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SURVIVAL, ACID–BASE BALANCE, AND GAPING RESPONSES OF THE ASIAN OYSTER *CRASSOSTREA ARIAKENSIS* AND THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* DURING CLAMPED EMERSION AND HYPOXIC IMMERSION

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ABSTRACT The eastern oyster *Crassostrea virginica* has a remarkable ability to withstand low oxygen conditions; however, many of the biochemical mechanisms by which that tolerance is accomplished remain poorly understood. In addition, little is known about hypoxia tolerances and adaptations of the Asian oyster *Crassostrea ariakensis*. By comparing these closely related species, we may learn more about the physiological mechanisms responsible for the hypoxia tolerance of *C. virginica*. We assessed the time to mortality and gaping responses in both species during hypoxia. Adult *C. ariakensis* died earlier than *C. virginica* and gaped more often and wider than *C. virginica*. Gaping by either species was associated with acidification of the ambient seawater. We also compared the hemolymph pH of emersed and clamped *C. virginica* and *C. ariakensis* between 0 h and 24 h. In both species, hemolymph pH declined over time, and *C. ariakensis* hemolymph became significantly more acidic throughout the study period than that of *C. virginica*. The infection levels of *Perkinsus marinus* observed in our samples were not correlated with hemolymph pH changes in either species. Our findings demonstrate fundamental differences in survival and suggest important physiological differences exist between these species.

KEY WORDS: acid–base balance, *Crassostrea virginica*, *Crassostrea ariakensis*, emersion, hypoxia, survival

INTRODUCTION

Low dissolved oxygen levels are common in estuarine systems such as the Chesapeake Bay, particularly in summer months, when considerable oxygen depletion occurs in the deeper stratified waters (Taft et al. 1980, Breitburg 1992). When this depletion results in an oxygen concentration less than 2 mg/L, the environment is classified as hypoxic. The temporal and spatial severity of hypoxic episodes in the Chesapeake Bay has been exacerbated by anthropogenic sources such as nutrient input and contaminants (Mackiernan et al. 1983, Diaz 2001, Hagy et al. 2004, Kemp et al. 2005, Diaz & Rosenberg 2008). Because near-bottom waters are more prone to hypoxia as result of decomposition of organic matter and stratification, benthic communities are affected disproportionately by hypoxia (Weisburg et al. 1997, Rabalais et al. 2002, Dauer et al. 2008). Sessile benthic organisms, such as oysters, are particularly vulnerable to low oxygen because they cannot move to evade hypoxic events.

Many sessile members of the benthic community, including the eastern oyster *Crassostrea virginica* (Gmelin, 1791), have evolved metabolic adaptations that facilitate survival in low-oxygen environments. One such adaptation in *C. virginica* is its demonstrated ability to decrease its oxygen consumption and reduce its metabolic rate up to 90% when exposed to hypoxia and anoxia (Shumway 1982; Shumway & Koehn 1982, de Zwaan 1983, Willson & Burnett 2000). Furthermore, during exposure to a hostile environment (e.g., hypoxia, anoxia, freshwater) or predators, *C. virginica* and other bivalves often close their valves for extended time periods to isolate their tissues from the stressful external environment. This, however, has been shown to induce hypoxia and acidosis within the hemolymph and other tissues (Moon & Pritchard 1970, de Zwaan & Wijsman 1976, Widdows et al. 1979, Akberali & Black 1980, Akberali & Trueman 1985, Truchot 1990, Byrne et al. 1990, Cochran & Burnett 1996, Paynter 1996, Burnett 1997, Michaelidis et al. 2005a). Reducing metabolic rate can decrease the accumulation of metabolic byproducts, thus limiting acidosis, and some bivalves, including oysters, are able to mobilize carbonate from their calcium carbonate shells, increasing the buffering capacity of the hemolymph and other tissues (Dugal 1939, Crenshaw & Neff 1969, Byrne et al. 1989, Dwyer & Burnett 1996, Michaelidis et al. 2005b).

