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Research Article

Effect of acute salinity changes on hemolymph osmolality and clearance rate of the non-native mussel, *Perna viridis*, and the native oyster, *Crassostrea virginica*, in Southwest Florida

Katherine McFarland, Ludovic Donaghy and Aswani K. Volety*

Florida Gulf Coast University, Vester Marine Field Station 10501 FGCU Blvd South, Fort Myers, FL 33965, USA

E-mail: kmcfarland@fgcu.edu (KM), ldonaghy@fgcu.edu (LD), avolety@fgcu.edu (AKV)

**Corresponding author*

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Abstract

The green mussel *Perna viridis* is a recent invader to Southwest Florida and, though it is currently found only in high salinity areas, concerns abound that it may threaten native oysters. The objective of this study was to compare the responses of *P. viridis* and the native eastern oyster, *Crassostrea virginica,* to acute salinity changes by measuring hemolymph osmolality and clearance rate of algal cells over time. The osmolality of hemolymph *in vivo* and surrounding water were assessed regularly over a one-week period at seven test salinities ranging from 5 to 35. While oysters reached osmotic equilibrium at all salinities within 144 hours, hemolymph of green mussels remained hyperosmotic at salinities ≤ 10. Clearance rates of algae by *P. viridis* and *C. virginica* held in static tanks at four salinities (10, 15, 25, and 35) were measured, employing flow cytometry. At salinities of 25 and 35, green mussel clearance rates were approximately double those of oysters. Unlike native oysters, green mussel clearance rates decreased by an order of magnitude at salinities of 10 and 15. Further, at salinities of 10 and 15, *P. viridis* tended to close their valves. In a specific test of this behavior, 100% of mussels remained open at salinities of 25 and 35. At salinities of 10 and 15, mussels increasingly closed their valves over time, and within 120 hours of exposure all were either closed or dead. The chief concern about *P. viridis* is that it might compete with native bivalves for food and space. However, our results suggest that this recent invader may be salinity-limited, providing *C. virginica* with a refuge from competition in estuaries that experience acute periods of low salinity.

Key words: osmoconformer; valve closure; osmotic stress; filtration; non-native species

Introduction

The green mussel *Perna viridis* (Linnaeus, 1758) was recently introduced to the coastal waters of Southwest Florida, where it was first reported in Tampa Bay in 1999 (Benson et al. 2001; Ingrao et al. 2001). Its native range is the Indo-Pacific along the coasts of India and Southeast Asia, in habitats ranging from oceanic to estuarine waters (Wong and Cheung 2001; Rajagopal et al. 2006). Green mussels were first observed in the South Caribbean on the coast of Trinidad in the mid-1990s (Agard et al. 1992), and they have since spread throughout the Caribbean and South Atlantic Basin (Hawkins et al. 1998; Baker et al. 2007). It is thought that U.S. populations of green mussels originated from the Trinidad area and were likely introduced via ballast water or biofouling on the hulls of ships (Baker et al. 2007). Green mussels have been found growing in densities as great as 4000 individuals m^{-2} on hard substrate, including other bivalves (Wong and Cheung 1999; Fajans and Baker 2005). Due to their rapid growth rates and a high tolerance to a wide range of environmental conditions, they are a significant biofouling organism in water cooling systems throughout the Indo-Pacific (Masilamoni et al. 1997; Rajagopal et al. 2006). These traits also increase their potential to out-compete local bivalves when introduced elsewhere (Lee 1986).

In Florida, *P. viridis* co-occurs with the native eastern oyster, *Crassostrea virginica* (Gmelin, 1791), and was found growing on top of *C. virginica*, causing high mortality of the oysters (Baker et al. 2007). As of 2002, green mussels

have displaced approximately half of the native oyster populations in Tampa Bay (Baker et al. 2006). *Crassostrea virginica* is a keystone species and provides important ecosystem services in estuaries: oysters play an important role in benthic-pelagic coupling; they create complex three-dimensional structures that serve as habitat for invertebrates and fishes (Wells 1961; Henderson and O'Neil 2003; Peterson et al. 2003; Tolley et al. 2005; Tolley and Volety 2005; Abeels et al. 2012); and oysters and the reefs they build protect and stabilize shorelines and sediments in estuaries. In contrast, green mussels tend to form a less rugose, two-dimensional mat over the substrate, attaching to each other and to hard surfaces by means of byssal threads. Thus, where green mussels displace oysters, they do not provide the same quality habitat that is vital for the survival of many estuarine species (Tolley and Volety 2005; Baker et al. 2007; Abeels et al. 2012).

