation of dissolved gases, and (4) density and viscosity (Kinne 1964) can all affect the functional properties of bivalve molluscs. Although ocean salinity is typically at or near 35 ppt (Dame 1996), in brackish water bivalves must change to hyperosmostic regulation of their body fluids (Deaton et al. 1989). A salinity tolerance study done by Castagna and Chanley (1973) examined 36 species of bivalves. Their study found that small incremental changes in salinity allow species to broaden their tolerance threshold.

Little is known about the ecology and life history of *M. charruana*. In this study we investigated several aspects of its salinity tolerance that mimic a variety of salinity conditions that *M. charruana* may experience. Specifically, we addressed the following questions: In what range of salinities is survival possible for *M. charruana* if slowly adjusted to test salinities? In what range of salinities can these mussels survive when experiencing rapid changes of salinity? In what range of salinities can *M. charruana* survive with temporary, rapid changes of salinity (6-h duration)? Furthermore, we tested for differences in salinity tolerance between large and small mussels in all 3 experiments. These results are discussed with regard to how salinity, in general, can influence invasion success.

MATERIALS AND METHODS

Collection

Mytella charruana were collected from floating docks along the northeast coast of Florida (Fig. 1, Table 1). The location of the collections varied in this region, because each experiment required 600 individuals, and 1 site did not have sufficient mussels for all trials.

Mussels were transported to the laboratory in Orlando, FL, in insulated coolers filled with enough water to cover all collected mussels. A thermometer was placed in the cooler and was regularly checked. Temperature varied no more than 2°C during transport. All mussels were placed in large aquaria (76–151 L) with circulating water from the collection site. Individuals were not disturbed for the next 7–10 days, except for the removal of dead mussels. Salinity was checked daily with a portable refractometer and maintained at collection site salinity with either deionized water or a mixture of deionized water and collection site water. Experiments were conducted from January 13, 2008, through January 6, 2009 (see Table 1 for details).

Experimental Setup and Feeding Regimen Common to All Experiments

For all experiments, *M. charruana* were randomly selected from the holding tanks. Shell lengths were measured with digital calipers, and mussels were placed into 2 categories: large mussels (≥19.0 mm) and small mussels (<19.0 mm). Fifteen individuals were placed in each plastic tank (HerpHaven Kritter Keepers, (Lee's Aquarium & Pet Products. San Marcos, California) 23 × 15 × 17 cm) with an air pump for aeration. Prior to starting all experiments, salinity treatments were randomly assigned to each tank. Water temperature in all tanks was maintained at 19–20°C. Mussels were exposed to light for 12–14 h each day.