Although the hypoxia tolerance of *Crassostrea virginica* has been well documented, the adaptations that give rise to this tolerance are not completely understood. Conversely, *Crassostrea ariakensis* (Fujita, 1913), although closely related to *C. virginica* (Foighil et al. 1995, Reece et al. 2008), has shown poor hypoxia tolerance as spat (Matsche & Barker 2006) and larvae (North et al. 2006). In this study, we compare the gaping behavior (gape distance, gape frequency, and role of gaping on ambient water pH) and time to mortality of adult *C. ariakensis* and *C. virginica* during hypoxic exposure. In addition, because prolonged valve closure results in pallial and tissue hypoxia (Moon & Pritchard 1970, Truchot 1990), and differences in mortality and gaping likely indicate different physiological responses to low oxygen, we assessed the change in hemolymph pH over time in both *C. ariakensis* and *C. virginica* during clamped emersion. Because it has been reported that *Perkinsus marinus* infection may impair the ability of *C. virginica* to maintain acid–base homeostasis (Dwyer & Burnett 1996), we also tested for any correlations between *P. marinus* infection and hemolymph acid–base balance in *C. virginica* and *C. ariakensis*. Findings from this research may shed light on the behavioral and biochemical responses to hypoxia in bivalves and thereby aid in understanding the physiological basis for hypoxia tolerance.

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MATERIALS AND METHODS

Oyster Collection

Specimens of both *Crassostrea ariakensis* and *Crassostrea virginica* were obtained from the University of Maryland's Center for Environmental Science at Horn Point Laboratory Oyster Hatchery in Cambridge, Maryland. Oyster shell height for mortality studies (next section) was approximately 80 mm and, for all other studies, was approximately 100 mm. Oysters were transported to the University of Maryland College Park campus and acclimated without food for a week in aerated seawater (25°C; salinity, 15) before testing. For biosecurity reasons, triploid oysters were available during the mortality study, so we quantified hypoxic mortality in both triploid and diploid oysters. In all other experiments, only diploid oysters of both species were studied.

Hypoxia-Induced Mortality

One-liter glass Mason jars each containing a single oyster were filled with artificial Seawater (approximately 20°C; salinity, 10) and was subsequently sparged with nitrogen gas until the dissolved oxygen level was 0.5 mg/L. Dissolved oxygen was measured using a benchtop oxygen probe (model 5000; Yellow Springs Instrument Company, Yellow Springs, OH). After sparging, the chambers were quickly sealed. Control chambers included oysters immersed in normoxic water as well as chambers containing seawater only that were opened at 1, 3, 5, and 15 days to confirm the low dissolved oxygen level during the course of the experiment.

Oyster mortality was assessed at least daily by examining oyster responsiveness. Any oyster that was observed to be gaping was tested for responsiveness by tapping on the jar with a large metal bolt or jostling the chamber; if the oyster remained gaping, we classified the unresponsive oyster as dead and recorded the date and time. If the oyster responded, it remained in the study. Dry weight was measured by placing oyster tissue in a drying oven at 60°C for 72 h, and shell height was recorded for each oyster.