Salinity is one of the most important environmental factors that affects dispersal and settlement of bivalve mollusks (Vakily 1989; Shumway 1996; Berger and Kharazova 1997). In Southwest Florida estuaries, average salinities range from 28 to 38 during the dry winter season and from 0 to 10 during the wet summer season (Barnes et al. 2007; Volety et al. 2009). In addition, locally managed freshwater releases for flood control induce large variations in salinity in some estuaries. In Estero Bay, Florida, *P. viridis* is largely restricted to the mouth of the estuary, where salinities are high, and to coastal buoys in adjacent marine waters (A. K. Volety, personal observation). However, the ability of green mussels to spread throughout Estero Bay is currently unknown.

Marine mollusks generally have a limited ability for internal osmotic regulation (Henry et al. 1980). Valve closure is the first and main response to a stressful salinity change. This behavioral response aids in preventing osmotic stress by secluding the animal from the external environment, but can only provide short-term protection from adverse conditions (Shumway 1977a, b; Shumway et al. 1977). Valve closure can allow for the internal osmotic concentration to remain high when external salinity is low and may prevent a rapid decrease in internal osmotic concentration leading to osmotic shock (Davenport 1979). Internal fluid (hemolymph) osmolality may therefore be used as an indicator to characterize the response of individual bivalves to changes in salinity. However, bivalves are filter-feeders, and valve closure and metabolic changes associated with salinity stress also alters filtration activity. Therefore, clearance rate, defined as the volume of water cleared of particles in a given time interval (Bayne et al. 1976; Hildreth and Crisp 1976), has been used to quantify physiological effects of environmental stressors, including heavy metals (Chong and Wang 2001; Anandraj et al. 2002), salinity (Bayne 1973), hypoxia (Norkko et al. 2005; Wang et al. 2005), quantity/quality of food (Bayne et al. 1987) and harmful algae (Hégaret et al. 2007). Thus, changes in hemolymph osmolality, clearance rate, and valve closure in response to acute salinity decreases can be used to help predict not only the response of *P. viridis* to reduced salinities encountered during the wet season, but also their potential to spread.

The goal of this study was to compare the ability of the invasive green mussel and the native oyster to cope with the extreme variation of salinity that occurs in many Southwest Florida estuaries. We therefore measured hemolymph osmolality, phytoplankton clearance rates, and valve opening/closing behavior of test animals subjected to acute changes in salinity.

Methods

Collection and maintenance of organisms

Green mussels were collected from New Pass Bridge in Estero Bay, Florida (26°22′40.75″N; 81°51′39.30″W), and oysters were collected from a reef inside the pass (26°22′35.25″N; 81°50′ 36.46″W), between August 2010 and March 2011. Bivalves were cleaned of epiphytic growth and kept in holding tanks with recirculating water at a salinity of 30 and temperature of 21–23°C, approximating the conditions at which they were collected, for one week prior to the beginning of the experiments. Test animals were fed Shellfish Diet ® (Reed Mariculture Inc., Waring, Texas, USA) at a rate of ~1 mL per individual per day. Green mussels and oysters used in this study had shell lengths of 90–110 mm and 80–100 mm, respectively.

Growth and maintenance of algae cultures used in clearance experiments

The microalga *Isochrysis sp.* (strain 1324) was obtained from the National Center for Marine Algae and Microbiota (East Boothbay, Maine, USA) and grown in f/2 media at a salinity of 30

using a 12:12 hour light/dark cycle. All media were made with 0.1µm filtered seawater, adjusted with DI water or Instant Ocean ™ (Aquarium Systems Inc., Cincinnati, Ohio, USA) to achieve optimal salinities, and were autoclaved prior to use. Temperature was kept constant at 25°C until densities reached 10^6 cells/mL. Concentrations of algal cells were determined prior to each experiment using a hemocytometer.

Effect of acute salinity change on hemolymph osmolality

To test the effect of acute salinity changes on hemolymph osmolality in *P. viridis* and *C. virginica*, six tanks, three containing 20 oysters and three containing 20 mussels were used for each salinity treatment. Treatment salinities were 5, 10, 15, 20, 25, 30, and 35. Exposures were conducted in a static system held at ambient temperature. Mussels were fed daily and water changes completed every 2 days. Mortality was monitored daily with the immediate removal of dead animals to prevent degradation of the water quality.

Paired hemolymph and water samples were taken from each tank at the beginning of the experiment and then at 1, 4, 8, 12, 24, 48, 96, and 144 hours thereafter. Hemolymph was sampled by withdrawing 500 µL from the adductor muscle using a 1-mL syringe with a 27-gauge needle. Each sample was analyzed under a microscope to ensure hemocytes were present, and that there was no contamination with tissue debris. Once checked, samples were frozen for later analysis. Water samples were likewise frozen for later analysis. No bivalves were bled more than once, and bivalves were not returned to experimental tanks following sampling. At the end of the experiment, samples were analyzed in a VaporPro® vapor pressure osmometer (Wescor Inc., Logan, Utah, USA) to determine osmolality of the hemolymph and corresponding tank water.