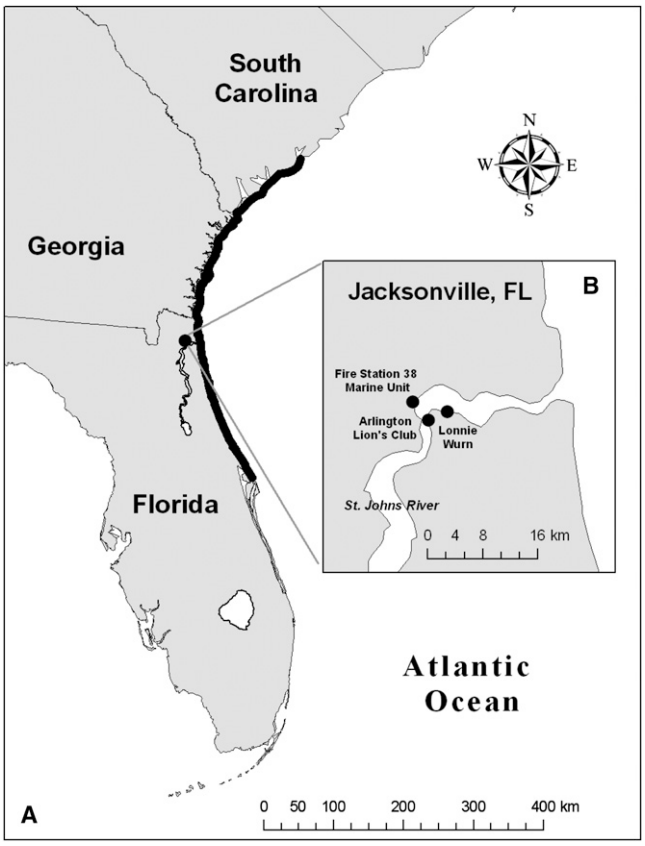

Figure 1. (A) Range of *Mytella charruana* in the southeastern United States as of December 2009. (B) Collection sites for *M. charruana* in Jacksonville, FL.

Each tank contained 2 L water in a ratio of 25% collection site water:75% artificial seawater (Instant Ocean salts and deionized water). A 100% water change occurred every third day. For the large mussel trials, a clay tile was placed on the bottom of each tank on which mussels could attach with their byssal threads. During each water change, the tiles with mussels were removed from the tank to reduce stress on mussels. This was not done for the small mussel trials because many of these mussels attached to the sides of the tanks. Mussel mortality was checked daily. If mussels remained gaping and did not respond to physical stimulation, they were considered dead and removed from tanks.

All mussels were fed daily with an algal paste that contained *Chaetoceros-B, Phaeodactylum tricornutum,* and *Nannochloropsis oculata* from Innovation Aquaculture (SPAT formula brand). Large mussels were given 10 drops of a 10% solution (0.5 mL) per tank (2 L) with a disposable pipette per 15 mussels, whereas small mussels were given 5 drops per 15 mussels. Volume of food per tank was reduced with mussel mortality. Three types of experiments were run: adjustment, permanent shock, and 6-h temporary shock. All trials were separately conducted with large mussels and small mussels.

Adjustment Experiments

Salinity at the three Jacksonville, FL, sites ranged from 10–31 ppt. Trials were run at the following salinities: 0, 2, 5, 14, 23,

TABLE 1.

Experimental conditions and results for each experiment.

Experiment	A: Adjustment	B: Adjustment	C: Permanent Shock	D: Permanent Shock	E: 6-Hour Shock	F: 6-Hour Shock
Mussel size range, mm	19.5–53.9 Large	3.0–19 Small	19.0–45.4 Large	4.9–18.6 Small	21.1–51.6 Large	3.6–18.9 Small
Collection date	1/13/2008	2/9/2008	5/11/2008	1/6/2009	5/27/2008	1/6/2009
Collection site	Arlington Lions Club dock (Jacksonville, FL)	Arlington Lions Club dock (Jacksonville, FL)	Lonnie Wurn dock (Jacksonville, FL)	Fire Station 38 Marine Unit (Jacksonville, FL)	Lonnie Wurn dock (Jacksonville, FL)	Fire Station 38 Marine Unit (Jacksonville, FL)
GPS coordinates	30°22′41.46″N, 81°37′14.28″W	30°22′41.46″N, 81°37′14.28″W	30°22′31.38″N, 81°35′8.58″W	30°23′14.40″N, 81°38′17.94″W	30°22′31.38″N, 81°35′8.58″W	30°23′14.40″N, 81°38′17.94″W
Collection salinity, ppt	13.0	10.0	31.0	15.0	31.0	15.0
Collection water temperature, °C	20	19	28	18	28	18
Holding period, days	10	7	10	10	10	10
Acclimation period, days	15	15	0	0	0	0
Experiment period, days	28	28	12	12	12	12
Salinities tested, ppt	0, 2, 5, 14, 23, 31, 40, 45	0, 2, 5, 14, 23, 31, 40, 45	0, 2, 5, 14, 23, 31, 40, 45	0, 2, 5, 14, 23, 31, 40, 45	0, 2, 5, 14, 23, 31, 40, 45	0, 2, 5, 14, 23, 31, 40, 45
Mussels per tank	15	15	15	15	15	15
Tanks per salinity treatment	5	5	5	5	5	5
Kaplan-Meier	$P < 0.0001$	$P < 0.0002$	$P < 0.0001$	$P < 0.0001$	$P = 0.2892$	$P = 0.3166$
One-way analysis of variance	$F = 8.877$, $P < 0.0001$	$F = 7.213$, $P < 0.0001$	$F = 70.141$, $P < 0.0001$	$F = 27.863$, $P < 0.0001$	$F = 0.320$, $P = 0.939$	$F = 0.267$, $P = 0.973$