Hypoxia Gaping Response

After the acclimation period, oysters were soaked in 9:1 water–chlorine bleach (HOCl) solution, rinsed in freshwater, and scrubbed to remove epifaunal and burrowing organisms. Individual oysters were then placed into half-inch Plexiglas respiration chambers (approximately 1,790 mL) filled with artificial seawater of salinity 15 at 25°C. The seawater within the chamber was monitored with a dissolved oxygen probe (model 550A; Yellow Springs Instrument Company) and sparged with nitrogen gas until the oxygen concentration was less than 0.5 mg/L. The chamber was then sealed immediately. The gaping behavior was assessed by visual inspection at the ventral shell margin at 5 min, 10 min, 30 min, 1 h, and 2 h, and then every other hour for the first 12 h, and every 24 h until the oysters did not respond to the external stimulus of knocking on the exterior of the chamber, and were then classified as dead. At each observation interval, the presence or absence of gaping and the gape width, defined as the distance (in millimeters) between the left and right valve of a gaping oyster at the ventral shell margin, was recorded. In addition, at each sample period between 8 h and 72 h after hypoxic immersion, the pH of the seawater surrounding each live oyster was analyzed using a Cole Parmer combination micro pH electrode and Orion Two Star pH meter. Controls included chambers with sparged seawater without oysters, with sparged seawater with either *Crassostrea ariakensis* or *Crassostrea virginica* valves (to account for any bacteria or cryptogenic species not removed during bleaching and scrubbing), and with live oysters immersed in normoxic chambers.

Clamped Emersion: Hemolymph Extraction and Analysis

Oysters were clamped closed at the ventral shell margin using 2-inch binder clips while underwater to prevent normal ventilation and/or gaping, and placed on the laboratory bench at 25°C. Oysters were sacrificed at 0, 2, 4, 6, 8, 10, 12, or 24 h. Hemolymph was sampled by quickly notching the anterior and posterior edges using a grinding wheel (approximately 5–15 sec) and draining the pallial fluid. A 5-mL glass syringe with a 19-gauge needle was inserted immediately into the adductor muscle sinus to collect hemolymph. As the extracted hemolymph was decanted into the bottom of a 1.5-mL Eppendorf tube, hemolymph pH was measured immediately using a Cole Parmer combination micro pH electrode/Orion Two Star pH meter. Each oyster was sampled only once to limit stress, which has been shown to influence hemolymph pH (Jones et al. 1993).

Diagnosis of **Perkinsus marinus** *for Hemolymph Studies*

Because infection by *Perkinsus marinus* (Mackin, Owen & Collier, 1950) Levine, 1978 has been shown to influence the ability of *Crassostrea virginica* to maintain an acid–base balance (Dwyer & Burnett 1996), all oysters were diagnosed for *P. marinus*. Gill, mantle, and rectal tissue samples were excised and incubated in Ray's fluid thioglycolate media and analyzed 5–7 days later in accordance with the procedures of Ray (1952, 1966), as modified by Burreson (2009). Disease intensity was quantified on a scale of 0–5 based on the microscopic abundance of *P. marinus* cells (Burreson 2009). Any oyster with a *P. marinus* score greater than 1 was excluded from hemolymph analysis. Diagnosing *Perkinsus marinus* is a lethal technique and hence was performed after pH analysis. Because *C. virginica* had higher *P. marinus* scores than *Crassostrea ariakensis*, more samples of *C. virginica* were excluded from analysis than *C. ariakensis*, resulting in an unequal number of replicates.

Statistical Analysis

We used a 2-way analysis of variance to compare time to mortality between species and ploidy rather than an Analysis of Covariance because there was little variation in oyster dry weight (0.9–1.1 g) and samples were not significantly different than 1 g.

For the gaping study, repeated-measures analysis of variance was performed to determine whether *Crassostrea ariakensis* and *Crassostrea virginica* gape width differed during the first 72 h of hypoxic exposure and whether the pH of the ambient seawater surrounding oysters that were gaping was less than that of oysters that were not gaping. A 1-way analysis of variance was used to test whether gaping frequency differed between species.

For the hemolymph study, a 2-way fixed-factor analysis of variance was performed to determine whether species or time emersed affected hemolymph pH, or whether there was an interaction between time and species. We also performed 2-sided

contrasts between each species at each time level to determine at what times these species' hemolymph deviated from one another.

To test the influence of *Perkinsus marinus* infection on acid–base balance, a 3-way analysis of variance was used to compare the hemolymph pH of oysters with low-moderate and moderate Dermo (infection intensity, 3; on a scale from 0–5 (Burreson 2009)) with that of oysters with no to light infection rates (infection intensity, ≤1 (Burreson 2009)) for 0–24 h in both species.