To compare the responses of *P. viridis* and *C. virginica* to different salinities over time, a repeated measures ANOVA was employed with two among-subjects factors (salinity and species) and one within-subjects factor (time). The dependent variable was the difference between the osmolality of a given hemolymph sample and the corresponding water sample. Due to violations of the assumptions of normality and homoscedasticity, the analysis was performed on rank-transformed values. Significant interactions were examined graphically.

Effects of an acute salinity change on clearance rates

Clearance rates were assessed at four salinities (10, 15, 25, and 35) to compare the effect of acute salinity changes on *P. viridis* and *C. virginica*. Prior to the experiment, bivalves were given a 2-day adjustment period to allow time for valves to open. They were fed on the first day and fasted on the second. Clearance rates of oysters and mussels were measured following the method described by Shumway et al. (1985). For each species and salinity treatment, five 2-L tanks were used, each containing one individual bivalve. Five control tanks, containing only water and empty shell, were also set up for each salinity level to provide a measure of passive cell loss from the water column. The alga *Isochrysis sp.* was added to each test tank at a concentration of 20,000 cells mL^{-1} and was kept in suspension with gentle aeration. Water samples were then drawn from each tank at 0, 30 and 60 minutes. The experiment was repeated on 3 different days within a one-week period, and was carried out at ambient temperature (21–23°C). After each trial, whole dry tissue weight was measured for each individual by drying at 60°C for 72 hours, and shell length was recorded.

At each sampling time, microalgal concentration was determined using a Cytomics FC 500 flow cytometer (BD Biosciences Inc., San Jose, California, USA). Algal cells were differentiated and counted based on internal complexity (SSC parameter) and chlorophyll fluorescence (FL3 parameter). Clearance rates were calculated using the equation $CR = V \times (\ln(C_0/C_t)/t - A)$ (Coughlan 1969) where V is the volume of water in each jar, C_0 is the initial concentration before the bivalve is placed in the jar, C_t is the concentration at sampling time t, t is the time, and A is the average algal cell loss in control jars, used to account for any decrease in phytoplankton concentration due to sinking or adhering to the sides of the jar. Clearance rates were normalized per gram of whole dry tissue weight ($L\ h^{-1}g^{-1}$) in order to compare among individuals and between species.

Clearance rates were compared using a repeated measures design with two among-subjects factors (species and salinity) and one within-subjects factor (sampling time). The day on which each exposure was conducted was treated as a random block factor, but upon determining that there were no significant interactions involving the day and no significant effect of day, the data for

Table 1. Comparisons of the difference between hemolymph and water osmolality in *Perna viridis* and *Crassostrea virginica* at different salinities over a one-week period.

	df	Mean Square	F	p
Among Tanks				
Species	1	32060.017	14.621	0.001*
Salinity	6	387357.443	179.652	<0.0005*
Species×Salinity	6	11695.833	5.334	0.001*
Tank(Species×Salinity)	28	2192.763	1.011	0.456
Between Tanks				
Time	8	60292.913	27.791	< 0.0005*
Time×Species	8	16719.959	7.707	< 0.0005*
Time×Salinity	48	12130.783	5.591	< 0.0005*
Time×Species×Salinity	48	6028.513	2.779	< 0.0005*
Time×Tank(Species×Salinity)	223	2169.513		

*shows significance

all days were pooled. Due to violations of the assumptions of normality and homoscedasticity, the analysis was performed on rank-transformed data. Significant interactions were further examined graphically and analyzed using Tukey's HSD to identify specific differences among treatments for each species separately.

Valve Closure

Valve closure was monitored to help define filtering capacity and determine how much valve closure disrupted clearance rates in *P. viridis*. Following an accommodation period of 3 days at a salinity of 30, mussels (n=10/tank) were placed in 40 L aquaria at salinities of 10, 15, 25 and 35, and were observed after 1, 8, and 24 h, then daily for 5 days. Valves were categorized as "closed" when no clear opening was observable; "slightly opened" when valves were partially open but gills were not protruding; "gaping" when valves were wide open and gills were protruding; and "dead" if a mussel was dead. Dead bivalves were removed from the tanks to prevent deterioration of water quality. Resulting counts were analyzed using two-way contingency tables for each time. Mortality only occurred in any treatment after 48 hours, so the dead category was only included in the analyses at 96 and 144 hours. Due to the small sample sizes, Fisher's exact test was used to detect significant deviations from independence of valve state and salinity.

Results

Effect of acute salinity change on hemolymph osmolality

Oysters and mussels differed in their osmotic responses to acute salinity change (Table 1). There was a significant three-way interaction among salinity, species, and time ($F_{48,223}$=7.07, P<0.0005), indicating that the two species reacted in different ways to the salinity changes over the course of the exposure. Hemolymph osmolality of oysters tracked treatment salinity, reaching osmotic equilibrium within the sampling period (144 h) at all salinities (Figure 1). In contrast, hemolymph osmolality of mussels only tracked test salinities ≥15. Below 15, hemolymph of mussels remained hyperosmotic relative to the external environment throughout the sampling period. Additionally, mortalities were observed in mussel tanks at salinities of 5 and 10 resulting in reduced numbers at the last sampling period. Oysters, however, were only hyperosmotic to the external water during the initial exposure at salinities of 5 and 10, and reached iso-osmotic conditions by 48 hours at a salinity of 10 and by 144 hours at 5. At salinities 15–35, oyster and mussel hemolymph conformed to the water osmolality within 24 hours.