Acclimation period was time in holding tanks prior to starting experiments. Adjustment period was number of days used to adjust to tested salinities at the start of adjustment experiments. Note that letters correspond to the experimental treatments presented in Figures 2 and 3.

31, 40, and 45 ppt. During a 15-day adjustment period, mussels went through a gradual change of salinity from their collection site salinity to the randomly assigned salinity treatments for their tank (Table 1). The amount of change in salinity (0–3 ppt) each day depended on the difference in the collection site and the experimental treatment salinity. Adjustment trials were run for 28 additional days after reaching treatment salinities.

Permanent Shock and 6-Hour Shock Experiments

Shock trials required no salinity adjustment and were run for a total of 12 days at the experimental salinities. Two types of shock trials were performed: permanent shock and 6-h temporary shock (6-h shock). For permanent shock trials, *M. charruana* remained in the predetermined shock salinity treatments (0–45 ppt) for the entire length of the trial after the initial 7–10 days in the holding tanks. For the 6-h shock trials, *M. charruana* were placed in their treatment salinities for 6 h at the start of the trials and then returned to the salinity at the collection site for the remainder of the 12 days (Table 1).

Statistics

Survival at the end of each experiment was analyzed with one-way analysis of variance using SPSS version 17.0.2 (SPSS Inc., Chicago, IL). This was followed by Tukey's *a posteriori* tests to determine significant differences among treatments. Kaplan-Meier survival analysis (Kaplan & Meier 1958) was used to compare survivorship throughout the entire length of each experiment (days until death) using pairwise comparisons to determine whether significant differences were present among treatments. The Kaplan-Meier survival analyses were done using JMP version 10 (SAS Institute, Cary, NC).

RESULTS

Adjustment Experiments

Mytella charruana exhibited significantly different rates of survival among the tested salinity treatments for both large and small mussels. The highest mean survival values in the large-mussel trial were at salinities between 2 ppt and 23 ppt (45% and higher; Fig. 2). In contrast, large mussels had 0% survival at the end of the experiments at 0 ppt and 45 ppt (Fig. 2). Trials containing small *M. charruana* followed a similar trend, with high mean survival at 2, 14, 23, and 40 ppt, and lowest survival at 0 and 45 ppt, with only 2% of the mussels remaining alive at 43 days (Fig. 3). Through Kaplan-Meier survival analysis, we were able to identify significant differences in the effects of