All statistics were performed using R (http://www.r-project.org/).

RESULTS

Mortality

For both diploid and triploids, *Crassostrea virginica* survived longer than *Crassostrea ariakensis* during hypoxic exposure ($P_{\text{diploid}} = 0.023$, $n_{C.\ ariakensis\ diploid} = 19$, $n_{C.\ virginica\ diploid} = 19$, $P_{\text{triploid}} < 0.001$, $n_{C.\ ariakensis\ triploid} = 19$, $n_{C.\ virginica\ triploid} = 14$). There was a significant interaction between species and ploidy on time to mortality ($P = 0.011$). Within *C. virginica*, there was no effect of ploidy on time to mortality; triploids exhibited a mean mortality day of 14.8 ± 1.1 (±SEM) compared with 17.0 ± 1.8 days for diploids ($P = 0.349$). However, triploid *C. ariakensis* exhibited a mean mortality day of 3.7 ± 0.3, which was significantly earlier than diploid *C. ariakensis* (12.2 ± 0.9 days; $P < 0.001$).

Gaping During Hypoxia

During hypoxic immersion, *Crassostrea ariakensis* gaped during 55.6 ± 7.1% (mean ± SEM) of the observation intervals throughout the study, which was significantly more often than *Crassostrea virginica* (17.1 ± 5.6%, $P < 0.001$, $n_{C.\ ariakensis} = 9$, $n_{C.\ virginica} = 9$; see Fig. 1A for gaping differences at each time level). Gaping during the first 6 h occurred almost exclusively by *C. ariakensis* because very few *C. virginica* gaped. During hours 6–48, about 20% of the *C. virginica* gaped whereas *C. ariakensis*