Effects of acute salinity change on clearance rates

There was a significant interaction between species and salinity ($F_{3,112}$ = 7.514, P < 0.0005),

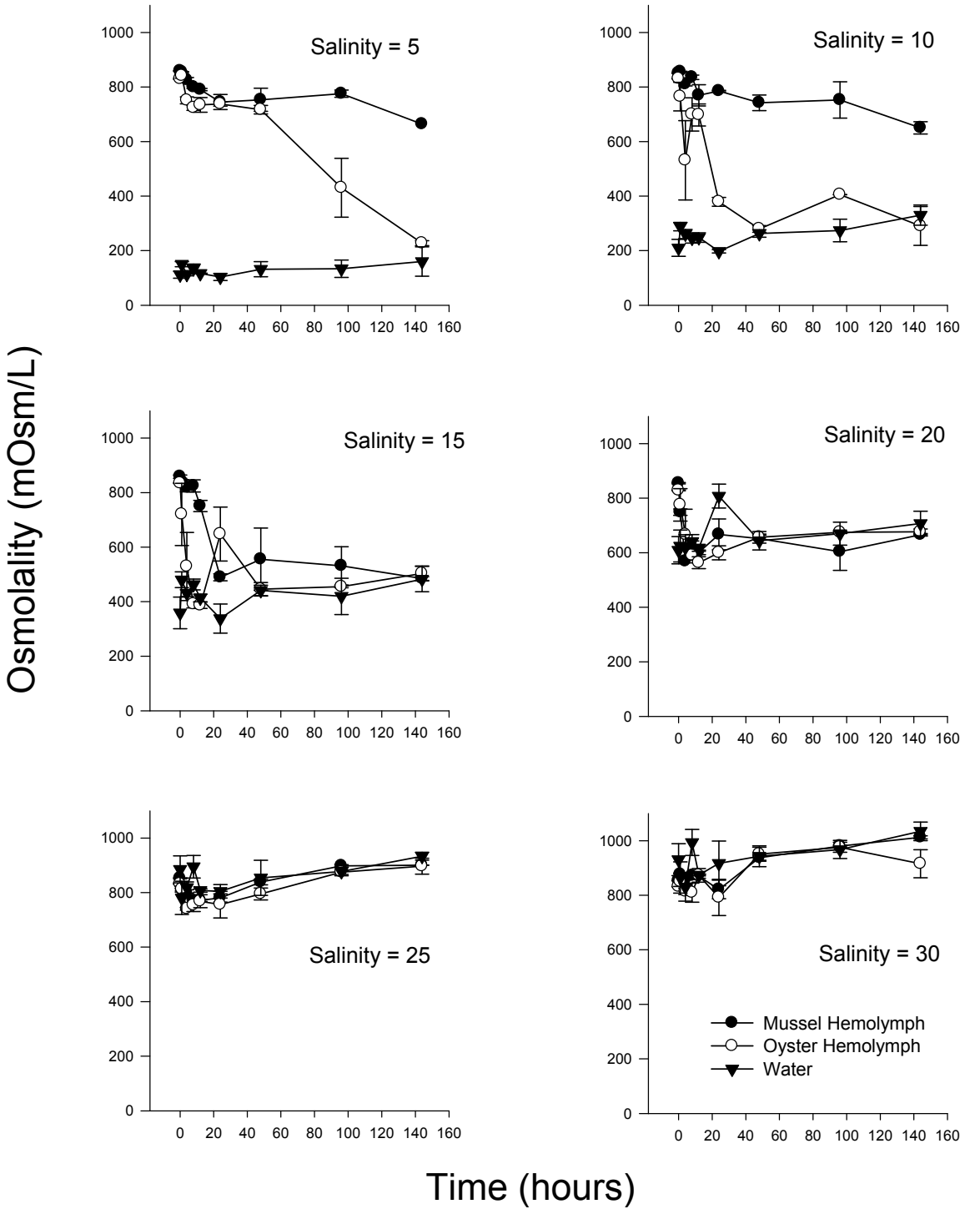

Figure 1. Hemolymph osmolality in green mussels and oysters at test salinities. Data expressed as mean values of three replicates for each sampling time and salinity. Bars represent standard error. Responses of oysters and mussels at salinity of 35 were similar to those at 30 and are not shown here.