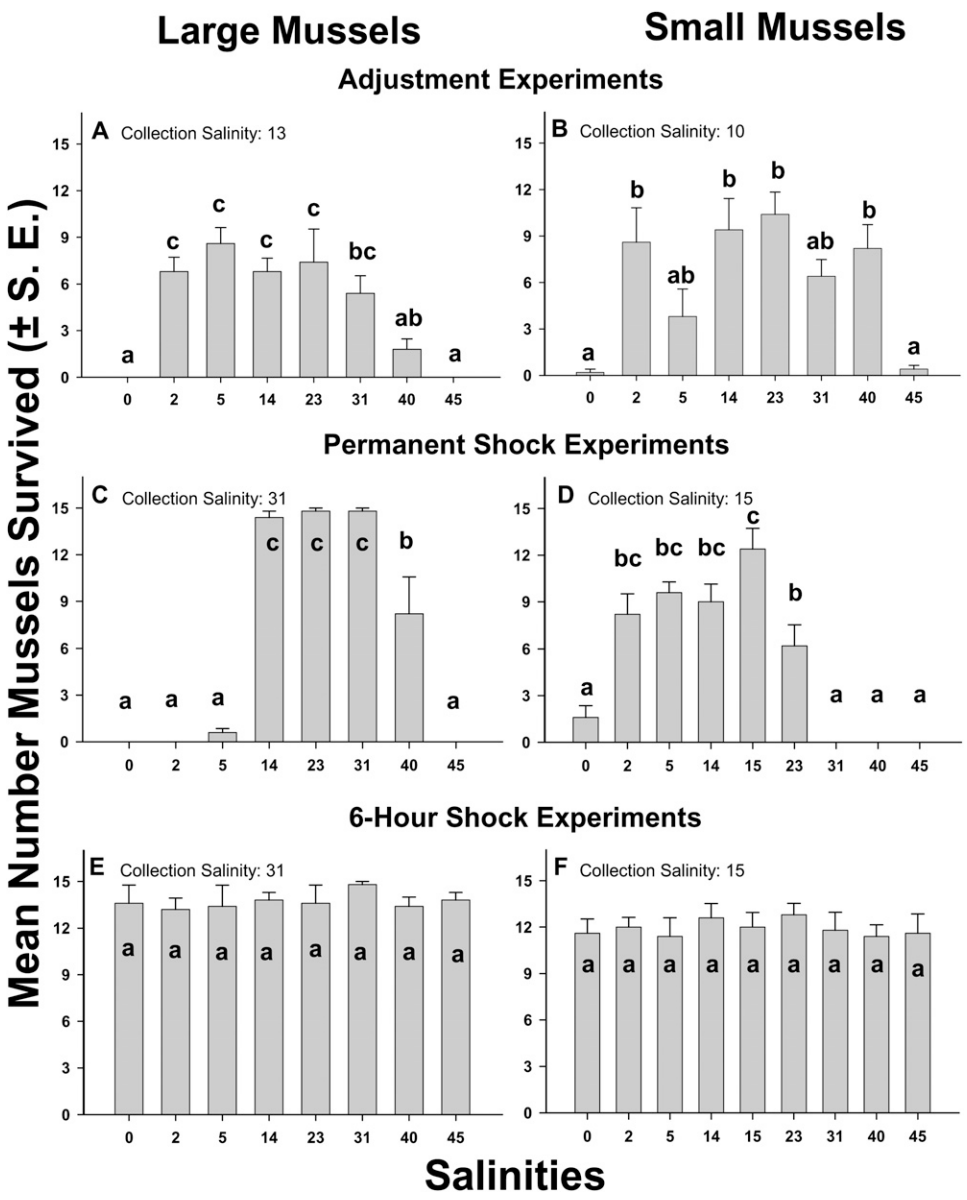

Figure 2. (A–F) Mean survival of *Mytella charruana*. Mean survival of *M. charruana* in adjustment experiments for large (A) and small (B) mussels. Mean survival of *M. charruana* in permanent shock experiments for large (C) and small (D) mussels. Mean survival of *M. charruana* after 6-hour shock experiments for large (E) and small (F) mussels. Salinity measured as parts per thousand. Treatments with different letters indicate significant differences at $P = 0.05$. See Table 1 for experiment details.

salinity treatments on survivorship over time within trials of both large and small mussels (Fig. 3). There were no significant differences between small *M. charruana* at salinities of 2, 14, 23, and 40 ppt (Fig. 3). Moreover, mussels in the two salinity extremes exhibited no significant difference between 0 ppt and 45 ppt, where survival was lowest. Large mussels had no significant differences in survival between 2–31 ppt, and showed a significant decline in survivorship at 40 ppt. The survival at 0 ppt and 45 ppt was significantly lower than all other treatments, but not significantly different from each other (Fig. 3).