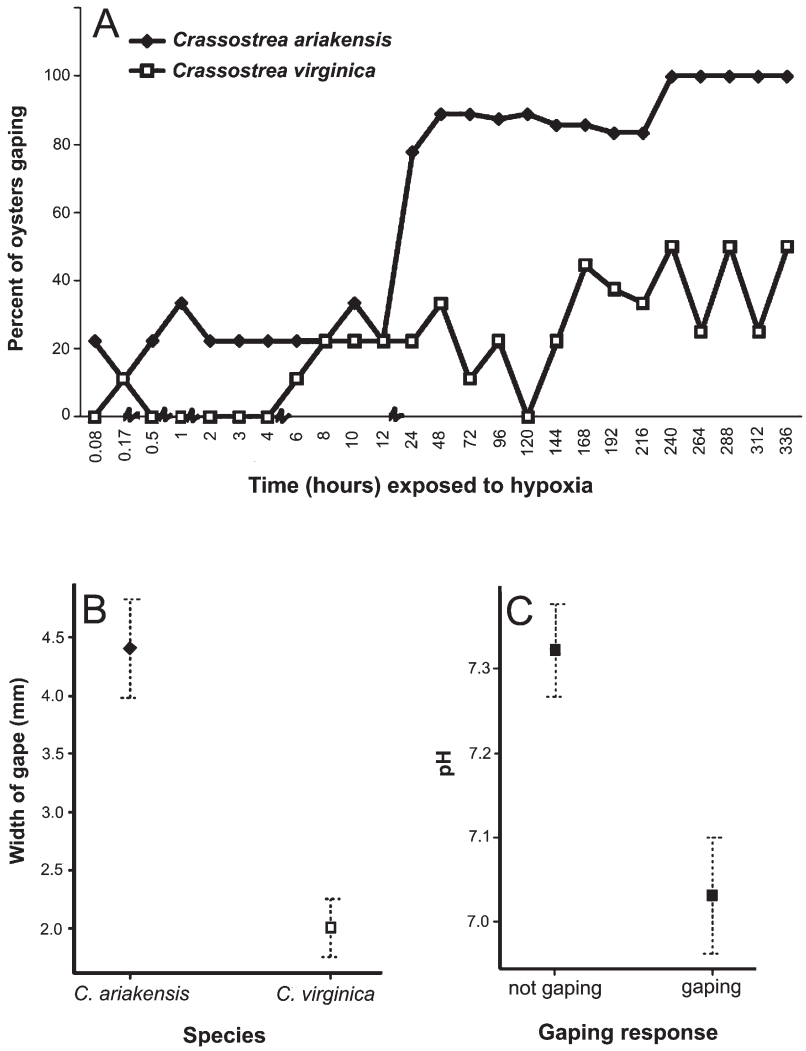

Figure 1. Gaping responses in *Crassostrea ariakensis* and *Crassostrea virginica* after hypoxic immersion. (A) The percent of oysters ($n = 9$) of each species gaping at each time level after hypoxic immersion within the first 14 days of hypoxic immersion. The *x*-axis represents the duration, in hours, that oysters were exposed to hypoxic water. (B) The average gape width (in millimeters) for each species during the first 72 h of hypoxic exposure, when they were exhibiting live gaping responses. (C) The mean pH of the water surrounding live gaping and nongaping oysters of both species after 8–72 h in hypoxic water. (B, C) Error bars indicate SEM.

gaping ranged from 20% to more than 80%. From 48 h to the end of the experiment, gaping by *C. ariakensis* remained above 90% and varied little whereas gaping by *C. virginica* generally remained in the 20–50% range and varied substantially. During the first 72 h, when the oysters did gape, *C. ariakensis* displayed a mean gape of 4.1 ± 0.4 mm, which was significantly wider than the 2.0 ± 0.7 mm gape of *C. virginica* ($P < 0.006$; Fig. 1B). However, when the oysters did not respond to external stimuli and were assumed to be dead, *C. ariakensis* and *C. virginica* gaped equally as wide ($\mu_{C.\ ariakensis} = 10.6 \pm 1.2$ mm, $\mu_{C.\ virginica} = 9.3 \pm 1.4$ mm, $P = 0.493$). In addition, the seawater surrounding live gaping oysters between 8–72 h, without regard to species, was significantly more acidic (pH 7.06 ± 0.08) than the seawater surrounding oysters that were not gaping (pH 7.35 ± 0.06. $P = 0.012$; Fig. 1C), but the pH of the seawater surrounding gaping *C. virginica* (7.11 ± 0.11) was not significantly different than that of gaping *C. ariakensis* (7.00 ± 0.09, $P = 0.205$).

Hemolymph pH After Emersion

The longer an oyster was emersed and clamped, the more acidic its hemolymph pH became for both species ($P < 0.001$; Fig. 2). The hemolymph pH of *Crassostrea ariakensis* became significantly more acidic than that of *Crassostrea virginica* ($P < 0.001$; Fig. 2). Despite the fact that there was no significant interaction between time emersed and species on hemolymph pH ($P = 0.293$), 2-sided *a priori* contrasts revealed that differences in hemolymph pH between *C. ariakensis* and *C. virginica* occurred at hours 8 ($P = 0.002$), 10 ($P < 0.001$), and 12 ($P = 0.035$) after being clamped (Fig. 2). Therefore, for both species, time clamped and emersed led to an acidic shift in hemolymph pH, with *C. ariakensis* exhibiting a hemolymph pH more than 0.6 less than *C. virginica* at 10 h ($\mu_{C.\ ariakensis} = 6.15 \pm 0.11$; $\mu_{C.\ virginica} = 6.78 \pm 0.16$).

Role of Perkinsus marinus *Infection on Acid–Base Balance*

Moderate Dermo (score, 3 of 5) was the highest intensity observed in any of samples. There was no significant interaction between clamped emersion time level, species, and Dermo infection level on hemolymph pH ($P = 0.140$). In addition, there were no significant 2-way interactions ($P > 0.05$). Furthermore, disease had no influence on hemolymph pH in either species ($P = 0.579$). Oysters with low infection had an average hemolymph pH of 6.75 ± 0.04, and oysters with greater infection levels exhibited a pH of 6.81 ± 0.05.