Table 2. Comparison of clearance rates (L $h^{-1}g^{-1}$) at different salinities and times for oysters and green mussels.

	df	Mean Square	F	p
Among Tanks				
Salinity	4	926249.0646	186.327	<0.0005*
Species	1	45816.067	9.217	0.003
Species×Salinity	3	37351.761	7.514	<0.0005*
Tank(Species×Salinity)	112	4971.047	2.853	<0.0005*
Between Tanks				
Time	1	1809.504	1.039	0.310
Time×Species	1	8.438	0.005	0.945
Time×Salinity	3	1909.293	1.096	0.354
Time×Species×Salinity	48	2794.737	1.604	0.192
Time×Tank(Species×Salinity)	223	1742.386		

*shows significance

Table 3. Results of Tukey's HSD multiple comparisons of salinities for green mussels. Underlining shows homogeneous groups.

Salinity	10	15	25	35
Mean	0.0927	0.0857	0.8963	0.8713

Table 4. Results of Tukey's HSD multiple comparisons of salinities for oysters. Underlining shows homogeneous groups.

Salinity	10	35	15	25
Mean	0.0927	0.3017	0.4110	0.3117

Table 5. Comparison of valve closure behavior over time.

	χ^2	df	p
Time			
1	61.818	6	< 0.0005*
8	52.286	6	< 0.0005*
24	41.667	6	< 0.0005*
36	46.667	6	< 0.0005*
48	40.000	6	< 0.0005*
96	48.200	9	< 0.0005*
120	60.779	9	< 0.0005*

*shows significance

indicating that the two species reacted in different ways to the different salinity treatments (Table 2). Clearance rates of green mussels ranged from 0.08 to 1.2 L $h^{-1}g^{-1}$ dry tissue weight (Figure 2), and were significantly lower at salinities 10 and 15 compared with 25 and 35 (Tukey's HSD, $p<0.0005$ for all significant pairwise comparisons) (Table 3). At 25 and 35, clearance rates of green mussels were 2–3 times higher than those of oysters, whereas at lower salinities (10 and 15), clearance rates for green mussels were similar to those of oysters. Clearance rates of oysters ranged from 0.09 to 0.43 L $h^{-1}g^{-1}$ dry tissue (Figure 2) and varied less among salinities (Table 4). The clearance rate at 25 was highest though it was similar to those at 15 and 35 (Tukey's HSD, $p=0.278$), and it was significantly higher than the clearance rate at 10 (Tukey's HSD, $p=0.009$), indicating that 25 may be at or near an optimal value for *C. virginica* (Table 4).

Valve Closure

Contingency table analysis indicated that valve behavior of *P. viridis* was dependent on salinity at all times (Table 5). Within 5 minutes of being

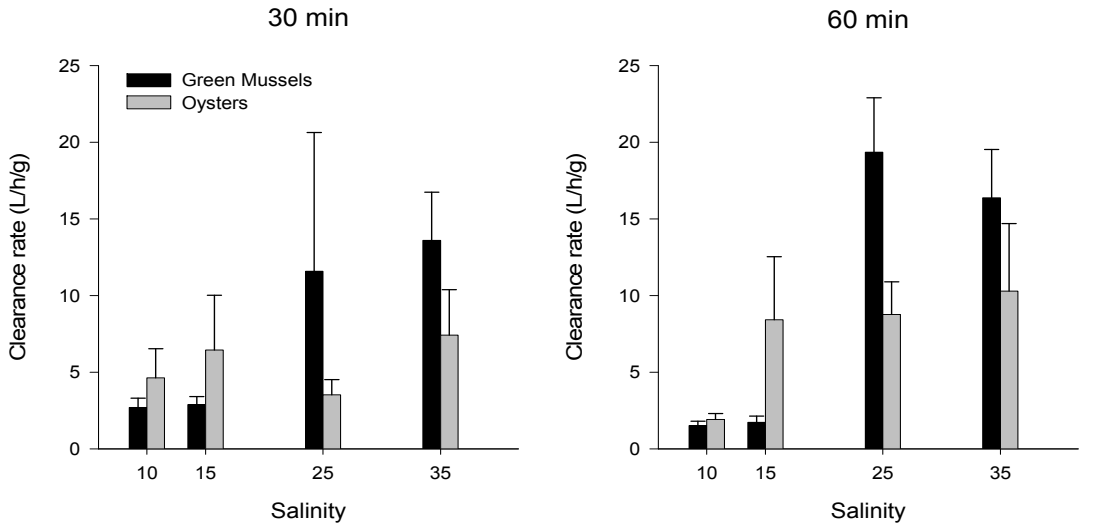

Figure 2. Mean clearance rates of pooled replicates for oysters and green mussels at 30 and 60 min. Bars represent standard error.

placed in the tank at a salinity of 35, 100% of the mussels were gaping with gills exposed to the external environment (Figure 3). At a salinity of 25, 50% of the mussels were partially opened (no gills protruding) within 1 hour, and within 8 hours 100% were gaping with gills exposed. At salinities of 15 and below none of the mussels showed the same gaping behavior with exposed gills observed at salinities of 25 and 35. In addition, mortalities were observed at salinities 10 and 15, 60% and 40% respectively with no mortalities observed at 25 and 35. After 48 hours at a salinity of 15, 100% of the mussels were partially opened, but this number decreased over time and by the end of the exposure (120 hours) all living mussels remained closed. At a salinity of 10 the number of mussels partially open peaked at 50% after 48 hours and at the end of the exposure all mussels were either dead or closed.