Permanent Shock Experiments

Large *M. charruana* had the highest final mean survival at salinities of 14, 23, and 31 ppt (collection salinity), and had significantly reduced or no survival at 0, 2, 5, 40, and 45 ppt (Fig. 2). Small mussels had the highest survival at 15 ppt (collection salinity), followed by 2, 5, and 14 ppt. Mussels in this trial had low survival at 0 ppt and no final survival at 31, 40, or 45 ppt (Fig. 2). Kaplan-Meier survival analysis showed 96% or higher survival, and hence similar slopes for large mussels at 14, 23, and 31 ppt, and significant decreases in slopes for 40 ppt followed by 5 ppt. For 0 ppt and 2 ppt, the survivorship curves were similar, with 100% mortality by day 11. Mussels in 45 ppt died significantly faster than all other mussels, with 100% mortality reached at day 8 (Fig. 3). Meanwhile, the survival analysis showed small mussels also had significant differences between treatments. The small mussels exposed to the highest salinities (40 ppt and 45 ppt) died faster than all other treatments, followed by mussels at 31 ppt (Fig. 3).

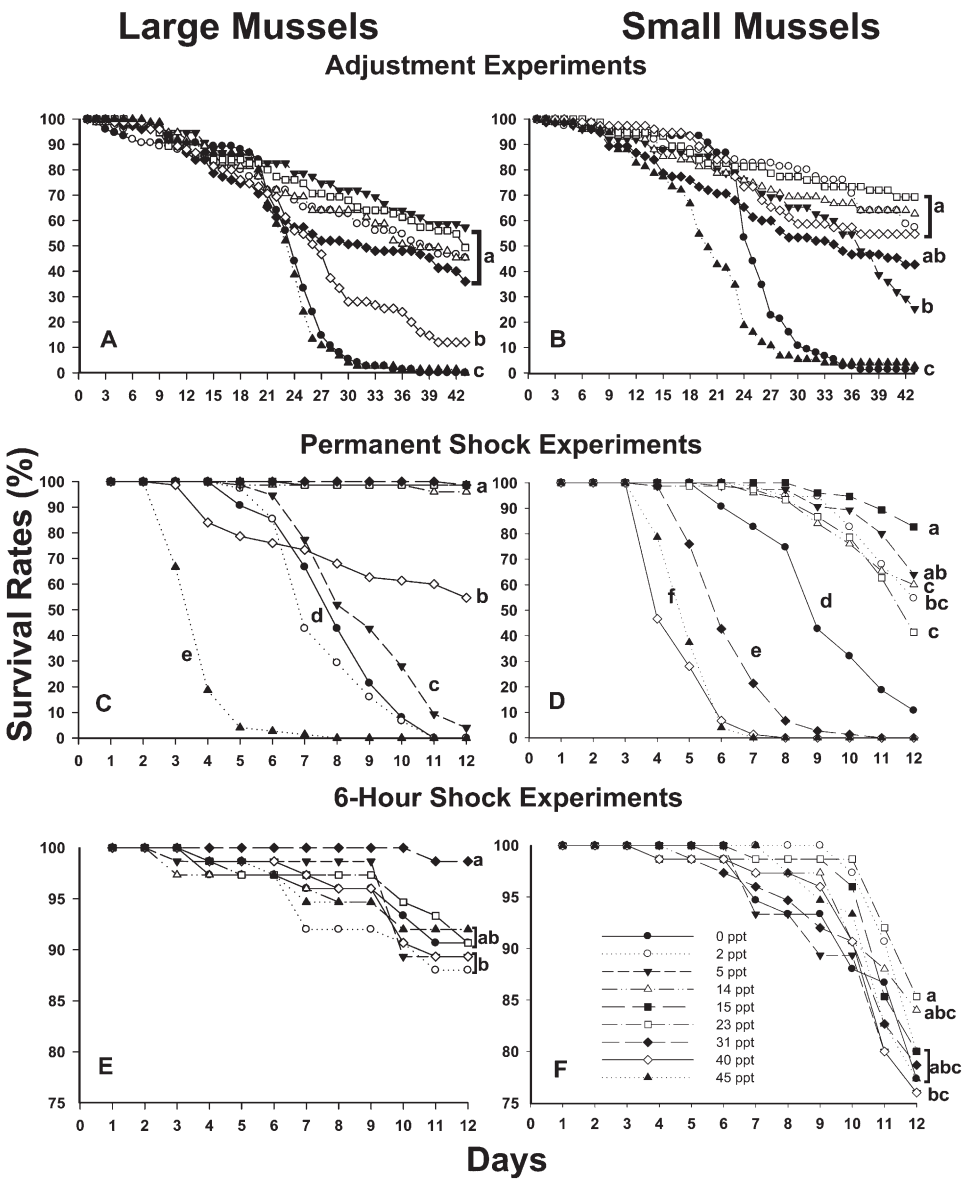

Figure 3. (A–F) Survivorship over time of *Mytella charruana*. Survival of *M. charruana* in adjustment experiments for large (A) and small (B) mussels. Survival of *M. charruana* in permanent shock experiments for large (C) and small (D) mussels. Survival of *M. charruana* after 6-hour shock experiments for large (E) and small (F) mussels. Note scale change in E and F. Treatments with different letters indicate significant differences at $P = 0.05$. See Table 1 for experiment details.

6-Hour Shock Experiments

Both large and small mussels had no significant difference in final survival among all tested salinities. The final mean survival values for large mussels was 88% or greater, with 31 ppt (collection salinity) having the highest final mean survival of 99% (Fig. 2). The final mean survival of small mussels was 76% or greater, with the highest final mean survival of 85% at 23 ppt (Fig. 2). There were significant differences in survival over time between treatments in the survival analysis for both large and small mussels (Fig. 3). Although high, survivorship was significantly reduced at 2 ppt and 40 ppt relative to 31 ppt for large mussels, and 40 ppt relative to 23 ppt for the small mussels (Fig. 3).

DISCUSSION