DISCUSSION

The oyster *Crassostrea ariakensis* showed substantially less tolerance to hypoxia than *Crassostrea virginica*. When gaping responses were not inhibited by clamping, *C. ariakensis* gaped wider and more often than *C. virginica* during hypoxic immersion. Furthermore, gaping was associated with the acidification of seawater surrounding the oyster in the chamber, regardless of species. When we prevented gaping by clamping the valves closed, *C. ariakensis* hemolymph pH declined to nearly 6.1 within 10 h, which was significantly more acidic than that of *C. virginica* during any period of the study (minimum at hour 12; 6.57 ± 0.15). In addition, at the level of infection in our samples, *Perkinsus marinus* infection did not influence the ability to maintain hemolymph acid–base balance in either species. Ultimately, our findings indicate these species differ in their ability to survive during hypoxia, and engage in different behavioral (gaping) and metabolic (hemolymph pH) responses to low-oxygen environments.

Our findings on adult *Crassostrea ariakensis* and *Crassostrea virginica* hypoxic survival are in agreement with other studies on spat (Matsche & Barker 2006) and larvae (North et al. 2006). When examining juvenile oyster mortality during hypoxia,

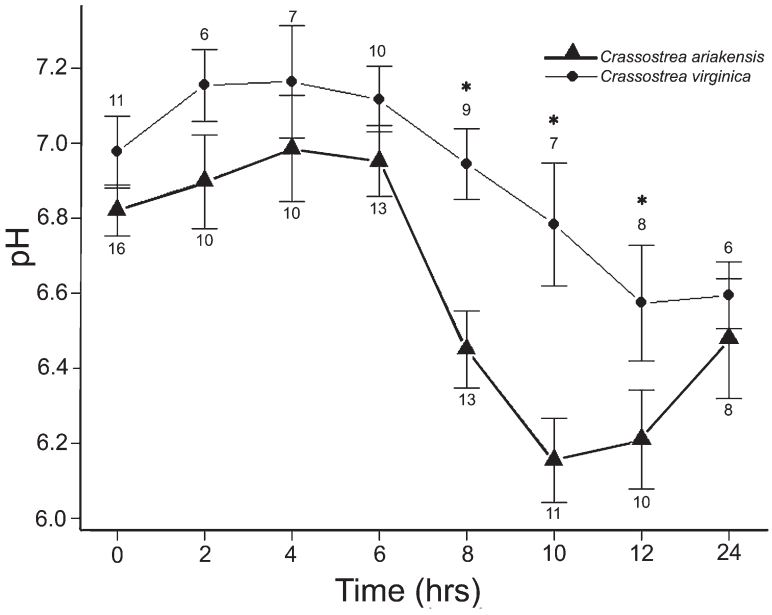

Figure 2. Hemolymph pH of *Crassostrea virginica* and *Crassostrea ariakensis* after clamped emersion. The *x*-axis represents the time, in hours, that the valves of each oyster were clamped closed and placed on a laboratory bench, preventing normal gaping. Error bars indicate SEM. Numbers above (*C. virginica*) and below (*C. ariakensis*) represent sample size at each time level. Asterisks represent statistical significance at an alpha of 0.05 using 2-sided contrasts between species at the indicated time levels.