Discussion

When exposed to salinities near or beyond their tolerance limits, the initial bivalve response is valve closure. This behavior temporarily isolates the animal from the external environment and prevents rapid changes in the osmotic concentration of their body fluids (Pierce 1971a; Shumway 1977a; Berger and Kharazova 1997). Although marine bivalves are typically considered to be osmoconformers, some possess a limited ability to regulate cell volume through the mobilization and loss of internal solutes in the form of free amino acids (Pierce 1971a, b; Pierce 1982; Kube et al. 2006). This release of solutes from the cells reduces water intake and prevents cell lysis (Pierce 1971a b; Pierce 1982). When external conditions are acceptable for the animal, and valves are open, the osmotic pressure of the external medium, intrapaleal fluid, and hemolymph reach equilibrium. During valve closure, normal physiological processes, such as clearance of the surrounding water column, are inhibited. If undesirable conditions persist, and the animal is not able to remain open long enough for sufficient gas and food exchange, the animal will eventually die. Thus, there is a tradeoff between the need to interact with the external environment and the need to be isolated from it. The aim of this study was to compare the ability of green mussels and native oysters to cope with salinity conditions prevailing in Southwest Florida estuaries by assessing their hemolymph osmolality, clearances rates, and valve closure behavior at decreased salinities. Within the context of this tradeoff, our results suggest that, although the geographic ranges of *P. viridis* and *C. virginica* now partially overlap, the difference