Nonnative species pose an ecological threat when they are introduced to new environments because of their potential to outcompete native species and alter the ecosystem (Pimentel et al. 2005). Although we currently have a good grasp on how marine species are typically transported (e.g., Carlton & Geller 1993, Chapman et al. 2003, Padilla & Williams 2004, Walters et al. 2006), it is less understood why some species are good invaders whereas others are not (Ehrlich 1986). This study examined the tolerances of large and small *M. charruana* under a range of salinity conditions that may be encountered in estuaries and nearshore coastal waters. Our results provide insights into why *M. charruana* has successfully invaded the eastern coast of the United States.

We found that *M. charruana* exhibited a very wide range of salinity tolerances, with limits for survival ranging from 2–40 ppt for long-term exposures, and an even wider range that included 0 ppt and 45 ppt for short-term fluctuations in salinity. The salinity tolerances we found would suggest that *M. charruana* could survive ocean transport either in ballast water or as hull fouling organisms. According to Carlton and Geller (1993), 71% of tested ballast water tanks contained adult bivalve molluscs. Although each vessel or ship is encouraged to perform open water ballast exchanges, it is difficult to enforce these regulations (Everett 2001, Murphy & Ruiz 2001, Baker et al. 2007). The ballast water exchange process involves replacing the brackish water collected at port with ocean water; this difference in salinity is primarily effective at killing species that prefer low salinities (United States Coast Guard 2009). Our study indicates that even small *M. charruana* should be able to survive in ballast tanks, where they would be able to withstand sudden change in salinity for prolonged periods of time.

The range and pattern of salinity tolerance found for this species indicate at least 3 reasons why *M. charruana* is a successful invader. First, as mentioned earlier, *M. charruana* can likely withstand transport in ballast water or attached to boat hulls. Second, after the mussels are released into new environments they may become established as a result of their ability to endure a wide range of salinities. Third, unlike marine invertebrates that have very limited salinity tolerance ranges as juveniles (Gillanders et al. 2003), we found that small *M. charruana* survived even better than large *M. charruana* at extreme salinities. In addition, *M. charruana* has the ability to adapt and survive variable conditions in new environments as a result of the extended spawning period and environmentally induced sex change allowing them to be more successful in establishment (Stenyakina et al. 2009).