Matsche and Barker (2006) found that *C. ariakensis* died approximately 35% earlier than *C. virginica*, which is similar to the 28% earlier mortality we observed in *C. ariakensis* compared with *C. virginica*. However, triploid *C. ariakensis* died 75% earlier than triploid *C. virginica* during hypoxia. Interestingly, the time to mortality was not statistically different for triploid or diploid *C. virginica*, but in *C. ariakensis* triploids died more than 3 times earlier than diploids. This result provides valuable information about differences in hypoxia tolerance based not only on species, but also on polyploidy. These findings on differential survival can be applied to restoration efforts. For instance, if *C. ariakensis* is introduced into the Chesapeake Bay or other hypoxia-prone regions, its introduction should be constrained to shallow, well-flushed areas that maintain high dissolved oxygen levels year-round. In addition, the difference in hypoxic survival between species may also explain early reports of *C. ariakensis* die-offs observed when placed intertidally (Kingsley-Smith & Luckenbach 2008, Kingsley-Smith et al. 2009) and may explain why *C. ariakensis* has such a short shelf life compared with *C. virginica* (Fisher 2006).

The oysters *Crassostrea ariakensis* and *Crassostrea virginica* demonstrated different gaping responses to hypoxia. Although, in general, *C. virginica* remained closed, *C. ariakensis* gaped more often and wider during hypoxic immersion. This difference is not likely the result of different ligament physiology and elasticity between species, as the gaping width of both species was larger and not significantly different between species in dead (unresponsive) oysters. Thus, both species appear mechanically capable of gaping to the same extent, yet exhibited different gaping widths during early hypoxia. Gaping was also correlated with the acidification of the seawater in the chamber. Thus, our results suggest that extensive gaping may be a strategy to reduce metabolic acidosis by allowing acidic products to escape from the tissues into the external environment.

When the valves are closed for any reason (e.g., during emersion, predator attack, or environmental stress such as hypoxia or pollution), metabolites can accumulate within tissues (Widdows et al. 1979, Akberali & Black 1980, Akberali & Trueman 1985). Because gaping may facilitate the release of acidic end products to the external environment, clamping shells likely inhibits this exchange and results in acidic and hypoxic tissues (Moon & Pritchard 1970, de Zwaan and Wijsman 1976, Akberali & Black 1980, Akberali & Trueman 1985, Truchot 1990). The lower hemolymph pH exhibited by clamped *Crassostrea ariakensis* in comparison with *Crassostrea virginica* is likely the result of several potential mechanisms: differing rates of metabolic byproduct accumulation, differing capabilities of carbonate buffering, and a combination of byproduct accumulation and buffering ability.

First, metabolic byproducts, including carbon dioxide, could be accumulating in the hemolymph and other tissues to a greater extent in *Crassostrea ariakensis*. Carbon dioxide is produced during aerobic respiration and can react with water to lower pH. During anaerobic respiration, oysters produce alanine and succinate, which although still acidic may be more advantageous for acid–base balance compared with the typical mammalian anaerobic end product of lactate (Collicutt & Hochachka 1977, Hochachka 1980, Eberlee & Storey 1984, Storey 1993). Differing metabolic rates or capabilities between *C. ariakensis* and *Crassostrea virginica* could explain differing tissue pH resulting from the accumulation of acidic metabolites. Studies by Harlan (2007) and Lombardi (2012) found that *C. ariakensis* and *C. virginica* adults had similar aerobic metabolic rates; however, differences may exist in the metabolic pathways and rate used during hypoxic stress. Burnett and Stickle (2001) found that the majority of *C. virginica* energy was obtained through anaerobic respiration during hypoxic stress. Furthermore, when exposed to hypoxic environments *C. virginica* has demonstrated an ability to reduce its aerobic metabolic rate to only 10% of its normoxic metabolic rate (Willson & Burnett 2000). However, little is known about the anaerobic or hypoxic-induced metabolic rate of *C. ariakensis*. In addition, Harlan (2007) proposed, based on findings of different anaerobic intermediate substrates and end products, that *C. ariakensis* and *C. virginica* species may engage in different anaerobic pathways. If *C. ariakensis* does not engage in substantial hypoxia-induced metabolic depression, or the hypoxic metabolism is less efficient, this could lead to the accumulation of more acidic metabolic byproducts, resulting in reduced hemolymph pH, which could lead to gaping as a means to release acids.