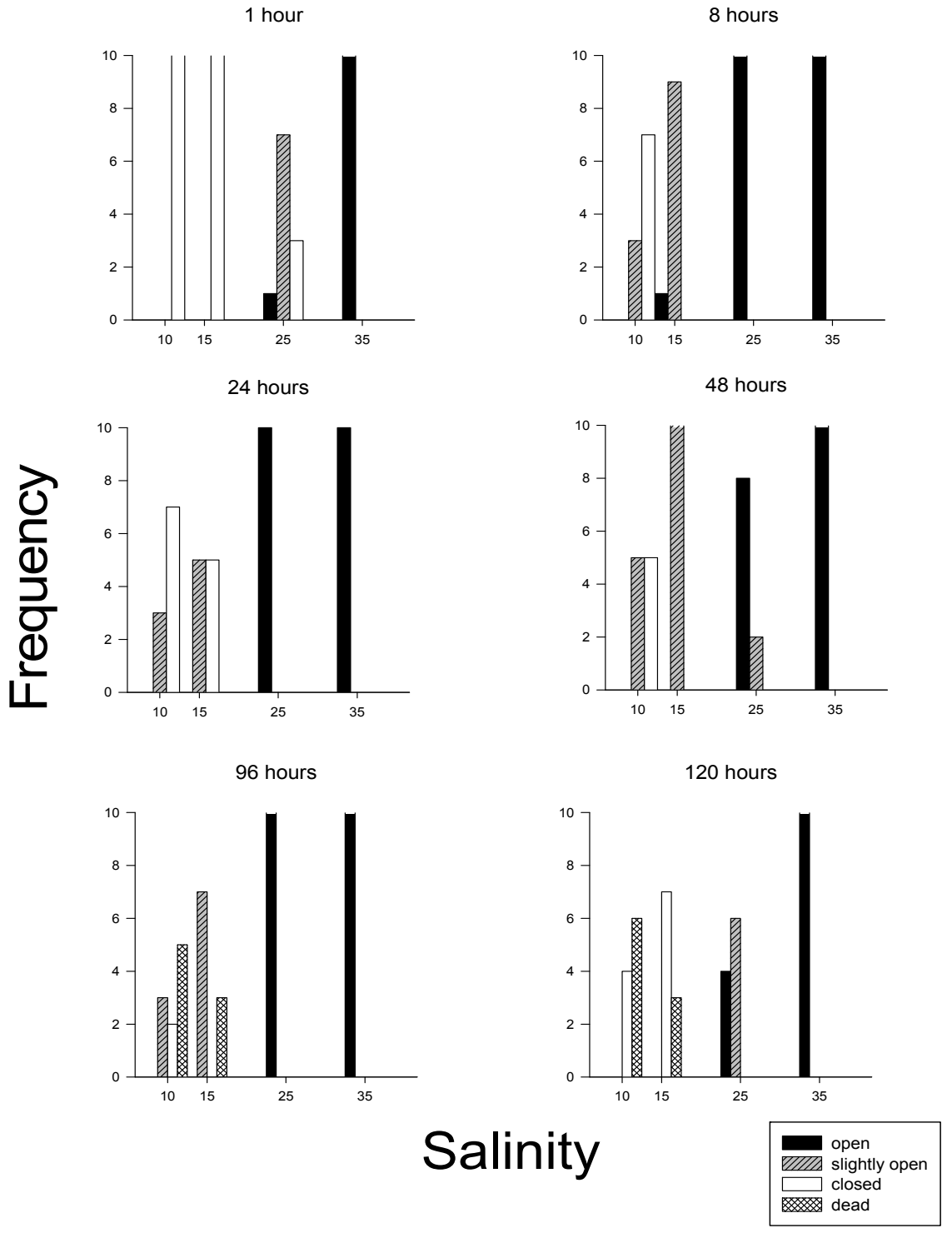

Figure 3. Results of the valve closure experiment in response to acute salinity decreases grouped by time. At all time periods valve closure response at salinities of 10 and 15 were significantly different from the response at 25 and 35 ($p < 0.0005$).

in the salinity tolerance of the two species may ultimately result in resource partitioning.

Hemolymph osmolality of the eastern oyster *C. virginica* tracked all treatment salinities (down to 5) within 1 week of exposure. Although the green mussel's hemolymph tracked salinities of 15 and above within 48 h, it remained hyperosmotic at salinities below 15, which is consistent with prolonged valve closure in response to stressful salinity conditions. Mussel mortalities observed at low salinities suggest that, not only are the mussels unable to reach osmotic equilibrium at these salinities, but their behavioral response to salinity stress overrides their need for food and gas exchange. However, the exposure to test salinities was acute in this study, and physiological and behavioral responses to gradual reductions in salinity may be different. Indeed, Shumway (1977a) reported that the ability of hemolymph to track external salinities was partially dependent on the rate of salinity change. Oysters are found throughout Estero Bay where they are subjected to large salinity fluctuations, ranging from 0–40 (Barnes et al. 2007; Volety et al. 2009), whereas, green mussels are currently only found in coastal waters and near the mouths of estuaries where salinities are high (A. K. Volety, personal observation), which is consistent with our results.

Bivalve feeding can be characterized by clearance rate, defined as the volume of water cleared of particles in a given time (Bayne et al. 1976; Hildreth and Crisp 1976) and helps quantify bivalve capacity to maintain critical physiological processes when exposed to environmental stressors. For both oysters and mussels, feeding can occur only when valves are open, exposing incurrent and outcurrent siphons to the external environment. Green mussels have previously been reported to exhibit higher clearance rates than other bivalves in their native range, and these elevated clearance rates have been linked to rapid growth (Hawkins et al. 1998). Our results were consistent with this in that clearance rates of mussels maintained at salinities occurring in their natural habitat (i.e., 25 and 35) were 2 to 3 times greater than that of oysters exposed to the same salinities. However, the clearance rates recorded for green mussels in this study (0.08–1.2 L h^{-1} g^{-1}) were an order of magnitude lower than those of mussels found in Hong Kong (8.6 to 9.7 L $h^{-1}g^{-1}$; Chong and Wang 2001), and were less than half of those of mussels found elsewhere in China (2.62 to 4.21 L h^{-1} g^{-1}; Wang et al. 2005). Similarly, clearance rates for oysters observed in this study (0.09 to 0.43 L $h^{-1}g^{-1}$) were also lower than previously reported in the literature which range from 1.21–2.18 L $h^{-1}g^{-1}$ (Riisgård 1988; Grizzle et al. 2008) and up to 6.4 L $h^{-1}g^{-1}$ under flow-through conditions (Newell and Koch 2004).

In the present study, although mussels and oysters were similar in shell lengths, dry tissue weight of mussels was nearly double that of oysters (3.14±0.22 and 1.90±0.10 g, respectively). Because increased tissue mass means greater metabolic demands, and therefore a higher demand for food, a higher clearance rate is expected. In addition to a greater somatic mass, green mussels grow in length at a rate more than double that of oysters: 10–13 mm/month (Lee et al. 1986; Walter 1982; Hawkins et al. 1998) and 2–5 mm/month (Volety et al. 2009) respectively. Increased growth rates will require more energy and thus a need for increased food intake, which is reflected by increased clearance rates.

The determination of clearance rates under different conditions provides valuable information about the ability of a species to inhabit new environments, which may aid in predicting the potential distribution of invading species. Although the results of this study show lower clearance rates than that previously reported in the literature for both species, it allows for a direct comparison between the two and can serve as a prediction for how they may alter feeding behaviour in response to decreased salinities. While comparison of clearance rates among studies is difficult due to variations in methodologies, environmental conditions and seasonality, the purpose of this study was to compare local differences in clearance rates of green mussels and oysters in response to depressed salinities that routinely occur in Southwest Florida estuaries. The trends observed were very clear in supporting a high tolerance to low salinities for oysters and low tolerance for green mussels.