Another successful bivalve mollusc invader in the southeastern United States is the green mussel *P. viridis* (Baker et al. 2007). Like *M. charruana*, *P. viridis* is a recent invader (1999) and the two species are frequently now found growing side by side (Boudreaux & Walters 2006, Gilg et al. in press). *Perna viridis* is native in the Persian Gulf and waters surrounding Sumatra, Borneo, Bali, and Sulawesi (Siddall 1980, Vakily 1989, Baker et al. 2007). Many studies on *P. viridis* show it to exhibit a broad temperature tolerance (10–42°C), and fast growth rates (Rajagopal 1991, Rajagopal et al. 2003, Rajagopal et al. 2006, Baker et al. 2007), and these traits typify why *P. viridis* is a good invader. Segnini de Bravo et al. (1998) found that the lower and upper lethal salinities of *P. viridis* are 0 ppt and 64 ppt, whereas those of *M. charruana* are 0 ppt and 45 ppt. Species such as *M. charruana* and *P. viridis* can survive in the horohalinicum, which serves as a barrier between fresh and marine species, where estuarine salinity ranges from 3–8 ppt, and where many species cannot survive (Deaton 1981). In addition, *P. viridis* can successfully adapt over the long term to salinities between 19 ppt and 44 ppt. In comparison, *M. charruana* has a slightly wider range (2–31 ppt) skewed toward lower salinities. It is currently unclear whether *M. charruana* or *P. viridis* would dominate in a habitat between 19–31 ppt if resources were limiting.

Because *M. charruana* has successfully entered and dispersed in coastal and estuarine waters of the southeastern United States, it should raise concern regarding the extent of environmental harm that might arise from this invasion. Alternating periods of high and low densities of *M. charruana* have been found in Mosquito Lagoon, part of the Indian River Lagoon system on the east coast of central Florida, since 2004. *M. charruana* have been found on dock pilings, along the shore, and attached to living oysters on intertidal reefs (L. Walters, pers. obs.). The Eastern oyster *Crassostrea virginica,* is considered a keystone species (Berquist et al. 2006) in many estuaries where it is the dominant bivalve providing structure, refuge, and food for fishes and invertebrates (Boudreaux et al. 2006, Barber et al. 2010). The optimal salinity range for *C. virginica* is 6–28 ppt (Castagna & Chanley 1973, Wilson et al. 2005); however, they have frequently been found living at higher and lower salinities (Buroker 1983). Our study indicates that *M. charruana* has a wider range of salinity tolerance than *C. virginica*. Sharp decreases or increases in salinity caused by significant wet and drought conditions might enable *M. charruana* to outcompete *C. virginica*. Such a change would alter the faunal composition of the estuary. This competition is further enhanced by recent data that suggest that *M. charruana* can survive during intertidal conditions (i.e., they can survive out of water for 12 or more hours; Nash & Walters, unpublished data). *M. charruana* also poses a potential problem for 2 native marine mussels in the southeastern United States: the ribbed mussel *Geukensia demissa* and the scorched mussel *Brachidontes exustus*. *M. charruana* has been found living in contact with both of these species along the southeastern Atlantic coast (L. Walters, pers. obs.). *G. demissa* can survive in salinities ranging from 6–70 ppt, and *B. exustus* also exhibits a broad salinity tolerance range (10–41 ppt) (Barber et al. 2005, Berquist et al. 2006). It is currently unknown whether *M. charruana* could outcompete or limit the survival of these native mussels. However, if salinity were to drop below 6 ppt (e.g., during storm events), niche space could open up for recruitment of *M. charruana*, because they can survive at salinities as low as 2 ppt.

In summary, *M. charruana* has a wide salinity tolerance in both large and small size classes. Our trials indicated that these mussels can survive large fluctuations in salinity for long and short periods of time. We need to follow the invasion of *M. charruana* carefully because this species has the potential to cause ecological damage. Knowing the limits of these mussels can help predict its potential range and aid our understanding of the role *M. charruana* will play in the estuaries where it is found.

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