Different hemolymph pH could also be explained by differences in the buffering capacities of the species, because *Crassostrea ariakensis* and *Crassostrea virginica* shells may have different properties, including density and load compression strengths (Newell et al. 2007; Lombardi et al. unpubl. data). Some molluscs, including *C. virginica*, react to metabolite-induced acidosis by mobilizing calcium carbonate from their valves to buffer their tissues (Dugal 1939, Akberali & Trueman 1985, Byrne & McMahon 1994, Dwyer & Burnett 1996, Michaelidis et al. 2005a). It is therefore possible that the difference in hemolymph pH between species can be explained not only by different rates of accumulating carbon dioxide and other acidic products, but also different rates of calcium carbonate mobilization into the hemolymph during clamped emersion.

Last, the difference in hemolymph pH between species may be a combination of differences in acidic metabolites and calcium buffering. For instance, Crenshaw and Neff (1969) reported increased calcium, carbon dioxide, and hydrogen ion concentrations when valves were closed in the hard clam *Mercenaria mercenaria* (Linnaeus, 1758). Further studies quantifying hemolymph biochemistry, and calorimetric anaerobic respiration studies during hypoxic exposure are needed to understand the mechanisms for the observed differences in hemolymph pH when clamped and emersed.

The oysters *Crassostrea ariakensis* and *Crassostrea virginica* have different life histories and preferred habitats, which may have contributed to the evolution of different mechanisms to respond to and to survive hypoxia. Populations of *C. virginica* have not only evolved in regions characterized by natural episodes of hypoxia, but in some southern regions, *C. virginica* reefs are mainly intertidal, and therefore the oysters routinely undergo regular tidal emersion. In contrast, *C. ariakensis* has been found in the sublittoral zone up to 10 m deep and has been reported to be unable to tolerate aerial emersion (see the review by Zhou and Allen (2003), Kingsley-Smith & Luckenbach 2008). These physiological differences observed during clamped emersion and hypoxia, coupled with differing habitats and distribution may help explain why *C. ariakensis* is not as well-adapted to hypoxia as *C. virginica*.

We found that at the level of disease observed in the samples, which is comparable with the level observed at most reefs in the Maryland portion of the Chesapeake Bay (Paynter et al. 2010;

Lombardi pers. obs.), *Perkinsus marinus* infection intensity did not influence the ability to maintain a hemolymph acid–base balance in either species. Although this finding differs from Dwyer and Burnett (1996), who found the *Crassostrea virginica* acid–base balance was impaired by *P. marinus* infection, this is likely a function of different infection intensity. In our samples, even the most infected oysters exhibited only an infection intensity of moderate, which equated to a score of 3 of 5 (Burreson 2009), whereas Dwyer and Burnett (1996) used oysters with a high infection intensity corresponding to a score of 4–6 on a scale of 0–6 (Quick & Mackin 1971). Thus, the influence of *P. marinus* infection on acid–base balance is likely a function of disease intensity, and at lower infection levels, as is typical in the upper Chesapeake Bay, there does not appear to be an effect in either *C. virginica* or *Crassostrea ariakensis*.

Comparative research into *Crassostrea ariakensis* and *Crassostrea virginica* physiology and biochemistry to identify convergences or divergences in responses may yield insight into adaptations responsible for the remarkable hypoxia tolerance of *C. virginica*. Our findings suggest that *C. ariakensis* is less hypoxia tolerant than *C. virginica*. Furthermore, *C. ariakensis* may be more dependent on gaping for acid–base homeostasis than *C. virginica*, because gaping was associated with the acidification of the external environment and *C. ariakensis* gaped both more often and wider, and its hemolymph pH was more affected by clamping. These findings indicate significant differences in the tolerance and physiological response of these oysters to low oxygen, which in turn may help illuminate adaptations enabling *C. virginica* to survive weeks without oxygen.

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