The valve closure behavior observed in this study can explain both reduced clearance rates and the inability to reach osmotic equilibrium when exposed to depressed salinities outside of its optimal range. Mussels remained closed at salinities of 15 and below which supports the hypothesis that mussels were unable to reach osmotic equilibrium due to valve closure. Mortalities observed at salinities of 10 and 15 also support the findings of the osmolality study in which mussels are unable to reach osmotic equilibrium resulting in death, likely due to an inability to open their valves and freely exchange gases.

Local *C. virginica* populations exhibit good growth rates, high recruitment at high densities, and the greatest survival at salinities of 10–28, but they are also successful at feeding in salinities as low as 5 (Loosanoff 1953; Volety et al. 2003). This suggests that oysters should not be restricted by valve closure, that is, they should have efficient clearance rates and the ability to reach osmotic equilibrium even at salinities as low as 10. Because the valve closure experiment was conducted only after observing a dramatic difference between oysters and mussels in both the hemolymph osmolality and clearance rate experiments, only green mussels were tested for valve closure at acute salinity decreases.

While overall *P. viridis* showed a low tolerance to decreased salinities, high clearance rates suggests that, under appropriate environmental conditions, the invasive green mussel could not only be the dominant grazer but, because of rapid growth, may also out compete native bivalves for space, potentially smothering smaller, slower growing species. A similar scenario has been observed with the zebra mussel *Dreissena polymorpha*, which has invaded fresh water lakes in the Northeastern U.S. and with high clearance rates, have impacted native bivalve populations by locally depleting food sources (Baker and Levinton 2003). However, when subjected to salinities prevailing within the estuaries in the wet season (10 and 15), oyster clearance rates were unaffected while mussel clearance rates decreased significantly, though it was similar to that of oysters. Wang et al. (2011) similarly reported a significant reduction in clearance rate of green mussels when salinity was reduced from 30 to 15. Segnini de Bravo et al. (1998) however, found that when subjected to a gradual decrease in salinity (decreasing by 2 every 2 days) their tolerance was expanded to salinities of 0–64. Additionally, Baker et al. (2006) found established green mussel populations throughout Tampa Bay in salinities as low as 14–16 suggesting some adaptive capabilities in invaded regions when compared to the reported salinities in its native range, 19–44 (Vakily 1989; Rajagopal et al. 2006). Seasonal trends in Estero Bay can be in the extreme between the wet and dry seasons with salinities dropping below 10 for extended periods of time in the summer months (Barnes et al. 2007; Volety et al. 2009), which may act as a natural limiting factor for green mussel spread.

Whether or not green mussels can obtain enough nutrition with reduced clearance rates at low salinities remains unclear. Due to their high metabolic needs, lower salinities might disproportionately reduce growth and reproduction relative to slower growing species, thus reducing the probability of long-term survival of local populations. For example, reduced growth rates have been observed for *P. viridis* when subjected to temperatures and salinities outside their optimal range (Chatterji et al. 1984), while oysters reach dense populations with high growth rates at salinities down 10 and are tolerant of the rapidly changes salinities within Estero Bay (Volety et al. 2003). Thus, decreased clearance rate and an inability to reach osmotic equilibrium at depressed salinities indicate that it is unlikely *P. viridis* will compete with oysters within estuaries where salinities frequently drop below 5–10 in the summer wet season, such as Estero Bay.

In Estero Bay, Florida *P. viridis* and *C. virginica* currently occupy distinct habitats that can be partially defined by salinity. Although the spread of *P. viridis* may not be of immediate concern locally, there have been a few isolated sightings of adult green mussels on oyster reefs within the Caloosahatchee and Estero Bay estuaries (Volety et al. unpublished). Green mussels do not form permanent reefs and lack the extensive three-dimensional structure created by oyster reefs. If *P. viridis* is able to thrive under estuarine conditions and displace the native oyster, it could reduce essential habitat for many ecologically and economically important species. The loss of oyster reefs due to invasion of green mussels would not only destroy habitat but also reduce ecological benefits such as shoreline protection and sedimentation. Although results of the present study demonstrate a limited tolerance of green mussels to low salinity, these experiments have been conducted as acute exposure events. It is unclear what effects would be seen if salinity was gradually decreased over time. In some portions of the estuary, a gradual salinity decrease is more common than sudden changes, which could allow settlement and higher survival. If their spread continues and green mussel populations begin to threaten oyster reefs, this information could be used by resource managers to predict areas within the estuary that are vulnerable to green mussels invasion. Future studies should include measurements of these physiological responses during prolonged exposure to decreased salinities following a gradual salinity decrease. In addition, investigation of the effects of other factors such as temperature and desiccation (two

major stressors observed among the shallow oyster reefs within the bay) on green mussel survival will allow for a more accurate prediction of their potential spread and allow for the estimation of potential competition with native bivalves under environmentally realistic conditions.

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